Low-temperature calorimetry of biological macromolecules

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This review shows the modern methods of calorimetry to be highly informative in studying the conformational properties of biopolymers when applied over a broad range of temperatures including the liquid-helium region, i.e., at temperatures far from the region of functioning of the macromolecules. Such an approach is typical in solving problems of the physics of the condensed state, since it allows one to reveal the characteristic features of a material by analyzing its physical properties under extreme external conditions of the environment (low and high temperatures, high pressures, etc.). This article collects and classifies the experimental data on the heat capacity of amino acids, peptides, polypeptides, proteins, and nucleic acids, and correlates them with the existing theories of the heat capacity of highly anisotropic structures at low temperatures. The observed low-temperature phase transitions in biopolymer-solvent systems are described, and the thermal properties of biopolymer chains are discussed in the light of the effect of the solvent and of dissolved ions of salts.

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CONTENTS

"The problem is as follows: does the current knowledge of the laws of inanimate nature suffice to describe all the phenomena characteristic of the living world?... In chains of sufficient length made of atoms alternating according to definite rules, we have no data to deny that a new property might not arise that is analogous to that of autoreproduction in living nature. In individual atoms and in simple molecules such a property might be undetectable."

P. L. Kapitsa
("Experiment, theory, practice (articles, lectures)")

1. INTRODUCTION

The physics of biopolymers, especially the theory of biological macromolecules and their ordered supermolecular structures (fibers, nucleoprotein complexes, membranes, etc.) has been developing on the basis of the advances of theoretical solid state physics, statistical physics, and thermodynamics. Currently the front line of studies of the physical properties of the most important biopolymers (proteins and nucleic acids) is rather broad. This consists in the detailed x-ray structural analysis (at a resolution of ~1–2 Å) of the spatial structure of proteins and nucleic acids, their conformational features, the character of the intramolecular conformational transformations, and the establishment of the relationship between the structure of biopolymers and their function, etc. Especial attention has been paid in studying the physical properties of biopolymers to the molecular mobility of macromolecules (the so-called "dynamic" properties of the macromolecules of proteins and nucleic acids), to the nature of the forces that stabilize the spatial structure in solution, and to the intramolecular rearrangements of macromolecules caused by various external agencies (temperature, pH, pressure, etc.)

People study the thermal properties of biopolymers in solutions and in the "solid" state [oriented films, protein crystals in the mother liquor, and dehydrated (dried) specimens], and in particular, the heat capacity over a broad range of temperatures. This allows us to obtain experimental values of the fundamental thermodynamic parameters of these compounds and to relate them to the dynamic properties and to the conformational features of the macromolecules. The methods of thermal measurements of biological objects are

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being ever more widely applied in molecular-biological studies (see, e.g., the reviews[1-7]). This has arisen both from the invention of precise new-generation thermophysical instruments (including scanning differential microcalorimeters[7-13]), and from the development of configurational statistics and the statistical mechanics of macromolecules,[14-16] the statistical-thermodynamical theory of phase transitions in biopolymers,[15,17-21] and the theory of solutions of macromolecules.[22,23]

As this review will make evident, the modern methods of calorimetry prove extremely informative also when applied for studying the properties of biopolymers and their synthetic analogs over a broad temperature range including the liquid-helium region, i.e., at temperatures far removed from the region of biological function of the macromolecules. Such an approach is typical in solving problems of physics of the condensed state, since it allows one to reveal the characteristic features of a material by analyzing its physical properties under extreme external conditions of the medium (low and high temperatures, high pressures, etc.).

The heat capacity of the most important biopolymers at low temperatures (2 °K and up) is being studied intensively at present in several scientific centers in the USSR,[24-30] the USA,[31-45] and France.[46,47] By the sixties, the thermodynamic quantities had been obtained for practically all the amino acids (the 20 "building blocks" from which the structure of proteins is built) over the temperature range 10–350 °K. (This has involved mainly the efforts of the group of Professor Hutchens at the University of Chicago[31-37]).

The low-temperature heat capacity has been studied for a series of synthetic polypeptides that model the structures of proteins,[40-44,46,47] and for globular[3] fibrous proteins.[24-27,48] Studies have begun on the low-temperature heat capacity of nucleic acids (DNA).[29,30] The physics of biopolymers at low temperatures is currently arising as a new field of studies in molecular biophysics (we might call this field "molecular cryobiophysics"). It is based on the enhanced experimental potentialities of the new precision calorimeters, the sharp decline in the expenditure of costly and difficult to obtain materials, the development of electronic systems for experiment automation, and the rapid, accurate processing of data on computers. It is of no small importance that the theories created for the quantum-mechanical description of the low-temperature thermal properties of strongly anisotropic solids (the so-called "layer and chain structures") describe well in a number of cases the behavior of biopolymers in the "solid" state at low temperatures.

Purpose of the review

This review attempts to assemble and discuss the varied experimental data on the physics of biopolymers, on the conformational analysis and on the thermal properties of macromolecules at low temperatures that might reveal new characteristic features of the structure of biopolymeric chains.

At the same time, the review tries for the first time to assemble and classify the experimental data on the heat capacity of amino acids, peptides, polypeptides, proteins, and nucleic acids and to correlate them with the existing theories of the heat capacity of highly anisotropic structures at low temperatures.

We can ask the following questions in analyzing the problem of the behavior of biopolymer chains at low temperatures:

1) Which phenomena observable at low temperatures can reveal the specifics of structure of biopolymer chains?

2) Does the character of the temperature-dependence of the heat capacity differ for polypeptides of differing conformations? If so, can we employ heat-capacity data to infer the presence of various helical configurations in biopolymers?

3) Can we distinguish the active conformation of a macromolecule ("helix") from the biologically inactive-denatured form ("coil") by the behavior of their heat capacities in the low-temperature region? What is the entropy difference between the coil and helical conformations of macromolecules?

4) Can we calculate the entropy of a protein from its amino-acid composition?

5) What role does the solvent (water) play in the thermal properties of biopolymer chains at low temperatures?

6) What are the characteristics of the low-temperature phase transitions and thermal anomalies in biopolymers and what is their relation to the biological function (distinctive feature) of the given polymer?

Unfortunately, we have not been able to treat all these problems in equal detail, owing to lack of the pertinent experimental data. We hope that the latter will be obtained in the near future.

Before starting to present the concrete results of measuring the heat capacity of biomacromolecules in the temperature range 2–300 °K, let us take up briefly the features of the structure of biopolymers and their conformational properties and the fundamental assertions of the theory of heat capacity of highly anisotropic materials. This review also includes a description of the major methods applied in experimental studies of the low-temperature heat capacity of biopolymers. Analysis of the existing experimental data shows that the accuracy with which the heat capacity of biopolymers at low temperatures has been measured is rather high. Yet it can be substantially improved at the modern level of methodology of calorimetric experimentation.

2. FEATURES OF THE STRUCTURE OF BIOPOLYMERS AND THEIR CONFORMATIONAL PROPERTIES

a) Proteins

The macromolecules of proteins consist of polypeptide chains, that are packed in space in a strictly defined manner. The polypeptides possess an almost unique combination of structural features. Figure 1

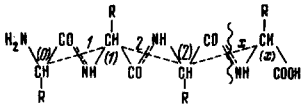


FIG. 1. Polypeptide chain. The partial double-bond character of the amide bonds is shown. The dotted line indicates the virtual bonds linking successive α-carbon atoms. The numbering of the residues (0), (1) ... (x) and of the virtual bonds linking them (1, 2, ..., x) is given.[16]

shows the chemical structure of a polypeptide chain. The repeating unit in polypeptides is the α-amino-acid residue (-NH-CHR-CO-), of which there are twenty. The strictly ordered sequence of different amino acids along the chain is the so-called primary structure of the protein. With rare exceptions, the polypeptides of living organisms have the L-configuration.[49]

The covalently bound amino-acid residues along the chain interact by formation of hydrogen bonds (Fig. 2) to yield a helical configuration with quite definite parameters (the number of residues per turn of the helix, pitch of the helix, etc.) (the secondary structure of macromolecules).[49] Figure 3 shows the α-helical conformation of the polypeptide chains of proteins.

Another example of a secondary structure of proteins is the conformation realized by forming hydrogen bonds between residues belonging to different polypeptide chains or by folding a single chain to form hydrogen bonds between the links (Fig. 4) (the so-called β-pleated structure).

However, this is not at all the highest level of organization of polypeptide chains. While in suspension in a solvent medium, the polypeptide chains, which contain regions having secondary structures, fold into dense, compact globules of definite geometric dimensions (the tertiary structure of proteins) (Fig. 5). The general principles of organization of the tertiary structure of globular proteins have been developed in Refs. 51–53.

Some problems of the statistical theory of biopolymers have been treated by I. M. Lifshits[19-21] and by his students.[54,55] He showed[19] that the phase state of a polymer chain is determined by the "lateral" or "volume" interactions of mutually remote links. Here he viewed the primary structure of the chain and the bonds between its links as fixed and constituting a "linear memory" in the system. This system attains equilibrium in the presence of the supplementary interaction forces with the restrictions imposed by the "linear memory". Depending on the number N of monomers and the temperature T, a model polymer chain having a "linear memory" can exist in one of three macroscopic states: 1) the quasigaseous coil state; 2) a peculiar two-phase state of a globule with a

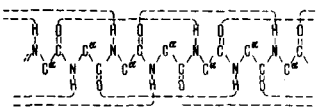


FIG. 2. Schematic drawing "in the plane" of a polypeptide chain in the α-helical conformation. The dotted lines indicate the hydrogen bonds.

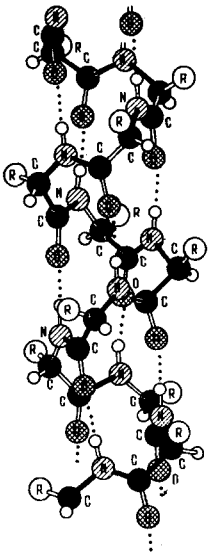


FIG. 3. The Pauling-Corey α-helix. Dotted lines: hydrogen bonds.

loose gaslike fringe; 3) the state of a globule having a sharp boundary. The phase transitions between these states can be of either the first or second order. A real protein globule is more complex, though undoubtedly Refs. 19–21, 54–55 bring us closer to an exact statistical-thermodynamic description of these complex systems. (Blyumenfel'd's monograph[56] makes a detailed analysis of Refs. 19–21 and connects the developed approach with fundamental problems of biophysics.)

The main distinctive feature of the structure of all globular proteins as manifested by diffraction patterns (at 1–2 Å resolution) is the dense packing of the chains. Practically all the space inside the region of the globular molecule is occupied by amino-acid residues of nonpolar (hydrophobic) nature. The free volume is negligibly small and practically inaccessible to the solvent (water). The ionic side-chains of the polar residues mainly lie at the surface of the particle. The configuration of the chain in the native state always distributes the residues in precisely this way.[57] In other words, the residues of the two types ("polar"

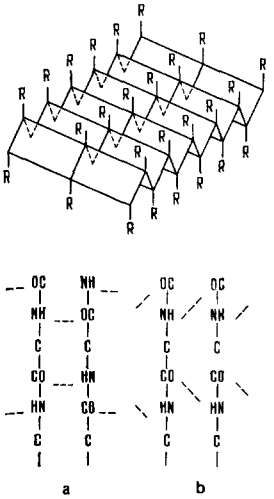


FIG. 4. Arrangement of the polypeptide chains in the β-structure. Antiparallel (a) and parallel (b) arrangements of the polypeptide chains in the β-pleated form.

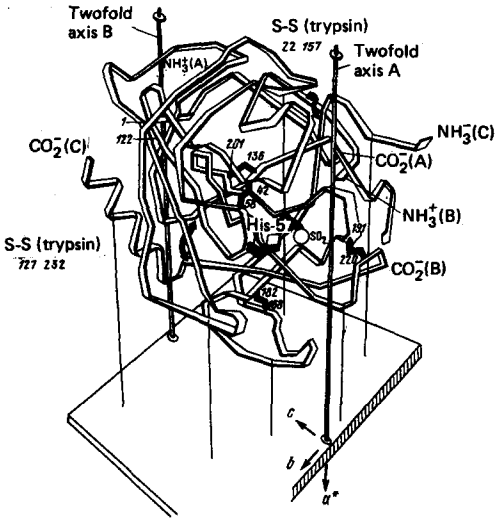


FIG. 5. Schematic drawing of the conformation of the polypeptide chains in A-chymotrypsin.[120]

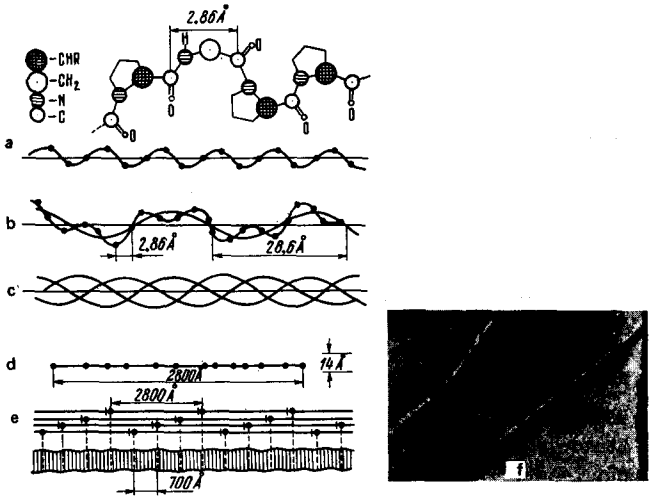


FIG. 6. The different levels of structural organization of collagen.[58] a) Peptide helix (primary structure); b) secondary structure; c) tertiary structure (triple superhelix); d) collagen molecule drawn as a rod 2800-Å long and 14 Å in diameter; e) packing of collagen molecules in the fiber (quaternary structure) with period 700 Å; f) electron micrograph of collagen fibers (taken in the Institute of Physics of the Academy of Sciences of the Georgian SSR by G. Natsvlishvili and G. Mikadze).

and "nonpolar") are arranged along the chain (the primary structure, or linear memory along the chain) in such a way as to satisfy these conditions while simultaneously ensuring high packing density of the residues in the globular state. The polar residues lying outside create an electrical layer that causes solubility in an aqueous medium and stabilizes the dispersed state with respect to coagulation.

We note also that the molecules of many proteins consist of several subunits, each of which amounts to an individual convoluted polypeptide chain possessing a tertiary structure. (This spatial combination of subunits into a complex molecule is called the quaternary structure.)

The fibrous proteins are an extremely interesting class of proteins. A typical representative is the connective-tissue protein collagen, which constitutes ~40% of all the proteins of the animal world. The macromolecules of collagen amount to three helical polypeptide chains wound into a superhelix reinforced by hydrogen bonds (Fig. 6). Each chain of the molecule consists of a regular sequence $(G\text{-}X\text{-}Y)_n$, where G is glycine, and X and Y are amino or imino acids. The collagen molecule is rather rigid and asymmetric: the diameter of the triple helix amounts to $d = 14$ Å, and the length is $l = 2800$ Å (the molecular weight $M \approx 360\,000$ daltons).[58] At present, the studies of Rich and Crick,[59] Ramachandran,[60] and Andreeva and her associates,[61] have found conditions and formulated criteria necessary and sufficient for the existence of the triple helix. We note that a regular network of interpeptide hydrogen bonds emerges as a universal element of the collagen structure. The molecules of the solvent (water) play an important role in preserving the helical conformation of collagen by forming an additional set of hydrogen bonds that stabilize the triple superhelix. The formation of the intramolecular ordered network of H-bonds has been shown to be accompanied by incorporation of water molecules into the structure. This incorporation becomes more effective as the network of hydrogen bonds becomes more ordered.[62]

b) Nucleic acids

Today everyone knows well that the DNA molecule is built of two long intertwined chains that form the Watson-Crick double helix.[63] Figure 7 shows schematically a fragment of the DNA chain consisting of about 10 nucleotide units. According to physical measurements, DNA chains consist on the average of 10 000 nucleotide units. (The molecular weight of some DNAs can be as much as 120×10^6 daltons.) The deoxypentose rings are linked by phosphate residues and form the backbone of the chain; the purine and pyrimidine rings constitute planar structures that lie perpendicular to the main axis of the chain. The purine and pyrimidine bases of one of the chains are linked by hydrogen bonds with the pyrimidine and purine bases of the complementary chain. (The structure and properties of the double helix of DNA have been excellently described in Watson's book.[50])

X-ray structural measurements have shown that the distance between the two DNA chains corresponds to the value calculated for a hydrogen bond linking a

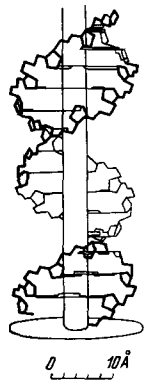


FIG. 7. Fragment of the Watson-Crick double helix (see Ref. 50).

purine with a pyrimidine base.[64] X-ray structural studies have also shown that the DNA molecule adopts different conformations in fibers and in solution. A large number of these conformations can exist in several ordered forms. The content in the fiber of water, salts, and cations used for neutralizing the phosphate groups are the main factors determining the conformation of the molecule. Transitions between different ordered forms of DNA are also observed in solutions when the properties of the solvent (water) are changed. For example, such a factor is the polarity of the solvent, which can be altered by adding different salts or organic solvents to the DNA solution (see Refs. 65–67).

Currently the coordinates of the atoms of the ordered forms of DNA have been refined and exact models have been built that describe the conformational properties of this molecule.[64] Great achievements have been made in the experimental and theoretical study of the process of melting (unwinding) of the double helix and transition of the macromolecules to the random-coil state.[68-70]

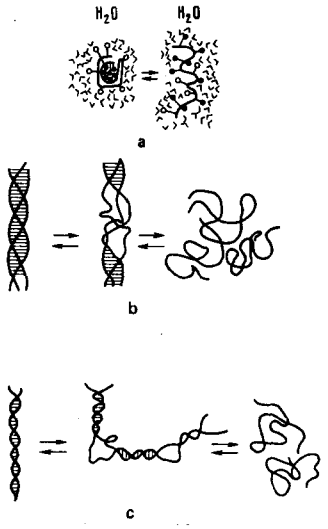


FIG. 8. Schematic diagram of the intramolecular phase transitions "globule-coil" (a) and "helix-coil" in collagen (b) and DNA (c). We show the fully helical, partly melted, and fully melted states of the moelcules with separated chains.

c) Characteristic features of thermal transitions in solutions of biopolymers over a broad temperature range

Biopolymers, which in the native state have a definite molecular conformation (this includes all the biopolymers treated in the previous section), can transform into a disordered random coil under appropriate conditions (increasing temperature, variation of pH or ionic strength, etc.). As we have seen, the energy balance that yields the native structure includes numerous chain-chain and chain-solvent interactions that involve chemical interactions created by the covalently bound atoms of the main chain as well as hydrogen bonds, electrostatic interactions, hydrophobic interactions, and dispersion (London) forces.

Temperature-induced intramolecular phase transitions such as the "helix-coil", "globule-coil" transitions, etc. (Fig. 8) are observed in a narrow temperature range and are characterized by high cooperativity (see Refs. 15–18). During these conformational transitions the balance of forces determining the native structure of the biopolymer completely breaks down, with a change not only in the intramolecular characteristics, but also in the state of all the surrounding solvent.

The studies of intramolecular melting in solutions of biopolymers that were begun in the fifties by Professor Doty in the USA[71] are currently being performed by varied physical methods—hydrodynamic, optical, and radiospectroscopic methods (see, e.g., Ref. 72).

As we have said, modern methods of calorimetry are successfully being applied for studying the thermodynamic quantities that characterize structural transformations in solutions of biopolymers over a broad temperature range.

For the sake of perspicuity, let us analyze the temperature dependence of solutions of biopolymers (we are considering a three-component system: biopolymer-water-ions of dissolved salts) in the range 2–400 °K (Fig. 9). (Here the heat capacity is given in arbitrary units.) We can distinguish four temperature regions in which detectable thermal effects are associated either directly with intramolecular conformational rearrangements of the biopolymer chains or with changes in the state of the water and of the salts dissolved in it.[29]

1) The interval that spans the temperatures 273.15–400 °K is the region exhibiting conformational transitions in solutions of proteins, nucleic acids, lipids, etc. "Intramolecular melting" of biopolymers is accompanied by absorption of heat. This process has been studied in detail for a number of proteins and nucleic acids by the recently developed methods of scanning differential microcalorimetry. The obtained information includes such important parameters as the temperature and heat of melting and the change in entropy and free energy. In a number of cases intramolecular melting of proteins and nucleic acids is accompanied by a change in the heat capacity (for details on application of microcalorimetry in studying

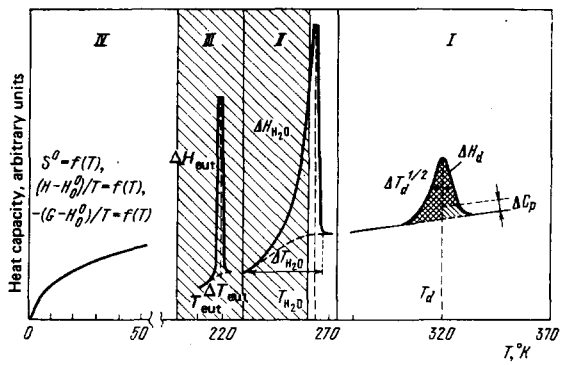


FIG. 9. Schematic diagram of the temperature-dependence of the heat capacity of solutions of biopolymers in the temperature range 2–400 °K.

conformational transitions, see the reviews of Sturtevant,[1] Andronikashvili,[2] Privalov,[4] McKnight and Karasz,[3] and Rialdi and Biltonen[5]).

A recent analysis by Sturtevant[73] shows that the large jump in heat capacity observed in the transition of macromolecules from the ordered to the coil state arises mainly from two contributions: exposure of the nonpolar groups of the macromolecules to the solvent (see Fig. 8a) and changes of the vibrational spectrum of the macromolecules in the random-coil state. Thus the study of the heat capacity of biopolymers over a broad temperature range becomes necessary for accounting for the various contributions to the size of the heat-capacity jump (ΔC_p) in the conformational transition.

2) A second temperature region (273–230 °K) reveals the features of the phase transition of water in the presence of the biopolymer. Upon determining the thermodynamic quantities that characterize the phase transition of water as spread out along the temperature axis (the temperature, enthalpy, and entropy of melting and the width of the melting range), we can infer the state of the water in biopolymer solutions while obtaining exact quantitative data on an important physicochemical parameter of the macromolecule such as its hydration.[29,74-75]

3) A third temperature range (250–210 °K) involves structural transitions that occur upon melting of the eutectic of the triple system biopolymer-water-salt formed during cooling of solutions. The thermodynamic properties characterizing such transitions (the temperature and enthalpy of melting of the eutectic mixture and the width of this transition) allow one to get quantitative data on the nature of the binding of ligands (the type of salt ions) that interact with the biopolymers.[29]

4) The fourth temperature region (liquid-helium temperatures) is interesting in obtaining the thermodynamic properties (heat capacities, entropies, enthalpies, free energies) for biopolymers of different conformations near absolute zero. This allows a complete thermodynamic description of the studied objects using the third law of thermodynamics.[24-47]

Moreover, the character of the temperature-dependence of the heat capacity of biopolymers at low temperatures allows one to study the region of the low-frequency vibrational spectrum (a temperature of ~1 °K corresponds to a maximum frequency of ~0.7 cm^{-1}) that is inaccessible to standard spectroscopic methods (infrared and Raman spectroscopy) that reveal the optical modes of vibrations.

We stress again that the nature of the thermal changes in each of these temperature regions and the quantitative values of all the thermodynamic characteristics arise from the conformational features of the macromolecules studied, from the concrete mechanisms of interaction of all three components (biopolymer-water-salt), and from their relative concentrations.

3. BRIEF REVIEW OF THE THEORY OF HEAT CAPACITY OF POLYMERS IN THE SOLID STATE

Wunderlich and Baur[76] have made a rather complete analysis of the theory of heat capacity as applied to polymers in the solid state. Hence we refer the reader to this book for more detailed acquaintance with the problem. (See also the monograph by Kosevich,[77] which has presented some special problems of crystal dynamics. In particular, it treats the dispersion laws of vibrations of highly anisotropic crystals, which give rise to a distinctive pattern of the low-temperature heat capacity of solids having layer structures.) Here we shall take up the major points and studies concerned with the theory of the heat capacity for crystals having a platy or fibrous (linear) structure.

As we know, at low enough temperatures the dimensions and shape of an object begin to play a substantial role in its thermal properties,[78] owing to the discrete nature of the vibrational energy levels manifested here.[78,79]

Strongly anisotropic crystals, e.g., crystals of organic substances having a chain structure of the molecules, correspond to an anisotropic continuum. In an anisotropic continuum the velocity of sound differs in different directions. A direction in space corresponds to more than one branch of vibrations, which generally differ both in polarization and in velocity of propagation.

In other words, the vibrational spectrum of a highly anisotropic crystal can contain more than one characteristic frequency. Then more than one characteristic temperature can figure in the thermodynamic expressions. Thus, for example, each individual direction in space can correspond to three Debye spectra having three different limiting frequencies, and correspondingly three different Debye temperatures: $\Theta_{3,1}$, $\Theta_{3,2}$, and $\Theta_{3,3}$.

A method of approximate calculation of the heat capacity of highly anisotropic crystals developed within the framework of the theory of the elastic continuum has been proposed by a set of authors (see Refs. 76, 77, 79–83). This approach is especially designed for calculating the heat capacity of a crystal of layer or chain structure in which the interaction forces between the layers or chains are several orders of magnitude weaker than in the plane of the layers or along the chains. At very low frequencies ($0 \leq \nu \leq \nu_D$) at which the weak interaction forces between the layers or chains still exert an effect and at which the wavelengths are large in comparison with the distance between the structural elements of the lattice, the spectral distribution density is proportional to ν^2. Hence the heat capacity of crystals of layer or chain structure obeys the Debye T^3 law at very low temperatures. However, at higher temperatures the weak interaction between the layers and chains now begins to play a subordinate role. The vibrational spectrum continues to degenerate into the spectrum of a monomolecular layer or one-dimensional chain.[76] Tarasov has proposed that it suffices to treat a layer as two-dimen-

sional in calculating the spectral distribution density in a certain interval above ν_D, and chains as a one-dimensional continuum.[81,82] According to Tarasov, the generalized frequency distribution function for a m-dimensional continuum has the following form

$$\rho(\nu)\,d\nu = 3m\,N\nu_{\max}^{-m}\,\nu^{m-1}\,d\nu. \tag{3.1}$$

He treated the noninteracting layers and chains on the basis of these frequency distributions to derive a generalized heat-capacity equation:

$$C_m = 3mR\left(\frac{T}{\Theta_m}\right)^m \int_0^{\Theta_m/T} \frac{(\Theta/T)^{m+1}\,e^{\Theta/T}}{(e^{\Theta/T}-1)^2}\,d(\Theta/T). \tag{3.2}$$

This implies three possible special cases: a) for the three-dimensional continuum of Debye; b) for layers or two-dimensional continua; c) for chains, or one-dimensional continua.

The expression for the heat capacity for strongly anisotropic solids (polymers in the solid state) with account for the preferential contribution of one-dimensional structures (chains) has the following form[76,82,85]:

$$\frac{C_{1,3}}{3R} = D_1\left(\frac{\Theta_1}{T}\right) - \frac{\Theta_3}{\Theta_1}\left[D_1\left(\frac{\Theta_3}{T}\right) - D_3\left(\frac{\Theta_3}{T}\right)\right],$$
$$D_n\left(\frac{\Theta_n}{T}\right) = n\left(\frac{T}{\Theta_n}\right)\int_0^{\Theta_n/T} X^{n+1}\,e^x\,(e^x-1)^{-2}\,dx. \tag{3.3}$$

Here R is the gas constant, D_n (Θ_n/T) is the Debye integral, and n is the dimensionality index. For solids allowing a preferential contribution of two-dimensional structures (layers) we have

$$\frac{C_{2,3}}{3R} = D_2\left(\frac{\Theta_2}{T}\right) - \left(\frac{\Theta_3}{\Theta_2}\right)^2\left[D_2\left(\frac{\Theta_3}{T}\right) - D_3\left(\frac{\Theta_3}{T}\right)\right]. \tag{3.4}$$

In line with what we have presented above, the heat capacity of a crystal made of chain molecules increases at first in proportion to T^3, then $\sim T$, and later it reaches its constant classical value.

We should note that many authors have employed the Tarasov approximation and were able to get remarkably good agreement between the calculated and the experimental values of the heat capacity (see the studies of Wunderlich[86] and of Reese and Tucker[87]). However, as Wunderlich and Baur[76] have emphasized, it remains unclear to what degree we can attribute this agreement to the correctness of the physical model. Tarasov's approximation allows one to calculate the lattice spectrum of stretching vibrations in the low-frequency region rather accurately, but it does not allow one to determine the analogous spectrum of the bending vibrations. Apparently[76] this is due to the fact that the theory of the one-dimensional continuum does not account, in deriving the spectral distribution function, for the fact that the bending vibrations are primarily associated with the deformation of the valence angles.[84] In other words, Tarasov's theory does not allow for the flexibility of the individual layers and correspondingly for the associated deformability of the valence angles (e.g., C-C-C). The forces that arise upon deforming the valence angles substantially alter the spectrum of bending vibrations. Thus the contribution of these vibrations to the heat capacity cannot be described by using the "two-dimensional" Debye function.[76]

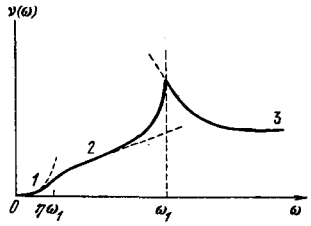


FIG. 10. Frequency distribution function of the bending vibrations of a highly anisotropic crystal.[77]

The most correct physical interpretation of the thermal properties of chain and layer structures at low temperatures has been given by I. M. Lifshits.[79,80] He showed that the "bending" waves with a dispersion law $\nu \sim k^2$ propagating in thin films and "needles"[1)] lead to distinctive temperature-dependences of the heat capacity at low enough temperatures.

In order to get a clearer picture of the physical pattern of the variation of thermal properties of chain and layer structures at low temperatures arising from the substantial reconstruction of the dispersion law of highly anisotropic crystals, let us turn to a schematic diagram taken from Kosevich's book[77] (Fig. 10). This diagram gives an overall picture of the frequency distribution function of the bending vibrations of a highly anisotropic crystal. Region *1* on the graph corresponds to the quadratic frequency-dependence $\nu(\omega)$, region *2* to a linear dependence, and region *3* to a constant value of $\nu(\omega)$ for $\omega \gg \omega_1$. One notes on the graph the "radical singularity" of the function $\nu(\omega)$ at the frequency $\omega = \omega_1$.[77]

Since the frequency distribution function for a highly anisotropic crystal is divided into several intervals, the low-temperature region will consist of several intervals having different temperature-dependences of the heat capacity of the crystal. Thus, for thin plates (Fig. 11) (films) at $T \ll \Theta_1$, Lifshits derived the equation

$$C = \frac{2\alpha k}{\delta^2\,(T/\Theta)}. \tag{3.5}$$

Here δ is the thickness of the film, we have $\alpha = (\pi\sqrt{3/12})\sqrt{1-\sigma}$ (where σ is Poisson's coefficient); when $\Theta_1 \ll T \ll \Theta$, we find $C \sim (T/\Theta)^3$.

For the heat capacity of thin needles (Fig. 12) we have

$$C = \nu\sqrt{\frac{T}{\Theta}}, \quad \nu = \frac{2k}{R}.$$

Just as in the case of a plate, for $\Theta_1 \ll T \ll \Theta$ we get the ordinary dependence $C \sim T^3$. Figure 12 shows the course of the heat capacity schematically.

Then I. M. Lifshits treated the heat capacity of real chain and layer structures with account for the interaction between the layers (or chains). As we have

[1)] The structure of a crystal is usually termed "acicular" when it consists of weakly interacting parallel linear chains. If the interaction of the atoms along an individual chain considerably exceeds the interaction between them in adjacent chains, then the dynamics of such a crystal resembles that of a layer crystal.[77]

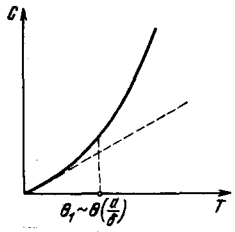


FIG. 11. Heat capacity of thin films according to Lifshits.[79]

stated above, it proved necessary in this case to allow for a dispersion law in the crystal, in which the interaction forces acting in one direction strongly differ from those acting in the two other directions.

In the temperature region in which the interaction between the layers is not essential, the "bending" waves give rise to a heat capacity $C \sim T$. At lower temperatures at which the interaction between the layers becomes essential, we have the following relationships [to the accuracy of the leading terms in (T/Θ)]:

$$C \sim T^3, \quad T \ll \Theta\left(\frac{\mu}{\alpha}\right), \qquad C \sim T^2, \quad \Theta\left(\frac{\mu}{\alpha}\right) \ll T \ll \Theta\sqrt{\frac{\beta}{\alpha}}. \tag{3.6}$$

Here μ is the shear modulus between the layers, β is Young's modulus in the Z direction (λ_{zzzz}), and α is Young's modulus in the plane of the layer (λ_{xxxx}).

An essential point is that the quadratic-dependence region can become rather broad for very small μ ($\mu \ll \beta, \alpha$). Moreover, as I. M. Lifshits showed, for chain structures an allowance for "bending waves" causes the noninteracting chains to approximate the law $C \sim \sqrt{T}$, which differs substantially from the equation derived by Tarasov for the case of noninteracting chains at very low temperatures without account for bending vibrations. We note also that a number of experimental data[83,88,89] agree well with Lifshits' theory.

Thus, taking account of vibrations with an ordinary dispersion law leads to a linear temperature-dependence of the heat capacity, while a supplementary inclusion of bending waves substantially alters the temperature-dependence of the heat capacity—it becomes proportional to the square root of the temperature. In Tarasov's theory the allowance for interaction is made by a rather formal combination of a three-dimensional with a one-dimensional continuum, whereas the fundamental method developed by I. M. Lifshits consists in seeking the dispersion law for the long-wavelength region of the vibration spectrum of a layer crystal as a whole in an approximation that accounts for the transverse rigidity of the atomic layers or chains, in addition to the equations of the theory of elasticity of a highly anisotropic solid (chain and layer structures).

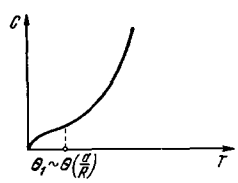


FIG. 12. Heat capacity of thin needles according to Lifshits.[80]

In solving problems concerning the behavior of the heat capacity of real polymeric molecules in the solid state, it also becomes necessary to account for the contribution to the heat capacity associated with the mobility of the side groups of the polymer links.[83]

The chemical structure of the polymer will be decisive in calculating the relationship between the skeletal heat capacity and the heat capacity involving the mobility of the side groups. The former makes the major contribution to the low-temperature heat capacity of the polymer, since the skeletal vibrations are acoustic vibrations and the individual links can be treated as point masses (especially for polymers having light side groups). The contribution of the other groups of vibrations arising from individual groups of atoms depends on the relationship of the masses of the atoms of the main chain and of the side group and on the temperature. These vibrations are characterized by considerably larger frequencies than for the skeletal vibrations. Consequently their contribution to the heat capacity becomes appreciable at moderately low temperatures[83] (see Refs. 88, 89, which give a calculation of the vibrational spectra of two very simple polymers, polyethylene and polytetrafluoroethylene).

4. CALORIMETRIC MEASUREMENTS

Currently most measurements of heat capacity are made by one of the two following methods:

a) *The "heat pulse" method,* which consists in imparting to a thermally isolated specimen a known amount of thermal energy (ΔQ) and measuring the temperature rise ΔT elicited by it. The heat capacity is

$$C = \lim_{\Delta T \to 0} \frac{\Delta Q}{\Delta T}.$$

In measuring the heat capacity one usually applies the approximation

$$C^*(\overline{T}) = \frac{\Delta Q}{\Delta T}.$$

Here $\overline{T}$ is the midpoint of the temperature interval ΔT. Usually one measures C_p, the heat capacity at constant pressure.

b) *The "continuous-heating" method,* in which one supplies heat to the specimen at the constant rate $dQ/dt = P$ and records the rate of temperature rise of the specimen. In this case the heat capacity is calculated by the formula

$$C = P\left|\left(\frac{dT}{dt}\right)\right.$$

As we have noted above, people have recently been applying differential scanning microcalorimeters in molecular biophysics and biology for studying the thermal properties of biopolymers. These instruments operate in a regime of uniform continuous heating (see the reviews[1-7]).

The continuous-heating method has a number of advantages. Thus, for example, it is most convenient for studying the heats of intramolecular phase transitions

and singular points on the curves of the temperature-dependence of the heat capacity, since the most important factor in such studies is high temperature resolution (small ΔT). Modern scanning differential calorimeters operating in a continuous-heating regime allow precise measurements on microgram amounts of a preparation, as is also highly important. The sensitivity of modern scanning microcalorimeters is as good as 10^{-7} W.

A merit of the heat-pulse method is that the specimen is practically in a state of thermal equilibrium before the onset of heating and after it has finished. Hence, the quantity $\Delta Q/\Delta T$ characterizes the properties of the specimen in a state of equilibrium. On the contrary, the continuous-heating method, in which a heat flux is continuously maintained in the specimen, allows one to determine the property of the specimen only in a nonequilibrium state. This is precisely why people employ modern calorimeters operating in the heat-pulse regime[90-93] for precise measurements of heat capacity, even in the face of high-sensitivity scanning calorimetry. The requirements of equilibrium conditions of performing calorimetric measurements become especially important if the investigator's problem is to determine the shape of the curves of the temperature-dependence of the heat capacity at low temperatures and to calculate the absolute values of the thermodynamic functions (change in enthalpy, entropy, free energy) of the material. Whenever one applies the continuous-heating method, one must perform the heat-capacity measurements in the temperature ranges of interest at three or more different heating rates in order then to extrapolate to zero heating rate. Only in such a case do the obtained results characterize the equilibrium properties of the specimen. This eliminates one of the fundamental objections against applying the continuous-heating method.

We shall not spend time on describing the different calorimeters applied in different laboratories to study biopolymers over a broad temperature range. For illustration, we shall restrict ourselves to describing the complex of low-temperature calorimetric apparatus of the Institute of Physics of the Academy of Sciences of the Georgian SSR (Figs. 13, 14). The distinctive

FIG. 13. Overall view of the low-temperature calorimetric apparatus of the Division of Biopolymer Physics of the Institute of Physics of the Academy of Sciences of the Georgian SSR.

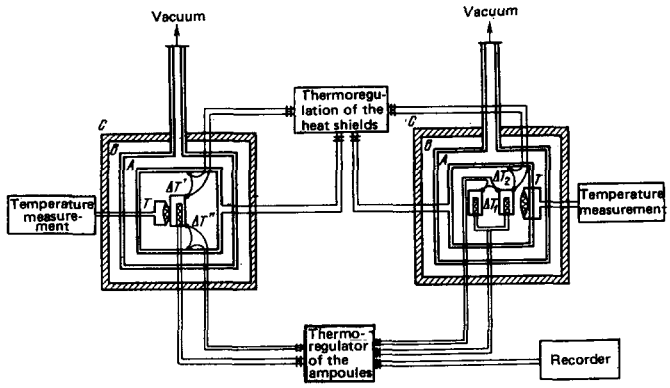


FIG. 14. Diagram of the complex of calorimetric apparatus, which allows study of the thermal properties of biological objects in the "heat-pulse" and "continuous heating" regimes over a broad range of temperatures. A—heat shields; B—vacuum jacket; C—cryostat; ΔT—detectors for temperature differences.[113]

feature of the employed apparatus is that one can perform a calorimetric experiment either by the "heat-pulse" method or in a "continuous-heating" regime. Here one uses a single electronic system for controlling the calorimetric experiment. One places in a metal cryostat containing a refrigerant (liquid nitrogen or liquid helium) either the vacuum jacket of an absolute adiabatic calorimeter (left-hand side of Figs. 14 and 15) or the vacuum jacket of a differential scanning microcalorimeter (right-hand side of Figs. 14 and 16). A set of connectors is used to attach one of the systems to the electronic blocks for automatic regulation of the thermal regime of the instruments. The electronic control system of the thermal regime of the calorimeter developed in our laboratory (see the studies of Monaselidze and Bakradze[10,11] and Kvavadze[4]) allows the attainment of good adiabaticity and full automation of calorimetric experimentation.

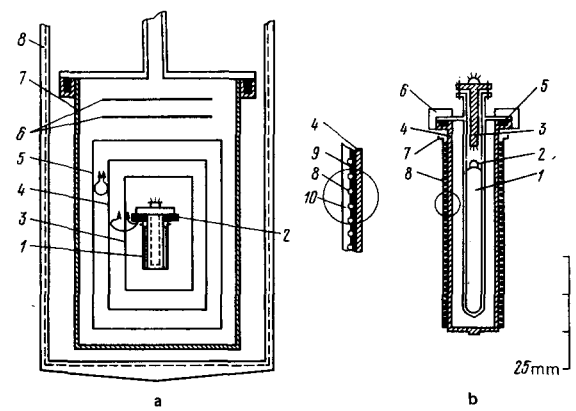


FIG. 15. Diagram of the vacuum adiabatic low-temperature calorimeter.[24,29] a) *1*—calorimetric ampoule containing the specimen; *2*—copper ring; *3*—heat shield; *4*, *5*—adiabatic shields with heaters; *6*—copper disks for equilibrating the temperature of the conducting leads; *7*—vacuum jacket; *8*—helium cryostat made of metal; b) calorimetric ampoule: *1*—capsule-type platinum resistance thermometer; *2*—gold-coated copper sleeve for the thermometer; *3*—spool for thermal equilibration of the leads; *4*—housing; *5*—gasket; *6*—adapter nut; *7*—heater leads; *8*—heater; *9*—insulating layer; *10*—silver cover.

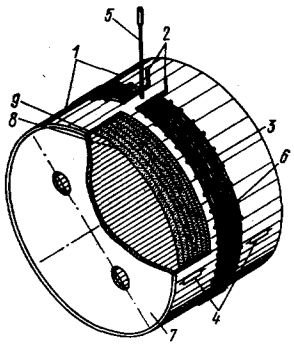


FIG. 16. Differential ampoule of the vacuum low-temperature scanning calorimeter.[94,29] *1*—containers for the measuring cells; *2*—leads for the heaters and thermocouples; *3*—heater; *4*—germanium resistance thermometers; *5*—pin for suspending the ampoule; *6*—thermobattery; *7*—cover; *8*—gold-coated copper disks for increasing the heat conductivity of the specimen: *9*—specimen.

A detailed description of the calorimeters is given in Refs. 10–12, 24, 29, and 94. Here we note that the volume of the ampoule of the adiabatic vacuum low-temperature calorimeter is 2 cm^3. Heat-capacity measurements are performed by the heat-pulse method every 1–4° with waiting for 1–2 hours for thermal equilibrium. The rms error of an individual heat-capacity measurement of specimens does not exceed ±1%, while the scatter of results in repeated measurements does not exceed ±0.5%. The data are processed on a computer.

The fundamental technical characteristics of the vacuum low-temperature scanning microcalorimeter employed in the Institute of Physics of the Academy of Sciences of the Georgian SSR are as follows: working temperature range 80–400 °K; heating rate 2–25 °/hour; working volume of the measuring cells 0.03 ml; sensitivity $\approx 10^{-7}$ W; accuracy of temperature measurement 0.1 °K (see Refs. 10–12, 29).

The fundamental technical characteristics of the low-temperature scanning differential calorimeter of the Institute of Physics of the Academy of Sciences of the Georgian SSR operating at liquid-helium temperature are as follows (see Refs. 94, 29): working temperature range 2–30 °K; heating rate 2–10°/hour; working volume of the measuring cells 10–25 cm^3; sensitivity 4×10^{-8} W; accuracy of temperature measurement ±0.005 °K. (One can find a description of other calorimetric instruments that are applied in various laboratories in the cited studies.[31,40,47,48,90,95] However, we should note that in most cases, to judge from the thermophysical characteristics of these instruments, they are inferior to the calorimeters that we have described.)

5. EXPERIMENTAL RESULTS

a) Heat capacity and thermodynamic properties of amino-acid residues over the temperature range 1.5 - 350 °K

Currently we possess data on the thermodynamic properties for practically all the amino-acid residues[31-37] in the solid state over the temperature range 10–350 °K (for *L*-alanine down to 1.5 °K).

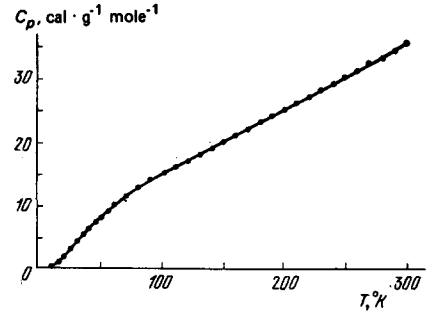


FIG. 17. Temperature-dependence of the heat capacity of crystalline *L*-proline.[34]

The experimental procedure of specimen preparation has involved preparation of the materials in the crystalline state with subsequent dehydration (removing water). Tests were made for crystallinity (study in the polarizing microscope) with analysis for the content of carbon, hydrogen, and nitrogen. Certain tests were also applied for biochemical activity of the preparations before and after performing the series of calorimetric experiments.[31-37]

The heat capacity of the crystalline amino acids was measured with the low-temperature adiabatic calorimeter (working ampoule volume ~100 cm^3) that the authors especially developed for studying biological objects, which operated in a heat-pulse regime (see Chap. 4 and Ref. 95). (However, we should note that the large working volume makes this calorimeter practically inapplicable for studying excessively expensive and scarce preparations of proteins, enzymes, nucleic acids, etc.)

As an example, Fig. 17 shows the temperature-dependence of the heat capacity of *L*-proline in the solid state as constructed from the data tabulated by Hutchens and his associates.[34]

This is a $C_p = f(T)$ relationship that is typical of all amino acids in the crystalline state. An exception is *L*-methionine, which shows a transition in the temperature range 180–350 °K with a peak in the heat capacity at 305.5 °K (Fig. 18). The entropy change in this transition was 4.2 cal · (°K)$^{-1}$ mole^{-1}.

Daurel and his associates[47] and Finegold and Cude[42,43] have also studied the heat capacity of *L*-alanine in the solid state at temperatures 1.5–300 °K and 1.5–20 °K.[42,43] The authors employed the classical Debye model and calculated the heat capacity of *L*-alanine in

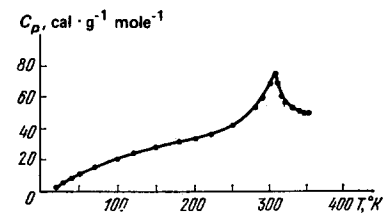


FIG. 18. Temperature-dependence of the heat capacity of crystalline *L*-methionine.[35]

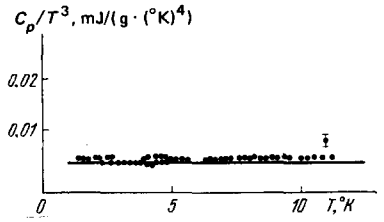


FIG. 19. The $C_p/T^3=f(T)$ relationship for crystalline L-alanine at low temperatures. Solid line: calculated by the Debye model ($r=2$).[47]

the solid state by the formula

$$C=\frac{12}{5}rkN\pi^4\left(\frac{T}{\Theta_D}\right)^3.$$

This proceeds from the idea that a molecular crystal should show a $C\sim T^3$ relationship in the low-temperature region. Figure 19 shows the experimentally determined function $C_p/T^3=f(T)$ for solid L-alanine. As we see from the variation, the crystals of the amino acid are fully described by the three-dimensional Debye model and they are characterized at low temperatures by a T^3 heat-capacity variation.

Table I gives the thermodynamic parameters: the heat capacity, entropy, and change in heat capacity and free energy for all the amino acids at the temperature 298.15 °K. We have compiled the table from the data of Refs. 31–37.

Thus the studies have been completed at present on the determination of the thermodynamic properties of the amino acids, which are the fundamental building blocks of protein polymer chains. The question has been posed of accounting for these properties in thermodynamic analysis of polypeptides and proteins, which posses a complex spatial structure, over a broad range of temperatures including that of liquid helium.

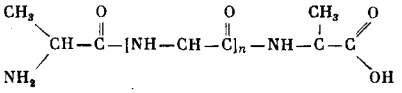
b) Heat capacity and thermodynamic properties of polypeptides over the temperature range 1.5 - 300 °K

Precision measurements of the low-temperature heat capacity of poly-L-alanine (PLA) in two conformations: α-helical and β-pleated, have been made

TABLE I. Thermodynamic parameters of amino acids at 298.15 °K (all values in cal·mole^{-1}, 1 cal = 4.186 J).

Amino acid	C_p	$S_0=\int_0^{298} C_p/T\,dT$	$\frac{H_0-H_0^0}{T}$	$-\frac{F_0-H_0^0}{T}$
Glycine	23.71	24.74	12.97	11.77
Alanine	29.22	30.88	16.06	14.82
Valine	40.35	42.75	22.11	20.64
Leucine	48.03	50.62	25.35	25.27
Isoleucine	45.00	49.71	24.90	24.81
Aspartic acid	37.09	40.66	20.69	19.96
Glutamic acid	41.84	44.98	23.06	21.92
Serine	32.40	35.65	18.16	17.49
Threonine	39.7	—	—	—
Lysine	57.10	63.21	31.72	31.49
Arginine	62.37	68.43	34.59	33.84
Histidine	59.64	65.99	33.16	32.83
Phenylalanine	48.52	51.06	25.61	25.45
Tyrosine	51.73	51.15	26.75	24.39
Tryptophan	56.92	60.00	29.83	30.17
Cysteine	—	—	—	—
Cystine	62.60	67.06	34.42	32.65
Methionine	69.32	55.32	28.14	27.18
Proline	36.13	39.21	19.59	19.62
Hydroxyproline	—	—	—	—

by Delhaes and his associates[46] in the Paul Pascal Research Center (Talence, France) in 1970, and by Finegold and Cude[40-43] at the University of Colorado, USA in 1972 (in the range 1.5–20 °K). They were repeated by the Franch group in 1975, but over a far broader temperature range (1.5–300 °K).[47] Poly-L-alanine has the following chemical structure:



(n is the number of monomers in the polypeptide chain).

Well-known difficulties exist in polymerizing PLA and in identifying the ordered forms of the polypeptide chain that arise. The conformation of the polypeptide chain of PLA was checked in independent experiments[40-43,47] by infrared spectroscopy (400–4000 cm^{-1}) and by x-ray diffraction.

Figure 20 shows the $C_p/T^3=f(T)$ relationship for PLA in the forms of an α-helix and a β-pleated structure.[40-43] The dots indicate the experimental values; the solid line corresponds to the model of Tarasov for "one-dimensional" (α-helix) and "two-dimensional" (β-form) structures in a three-dimensional continuum with allowance for an independent set of vibrators. The dotted line reflects the $C_p/T^3=f(T)$ relationship corresponding to the Tarasov model (without allowance for independent vibrations).

As we see from the presented graphs, the experimental values fit the Tarasov model only when one allows for a small number of independent oscillators having identical frequencies (Einstein oscillators). The authors view that these results can be employed to estimate the contribution of "one-dimensional" and "two-dimensional" structures to the heat capacity of a solid.

As we have noted, Daurel *et al.*[47] have studied the heat capacity of PLA in the α- and β-conformations over a broad temperature range (1–300 °K). The specimens contained 85 ± 15% of polypeptide chains in the α-form and 85 ± 15% in the β-form. They calculated the heat capacity by the classical Debye model for different values of the parameter r (the number of vibrators). Figure 21 shows the temperature-dependence of the heat capacity of PLA for the "pure" α- and β-conformations in the temperature range 1–300 °K.

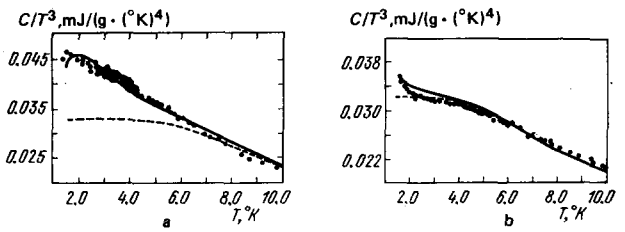


FIG. 20. The $C_p/T^3=f(T)$ relationship for poly-L-alanine in the the α- (a) and β- (b) conformations. Dots: experimental values. Dotted line: calculated by the Tarasov model. Solid line: calculated by the Tarasov model with allowance for the contribution of an independent set of vibrators.[41]

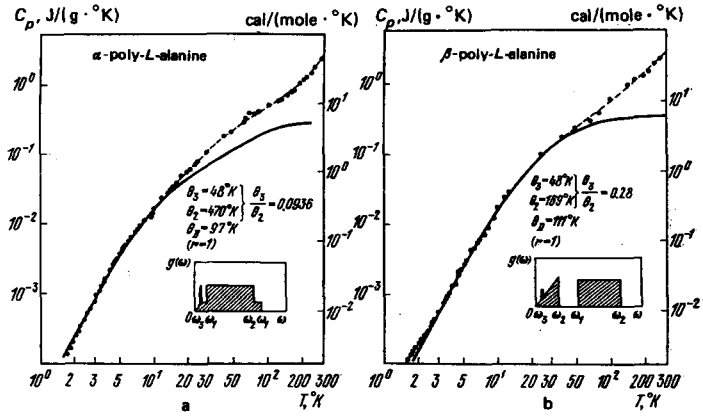


FIG. 21. Temperature-dependence for poly-L-alanine in the "pure α-helix" (a) and "pure β-structure" (b) conformations. Solid lines: calculated by Tarasov model [by Eqs. (3.3) and (3.4) for $C_{1,3}$ and $C_{2,3}$]. The vibrational spectrum for the two forms is also given.[47] ω is the vibration frequency.

The authors used a simplified Tarasov model to explain the obtained experimental data, and they calculated the heat capacity by Eqs. (3.3) and (3.4) for $C_{1,3}$ and $C_{2,3}$, while allowing the existence of only one form of vibrating units ($r=1$). This simplification allows one to treat the heat capacity that corresponds to the acoustic branches of vibrations alone.

Figure 21 compares the experimental and calculated data.

Figure 22 compares the experimental data obtained by Daurel and his associates[47] with the theoretical calculations of $C_p=f(T)$ performed by Fanconi *et al.*[96] and by Wunderlich and Baur[97] for layered, anisotropic polymeric materials.

As we see from the temperature dependencies, in the temperature range 80–150 °K the experimental data agree well with the theoretical calculations that allow for the specifics of the behavior of one-dimensional structures in a three-dimensional continuum. Interestingly, the data for the β-form differ more from the quantities calculated for one-dimensional structures. This indicates that one cannot identify the pleated β-form with a "one-dimensional" structure. Substantial deviations exist at low and high temperatures in the functions $C_p=f(T)$ from the theoretical values for the two forms of polypeptide chain. The deviations in the low-temperature region can result from taking into account incorrectly the features of the thermal properties of highly anisotropic materials involving the transverse rigidity of the individual atomic layers and chains and the unusual dispersion law for the long-wavelength part of the spectrum (see Chap. 3).

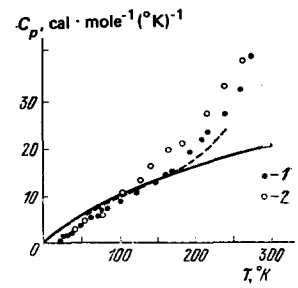


FIG. 22. Comparison of the experimental data for the temperature-dependence for poly-L-alanine with the theoretical calculations of Fanconi *et al.*[96] and of Wunderlich.[97] *1*—α-form, *2*—β-form; solid line—data of Ref. 96, dotted line—data of Ref. 97.

Fanconi and Feingold[44] at the Institute for Materials Research of the National Bureau of Standards (Washington) and at Drexel University (Philadelphia) have obtained interesting results in studying the low-temperature heat capacity of homopolymers. They studied the heat capacity of polyglycine II. As we know, polyglycine (PG), or poly-(-COCHRNH-), where $R=H$, can exist in the form of a β-pleated structure (PG-I) or in the form of a triple helix (PG-II). In turn, the latter can have parallel and antiparallel packings of the three polypeptide chains reinforced by interchain hydrogen bonds. The authors adopted the aim of obtaining by calculation the spectral distribution density for PG-II and comparing the theoretically calculated heat capacity with the experimental $C_p=f(T)$ relationship. To simplify the calculation, they assumed a parallel arrangement of the chains with respect to the axis of the helix, and they accounted for the force constants pertaining to the hydrogen bonds.

Figure 23 shows the spectral distribution density of PG-II. (See Ref. 44 for details on the method of calculation.) Frequencies above 350 cm^{-1} have little effect on the low-temperature heat capacity. Figure 24 shows the experimental and calculated variations of the heat capacity $C_p=f(T)$ and $C_p/T^3=f(T)$ for PG-II.

The presented temperature dependences show the

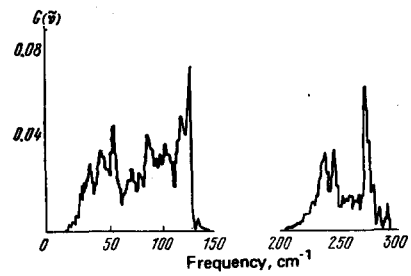


FIG. 23. Spectral density distribution for polyglycine II.[44]

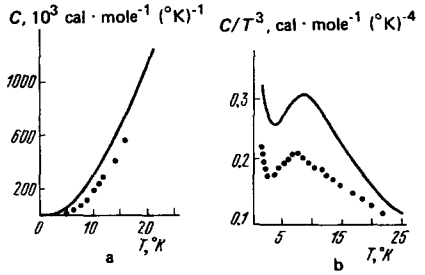


FIG. 24. Experimental (dots) and calculated relationships $C_p = f(T)$ (a) and $C_p/T^3 = f(T)$ (b) for polyglycine II.[44]

qualitative agreement of the experimental and calculated data to be good. Still, the authors deem it improbable that the discrepancy in the quantitative values might stem from the errors that usually accompany measurements of the heat capacity at very low temperatures. The maximum at a temperature near 8 °K was first noted for homopolymers. Indeed, the nature is currently unclear of the observed maximum, which corresponds to a frequency of ~5 cm^{-1}, and which therefore cannot be detected by infrared and Raman spectroscopy. In any case we can state that the PG-II conformation leads to features in the low-temperature heat capacity that have not been observed for poly-*L*-alanine in either the α- or the β-conformations. Hence they directly reflect the specifics of packing of the polypeptide chains and the nature of the bonds of the triple-helix conformation of PG-II.

Thus the existing data on the low-temperature heat capacity of polypeptides indicate the study of the thermodynamic properties of these compounds as holding promise for revealing their conformational properties at low temperatures. In turn, the latter must necessarily be taken into account in treating the structural features of biopolymers. It is an attractive idea to pose the problem of discovering the preferential conformation of polypeptide chains in biopolymers of unknown spatial structure from heat-capacity data.

c) Heat capacity and thermodynamic properties of globular proteins over the temperature range 10 - 350 °K

The globular proteins were the first objects of study for heat-capacity measurements on biopolymers. Yet data were lacking until the sixties on the heat capacity of these proteins over a broad range of temperatures. The major efforts of investigators were directed toward studying the thermodynamic properties characterizing the phase transition in dilute solutions of these proteins (see Chap. 2c and Refs. 1–7). Moreover, a methodology was developed of studying the state of the water in solutions of biopolymers by low-temperature calorimetry (in the temperature range from −50° to 20 °C) that was based on measuring the heat capacity of protein solutions in this temperature region.[74-75]

The first precision calorimetric measurements of the heat capacity of globular proteins in the solid state over a broad temperature range (10–350 °K) were performed in Hutchens' laboratory in 1968–1970.[38-39] The objects of study were Zn-insulin and chymotrypsinogen A.

The study of the thermodynamic parameters of Zn-

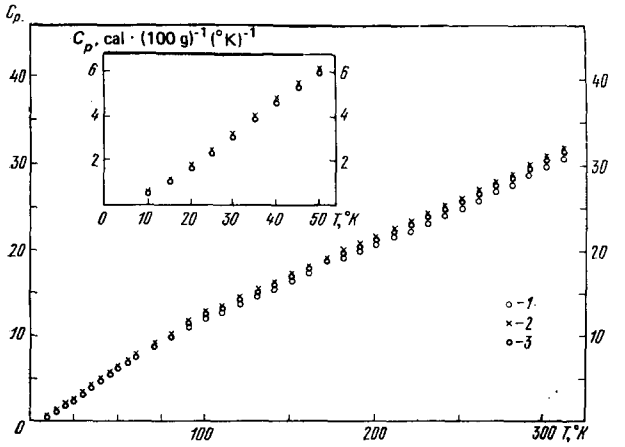


FIG. 25. Temperature-dependence of the heat capacity for Zn-insulin (*1*), chymotrypsinogen A (*2*), and serum albumin (*3*).[38] The low-temperature region of the relationship is shown separately.

insulin over a broad temperature range including liquid-helium temperatures was warranted by the following circumstances:

a) The structure of Zn-insulin molecules had been established at about that time by x-ray diffraction at a high enough resolution; b) preparations of Zn-insulin were obtained in crystalline, salt-free form (the experimental methods of preparing specimens for measuring the low-temperature heat capacity of globular proteins are described in detail in Ref. 38).

Besides Zn-insulin, they measured the low-temperature heat capacity for serum albumin and chymotrypsinogen A.

Figure 25 shows the temperature-dependence of the heat capacity of these proteins over a broad temperature range (10–350 °K). We see from the diagram that the temperature-dependence of the heat capacity has the same character for all the globular proteins in the solid state. Attention is called to the broad temperature interval in which the heat capacity varies according to a linear law (from 40 °K up).

The rest of the thermodynamic functions were calculated for these compounds from the heat-capacity data. We have summarized in Table II the data on the thermodynamic properties of the globular proteins in the solid state at the temperature 298.15 °K.

We see from Table II that the values of the heat capacity and entropy at 298.15 °K differ little for different proteins in the solid state. Moreover, since they had the values of the standard thermodynamic

TABLE II. Thermodynamic properties for globular proteins in the solid state at 298.15 °K (cal/100 g · °K).

Protein	C_p	$S_0 = \int_0^{298} \frac{C_p}{T} dT$	$\frac{H_0 - H_0^0}{T}$	$-\frac{F_0 - H_0^0}{T}$
Zn-insulin	29.96	31.44	15.85	15.85
Serum albumin	30.46	31.74	16.12	15.62
Chymotrypsinogen A	30.90	32.27	16.28	15.99

properties for all the amino-acid residues and knew the amino-acid composition of all the listed proteins, they could calculate the entropy change in the hypothetical reaction:

N amino-acid residues $\rightleftharpoons$ protein $+ (N+1)H_2O$. This makes it possible to estimate the entropy change in forming a peptide link by comparing the calculated and experimental data. It turned out that this quantity for all proteins amounts to $\Delta S \approx 9$ cal/°K · mole per peptide bond, and it coincides with the entropy change in forming a dipeptide.[38] The obtained result is quite remarkable, since it doesn't reflect at all the specifics of the structure of different proteins comprised in the conformational features of the polypeptide chains and their spatial packing.

Thus an attempt has been made to calculate the entropy for proteins in the solid state from their amino-acid composition and from experimental data on the heat capacity of amino acids and proteins in the solid state. However, evidently this approach is inapplicable in practice for calculating the entropy of proteins in dilute aqueous solutions, in which stabilization of the spatial structure of the protein is attained through a balance of the forces of the intramolecular and intermolecular bonds with the solvent (see Chap. 2, Sec. a).

As has become known from subsequent experiments,[29,73] the deviations in the heat capacities for different globular proteins in the solid state ($\varnothing C_p$) are far smaller than the difference between the heat capacities of a given protein existing in the helical and coil states in dilute aqueous solutions (ΔC_p). Thus, $(\varnothing C_p) = \pm 0.01$ cal/g °K, whereas $(\Delta C_p) = +0.1$ cal/g °K. Moreover, a considerable difference has been shown in the thermal properties and mechanisms of conformational transitions of protein macromolecules in dilute solutions and in the concentrated state.[98,99]

Analysis of the experimental data[29] shows that the formation of the spatial structure of any biopolymer in the process of sorption: solid (dry) specimen – moist film – isotropic solution–exists in direct relationship with the overall production of the hydration layers of the macromolecules. In other words, each "design" has its critical value of the volume fraction of solvent at which the polymer adopts its "equilibrium" ordered form.[29]

Hence, in low-temperature measurements of the heat capacity of biopolymers, one should pay much attention to a correct choice of the volume fractions of the solvent in the specimens, and the measurements should be performed under conditions in which the biopolymer contains the minimal amount of water of crystallization required to maintain the given conformation in the native state.

We shall illustrate that this is precisely so in the following sections on the basis of data on the low-temperature heat capacity of collagen and DNA obtained over a broad temperature range.

d) Thermodynamic properties of the fibrous protein collagen in the helical and coil states at temperatures 4 - 400 °K

Studies have been performed in the Institute of Physics of the Academy of Sciences of the Georgian SSR on the connective-tissue protein collagen in the helical and coil states over the temperature range 4–400 °K.[25-29]

As we have noted, the collagen molecule consists of three polypeptide chains that form a triple superhelix reinforced by intramolecular hydrogen bonds (see Chap. 2, Sec. a). In addition, the collagen macromolecule is stabilized by a chain of water of hydration "inscribed" in the triple helix.[100-102] Hence, in describing the thermodynamic properties of collagen in the helical and coil states, one must take into account not only the order-disorder transition of the polypeptide chains themselves, but also the order-disorder transition in the solvent (water).[101] The concrete thermodynamic problem was to study the low-temperature heat capacity of collagen and to determine the excess entropy value of collagen when existing in the coil state with respect to the same protein in the helical state.

Figure 26 shows the temperature-dependence of the heat capacity of native dehydrated collagen over the temperature range 4–320 °K. We note that the heat capacity of collagen had been measured earlier[39] from 10 °K up. We can view the results as agreeing well with one another.

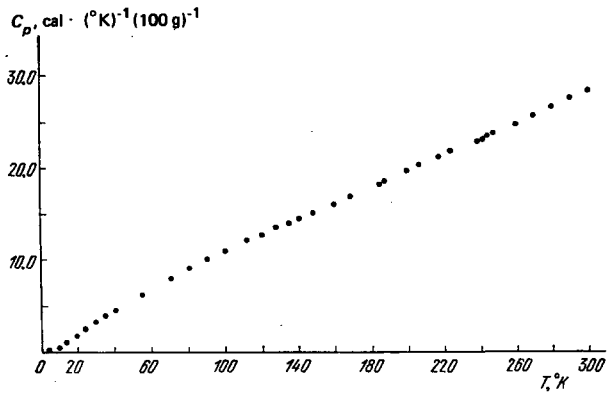


FIG. 26. Temperature-dependence of the heat capacity of native dehydrated collagen.

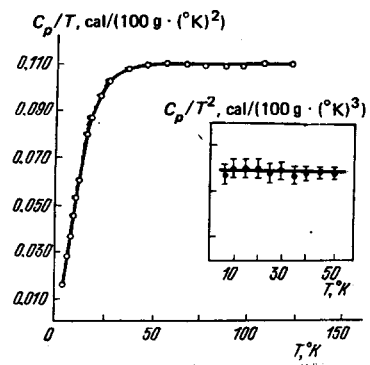


FIG. 27. The relationships $C_p/T = f(T)$ and $C_p/T^2 = f(T)$ for native dehydrated collagen (low-temperature region of the relationship).

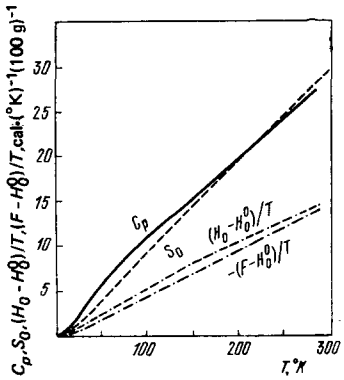


FIG. 28. Temperature-dependences of the thermodynamic properties for native dehydrated collagen.

In order to reveal the regularities in the temperature behavior of the heat capacity of collagen, we constructed the relationships $C_p/T = f(T)$ and $C_p/T^2 = f(T)$ for native, dehydrated films of the protein (Fig. 27). As we see from the diagram, the heat capacity varies over the interval 4–50 °K in proportion to the square of the temperature. A linear variation exists above 50 °K.

By using well-known relationships, we calculated from the heat-capacity data also the other thermodynamic properties for this protein (the entropy S_0, the enthalpy change $(H_0 - H_0^0)/T$, and the free energy $(F - H_0^0)/T$). Figure 28 shows the temperature-dependences of these quantities.

As we have noted, biopolymers maintain their native, biologically functional structure only in an aqueous medium. Hence, evidently, the fundamental point is to study the thermodynamic properties of biopolymers specifically in the hydrated state. The amount of structurally necessary water in collagen macromolecules existing in the helical state has been determined[101-102] by calorimetry and nuclear magnetic resonance. It amounts to 0.35 gH_2O per 1 g of protein [or ~5 H_2O molecules per tripeptide link (G-X-Y)]. This is precisely the ratio of water to protein that was taken in studying the low-temperature behavior of hydrated specimens of collagen (Fig. 29). Let us call attention to the sharp difference in the course of the heat-capacity curve of hydrated collagen (dots) and for the dehydrated protein (dotted line).

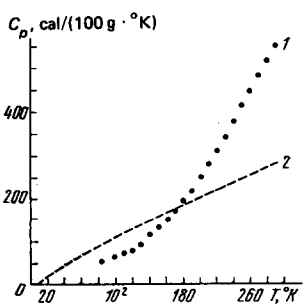


FIG. 29. Temperature-dependence of the heat capacity of hydrated (~5 H_2O molecules per tripeptide link) (*1*) and of dehydrated collagen (*2*).

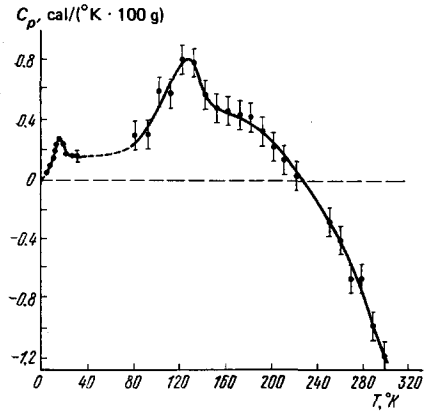


FIG. 30. Temperature-dependence of the difference of heat capacities between hydrated collagen specimens in the helix and coil states ($\Delta C_p = C_{coil} - C_{helix}$).

We have studied the temperature region 240–273 °K in considerable detail for proteins of different conformations (globular and fibrous) for specimens of different water concentrations (see Ref. 75). In all cases we were able to detect the critical concentration at which all the water in the macromolecules exists in the bound (not frozen out) state. The absence of heat absorption in this temperature region for collagen (Fig. 29) indicates that all the water added to the dehydrated protein, the presence of which has sharply altered the $C_p = f(T)$ relationship, "has become bound" to the collagen macromolecules and has become a constituent part of the triple helix of the protein.

Let us proceed to examine the low-temperature behavior of collagen when its molecules have been converted into the random-coil state by thermal denaturation. We note that here we measured the heat capacity of hydrated (0.35 gH_2O per 1 g protein) and dehydrated specimens.

Figure 30 shows the temperature-dependence of the difference between the heat capacities of hydrated denatured (coil) and hydrated native (helix) collagen in the temperature range 4–310 °K:

$$\Delta C_p = C_{coil} - C_{helix}.$$

Attention is called to three temperature regions in which the differences are especially sharply manifested in the heat capacities of collagen in the helical and coil states:

1) The difference in the heat capacities between the coil and the helix gradually rises from 4 °K, reaches a maximum at 15 °K, and then begins to decline.

2) the heat capacity of collagen in the coil state again begins at 70 °K to outstrip that of the native protein. At 120 °K, ΔC_p reaches a maximum.

3) At temperatures of 230–240 °K, the heat capacities of the native and of the denatured protein become equal, and thereafter ΔC_p becomes negative up to 310 °K, i.e., up to the temperature of onset of the anomalous growth in the heat capacity involving the melting of the native protein.

A completely different pattern is observed in study-

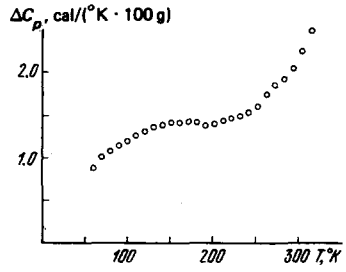


FIG. 31. $\Delta C_p = f(T)$ for dehydrated collagen.

ing the heat-capacity difference for dehydrated specimens in the helical and coil states. In this case $\Delta C_p = f(T)$ has the form depicted in Fig. 31.

Proceeding from the obtained results and considering the data of Ref. 80, we can state that the heat capacity for collagen in the solid state at low temperatures (4–50 °K) is determined by the vibrations of not only the individual atomic layers or chains, but substantially by the interactions between the individual polypeptide chains, since in this temperature range we have $C_p \sim T^2$. Apparently a slight slippage occurs between the chains with rising temperature. It quenches the bending vibrations and leads to a linear variation of the heat capacity while one-dimensionality is conserved.

The difference in the character of the temperature-dependence of the heat capacity for dehydrated and hydrated specimens of collagen shows how essential the specific interaction of the water molecules with the polypeptide chains is in the thermal properties of this protein. We show in Fig. 32 (see also Ref. 103) a diagram of the chain hydration of collagen and the possible orientations of the water molecules in the protein existing in the helical and coil states based on the geometric model of hydration of collagen proposed in Ref. 100. Whereas strict correlation exists in the native protein in the orientation of the water molecules along the axis of the macromolecule, collagen in the coil state has no correlation in the mutual arrangement of the water molecules—in the helix-coil conformational transition the "fourth helix" consisting of water molecules practically completely breaks down (from nuclear magnetic resonance data[101-102]). Figure 32 shows shows also the intermediate states of the H_2O chains (c, d, e), and shows the possible production of defects

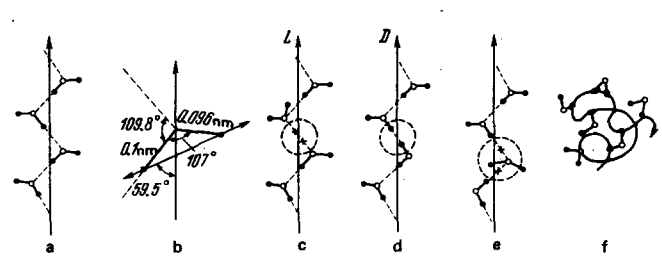


FIG. 32. Diagram of the arrangement of water molecules in the chains that constitute the "inner" hydration layer of the triple helix of collagen. a, b) Chains in the native protein (the arrow indicates the direction of the axis of the helix)[100]; c–e) Bjerrum defects; f) binding of water molecules to the separated polypeptide chains in the coil state.

caused by reorientation of the protons in the hydrogen bonds of H-O-H. Defects of this type (Bjerrum defects[104]) are realized in the structure of ice and they play a substantial role in its various properties. In particular, some authors think that they give rise to the thermal anomaly at 120 °K discovered in measuring the heat capacity of ice[105] (we recall also the studies of Onsager[106] on the electrical properties of ice).

We note that very recently a cooperative mechanism of reorientation of protons in chains of hydrogen-bonded molecules has been proposed to explain transport of protons through a biological membrane.[107] The difference between the entropy of hydrated collagen in the coil state and that of the protein in the helical state is positive throughout the temperature range. At 298 °K it amounts to $\Delta S = 0.8$ cal/(100 g)·°K (where $\Delta S = S_{coil} - S_{helix}$).

It seems highly essential to us that the difference in the thermodynamic properties of a protein in the helical and coil states stems to a considerable extent from the differing character of the interaction of the polypeptide chains with water molecules, and it is manifested at temperatures remote from that of the helix-coil intramolecular transition.

e) Heat capacity of DNA at low temperatures

We have emphasized that one must pay especial attention in studying the low-temperature heat capacity of biopolymers to a correct determination of the critical concentrations of solvent, since the configuration of the polymer chains in the native biopolymer is conserved only at a definite volume ratio of polymer to solvent. DNA also belongs to this class of biological macromolecules. A direct relationship of the conformation of DNA to the moisture content was demonstrated even in the early classical x-ray diffraction studies.[108] Any change in the conformation of DNA that ensues from changes in the external conditions of the medium arises either from direct action of the perturbing agent on the double helix (e.g., binding of ligands) or via the solvent molecules (change in ionic strength, pH, polarity, pressure, temperature, etc.). Hence the study of the state of the solvent molecules directly adjacent to the DNA macromolecule, i.e., the hydration of the double helix, is especially significant. The study of the low-temperature heat capacity of DNA performed in the Institute of Physics of the Academy of Sciences of the Georgian SSR[29,109-113] at different moisture contents has yielded data on the mechanisms of interaction of DNA with solvent molecules.

Figure 33 shows the temperature-dependence of the heat capacity of native DNA at different water contents. (Here and henceforth we give the heat-capacity data averaged over equal temperature intervals.[29])

We see that a water content of 18% H_2O does not lead to a deviation from a linear variation of the heat capacity of DNA nor to the appearance of heat absorption in the region of the phase transition of ice. This indicates that this amount of water is firmly bound to the DNA molecules. When we increased the water

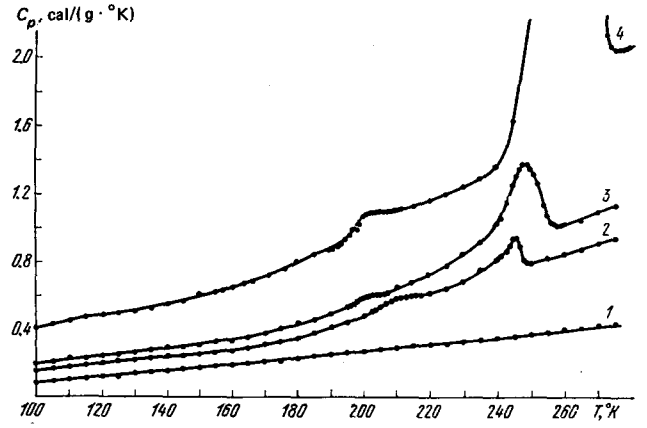


FIG. 33. Temperature-dependence of the heat capacity of native DNA at different water contents. *1*—18%; *2*—31%; *3*—37%; 4—61% H_2O by weight.

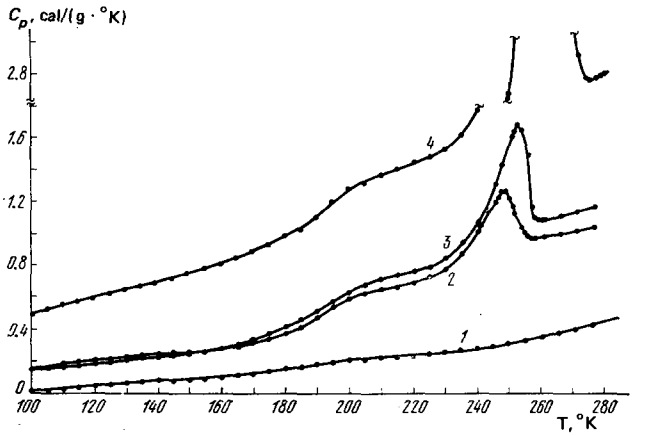


FIG. 34. Temperature-dependence of the heat capacity of denatured DNA at different water contents. *1*—6%; *2*—35%; *3*—39%; *4*—68% H_2O by weight.

content to 31% (curve *2* in Fig. 33), we observed a slight change in the heat capacity in the temperature range 200–220 °K, and then a heat absorption involving melting of a certain amount of water that had crystallized upon cooling. The temperature corresponding to the maximum of the heat-absorption peak at the given values of the water concentration is 245 °K. This is shifted by 28 ° from the melting point of pure water. Further increase in the water content manifests even more clearly the heat anomaly at 190–210 °K. At the same time it shifts the maximum of the heat-absorption peak toward 273 °K.

Figure 33 shows that the melting of the water in a DNA-H_2O system at low water concentration loses the character of a sharp phase transition, and it degenerates into a transition of the order-disorder type that is extended in temperature. We point out that the temperature interval of the extended transition and the character of the heat-absorption curve depend (at a particular moisture content) on the conformation of the biopolymer,[75] including the conformation of the polynucleotide chains. It has been shown[75] that the reason for the degeneration of the first-order phase transition is not the small amount of solvent (since decomposition into two phases, i.e., a sharp phase transition, is observed even in systems of several hundred particles), but the interaction of the water molecules with the biopolymer and its effect on the structure of the solvent.

The curves of the temperature-dependence of the heat capacity for denatured DNA preparations (curve *1* in Fig. 34) are considerably altered in character as compared with native preparations (curves *2* and *3*). In particular, the heat anomaly in the temperature range 200–210 °K is altered in character, i.e., the anomaly in the region where we assume that the relaxation transitions involve the vitrification of a certain fraction of the water.[29] For preparations of denatured DNA existing in a random-coil state, the heat capacity in this temperature interval varies very smoothly without a sharp jump. Here the shape of the heat-capacity curve (within the limits of experimental error) no longer depends on the thermal prehistory of the specimens, and in particular, on the rates of cooling of the solutions.

Figure 35 shows the dependences of the amount of bound water obtained from the calorimetric data on the total water content for native (curve *1*) and denatured (curve *2*) DNA preparations. In essence these variations amount to "sorption curves" in which one can distinguish three regions: a) an initial region (H_2O content up to 20%); b) the "sorption" region in which one observes a sharp increase in binding of water molecules with the polynucleotide chains of DNA (H_2O content 20–40%); and c) the region of H_2O concentrations from 40% up, in which the hydration curve reaches saturation.

According to the calorimetric data (see Fig. 35), in the initial stage of hydration ~7 molecules of H_2O bind to the DNA molecules per base pair. That is, presumably ~3 molecules of H_2O are deposited on each $>PO_2^-$ group and strongly bind to the oxygen atoms. Interestingly, the initial region of the hydration curve is the same for denatured and native DNA.

The sharp increase in the number of bound water molecules in the second stage of hydration (region b in Fig. 35) indicates the cooperative formation of the successive hydration layers. As we know, at these moisture contents the phosphate groups undergo a great

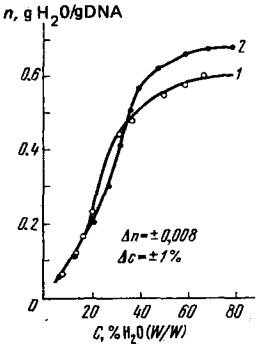


FIG. 35. Hydration curves of native (*1*) and denatured (*2*) DNA. *n* is the amount of bound water (g H_2O per g DNA), and *C* is the weight percent of water in the DNA specimens.

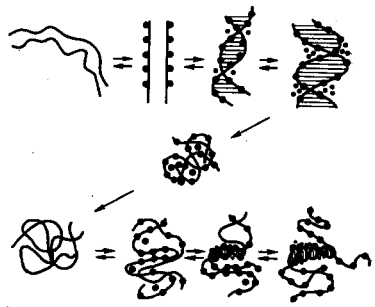


FIG. 36. Schematic diagram of the process of sorption of water in native, dehydrated DNA specimens (upper row) and in polynucleotide chains in the random-coil state (lower row). In this case drying was performed by rapid cooling of a solution of random coils with subsequent sublimation.

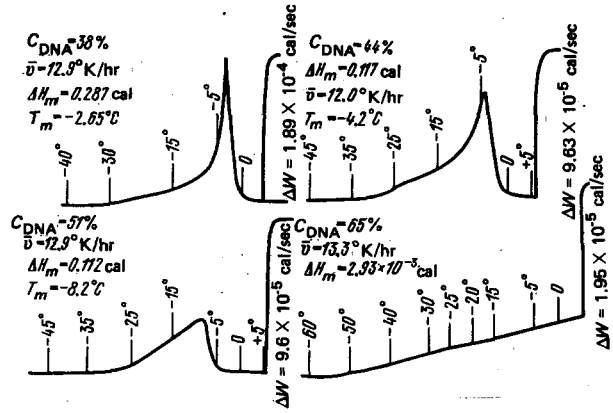


FIG. 37. Microcalorimetric recordings of the process of phase transition of water in the system Na-DNA–H_2O at different DNA concentrations.

unshielding, and twisting of the two polynucleotide chains begins with simultaneous onset of the vertical "stackings", or interactions between the bases (Fig. 36). At this stage of hydration the number of water molecules bound to native DNA increases to nine H_2O molecules per base pair. The cooperative formation of these hydration layers, which are build into the structure of the double helix, is caused by the appearance of "water bridges" in the grooves of the double helix. The possible existence of these bridges has been shown by direct calculation methods for DNA-hydrate (Na^+) complexes.[114] It is a highly essential point that the number of bound water molecules in this region of the hydration curve is smaller for denatured DNA preparations than for native (by 4± 1 H_2O molecules per base pair). Further increase in the moisture content of preparations of native DNA leads to further binding of 10 more H_2O molecules per base pair (region c, Fig. 35), which fill the grooves and form outer hydration layers. The complete saturation of all the hydration centers (26 H_2O molecules per base pair), the even greater twisting of the chains, and increase in the hydrophobic interactions of the stack of bases that occur at this hydration stage lead to establishment of the equilibrium ordered forms of the double helix of DNA. And here we must stress the highly important circumstance that the number of H_2O molecules bound to denatured DNA preparations at this latter "sorption" stage exceeds the hydration of native specimens.

We must note that the water in the hydration layers (the second and third fractions of the bound water) forms a structure possessing the helical symmetry of the DNA molecule. Thus it can undergo structural transitions, both at the temperatures of intramolecular melting of the double helix and at lower temperatures.[29]

Recently high-sensitivity low-temperature scanning microcalorimeters (LTSMs) have been applied for the first time in the Institute of Physics of the Academy of Sciences of the Georgian SSR in order to elucidate the mechanisms of interaction of DNA existing in the helical and coil states with the aqueous environment and with the ions of dissolved salts. The use of LTSMs permitted us not only to sharply diminish the expenditure of the studied preparations (by a factor of 10^3), but also to study the thermodynamic parameters of DNA and of the aqueous environment over a broad range of temperatures, moisture contents, and concentrations of dissolved ions of salts.

Figure 37 shows the microcalorimetric recordings of the heat absorption observed in the region of the ice-water phase transition during heating of frozen DNA-H_2O solutions of differing H_2O/DNA ratio. The microcalorimetric data allowed us to obtain precise results on the phase-transition temperature of the water and the enthalpy of the "degenerate" phase transition (Fig. 38). The critical values of the polymer concentration at which all the water existing in the DNA-H_2O system exists in the non-frozen-out (bound) hydration state were established with greater precision (see Fig. 37).

Here we should note that recently a number of studies have employed commercial low-temperature Type DSC-1, 2 microcalorimeters (from the Perkin-Elmer firm) for studying hydration of biopolymers. In spite of full automation, high sensitivity, and convenient operation, the thermophysical characteristics of these microcalorimeters do not satisfy the requirements imposed on instruments for studying biological objects. Thus, in a study[119] of the low-temperature thermal properties of elastin in scanning at the rate of 8°/min (!), the temperature of the phase transition

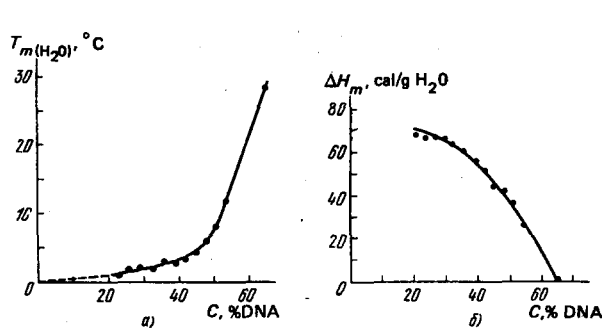


FIG. 38. Dependence of the temperature T_m (a) and the enthalpy ΔH_m (b) of the ice-water phase transition on the DNA concentration in the system Na-DNA–H_2O [temperatures in °C below zero are plotted in diagram (a)].

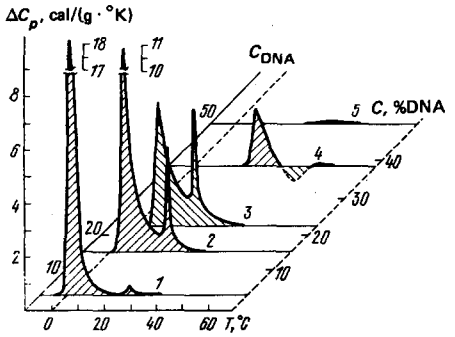


FIG. 39. Relationship of the excess heat capacity of the system Na-DNA–H_2O–NaCl to the temperature and the DNA concentration. The initial molarity of the solution is 0.2 M NaCl.

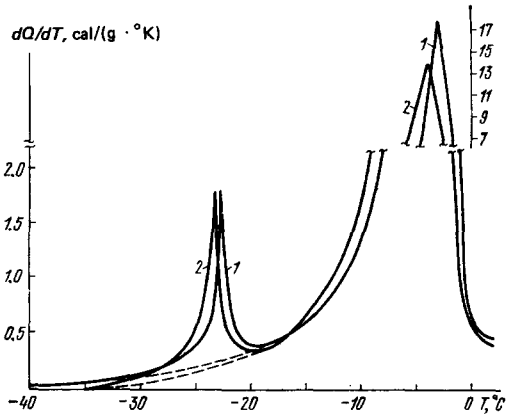


FIG. 40. Microcalorimetric recording of the processes of the ice-water phase transition and of eutectic melting in the system Na-DNA–H_2O–NaCl. *1*—native DNA, *2*—denatured DNA (1.35 mg DNA and 0.38 mg NaCl; total weight of specimen 2.8 mg).

of water proved to be +10 to +15 °C (!?). As is well known, nonequilibrium conditions of experimentation can be the source of artifacts in employing this apparatus for studying the thermal properties of biological macromolecules, and one must treat with caution the results obtained by using such apparatus. The scanning microcalorimeters of our country produced by the Special Design Office of Biological Instrument Construction in Pushchino (DASM-1) possess considerably better thermophysical characteristics (actually, in the restricted temperature interval 0–100 °C).

We have also used the LTSMs of the Institute of Physics of the Academy of Sciences of the Georgian SSR for a more detailed study of the thermal effects of the phase transition of water and the melting of eutectics in the three-component system DNA-H_2O-NaCl.

Figure 39 shows the temperature-dependence of the change in heat capacity of the three-component system Na-DNA–H_2O–NaCl at different concentrations of DNA.[112-113] We note the following characteristic features of the melting curves:

1) The temperature region near −21.3 °C in which heat absorption occurs corresponds to melting of the eutectic formed during cooling of the three-component system Na-DNA–H_2O–NaCl.

2) The process of melting of ice in the three-component system Na-DNA–H_2O–NaCl that follows the process of eutectic melting is "spread out" along the temperature axis. The transition "point" shifts toward lower temperatures with increasing DNA concentration, just as for two-component systems (cf. Fig. 37).

3) Increased DNA concentration (small volume fraction of solvent) leads to overlap of the heat-absorption peaks corresponding to melting of the eutectic and of ice. Ultimately it leads to the disappearance of the eutectic peak (curve *5* in Fig. 39).

4) The exothermal effect observed in the system Na-DNA–H_2O–NaCl at small values of the volume fraction of solvent in the temperature region −46° to −34 °C (curve *4* in Fig. 39) can be attributed to a recrystallization process (see Ref. 115, where a similar heat effect was discovered calorimetrically in other systems).

5) At a certain critical value of the volume fraction of the polyanion, the phase transitions observed in the low-temperature region in the system Na-DNA–H_2O–NaCl completely vanish. They are not detected at the highest sensitivities of the microcalorimetric apparatus. At these concentrations we can represent the state of aggregation of the three-component system in the low-temperature region as a mixture: Na-DNA–unfrozen (bound) water-NaCl.

Comparison of the variation with temperature of the excess heat capacity for dilute Na-DNA_{coil}–H_2O–NaCl and Na-DNA_{helix}–H_2O–NaCl solutions (Fig. 40) shows graphically the changes that have occurred in the three-component system upon thermal denaturation of the DNA, i.e., in the helix-coil transition. The eutectic melting peak is shifted toward lower temperatures (by 0.5°). The total width of the eutectic melting peak is increased by 2°. Calculation (see Ref. 29) shows that ≈ 40% of the ions are "rejected" into the solution during thermal denaturation of DNA (cf. the results of Ref. 116).

Thus the study of the low-temperature heat capacity of DNA at various moisture contents and concentrations of ions of dissolved salts has enabled us to obtain important results on the mechanisms of interaction of DNA macromolecules with the aqueous environment and with the ions of salts for the polynucleotide chains in the random-coil state.

At present studies have begun in the Institute of Physics of the Academy of Sciences of the Georgian SSR on the heat capacity of DNA at different moisture contents in the liquid-helium temperature region. Along this line, there are already some experimental data obtained in the Physicotechnical Institute of Low Temperatures of the Academy of Sciences of the Ukrainian SSR. Verkin and his associates[30] have studied the heat capacity of moist DNA films in the temperature range 4–200 °K. The authors arrive at some substantial conclusions on the features of the low-temperature heat capacity of DNA films based on the theory of heat capacity of chain and layer structures at low tempera-

tures.[79-80] They showed that the linear variation of the heat capacity in the region 4.2–180 °K is mainly due to participation in twisting vibrations of the backbone of the polymer and the side chains having large moments of inertia. However, as we have stressed above, drawing an unambiguous conclusion on the "conservation of the secondary structure of DNA in dry films" requires study at different moisture contents up to values that correspond to a complete hydration layer on the double helix.

f) Anomalies in the low-temperature heat capacity of melanin and of the tumor melanosome

The melanins are extremely widespread pigments of skin and hair to which the color of the latter is due. The chemical structure of the melanins has not been fully worked out.[117]

Study of the physical properties of the melanins has shown these compounds to be characterized by an amorphous structure with a high density of free spins (10^{19} g^{-1}).[118] The melanins also have been shown to exhibit a number of the unusual electrical properties found in certain amorphous materials.[45]

American investigators have studied the heat capacity of natural and synthetic melanins as well as of melanosomes [isolated from malignant tumor tissues (melanomas)] in the temperature range 1–5 °K.[45]

Figure 41 and Table III show the results of the studies, which revealed a number of interesting properties of melanin. Processing of the data by the usual equation $C_p = \gamma T + \beta T^3 + \delta T^5$ (where γ is the coefficient of the linear term of the electronic heat capacity, and β and δ are coefficients reflecting the contribution of the lattice to the heat capacity) (see Table III) shows that we can infer from the sizes of the coefficient β that the contribution of the lattice heat capacity is the same for all three materials. The values of the coefficient δ are also the same, though considerably smaller in absolute magnitude. Yet the coefficient of the linear electronic term proved amazingly large, and substantially elevated for amine-polymerized melanin, on the one hand, or the tumor melanosome, on the other, as compared with H_2O-polymerized melanin.

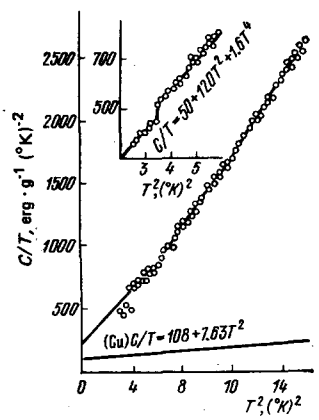


FIG. 41. The $C_p/T = f(T^2)$ relationship for synthetic melanin (in the center) and for the tumor melanosome (inset) (the heat capacity of copper is given for comparison).[45]

TABLE III. Low-temperature heat capacity of melanin.

	γ, erg·g^{-1}·K^{-2}	β, erg·g^{-1}·K^{-4}	δ, erg·g^{-1}·K^{-6}	T_0, K	ΔC, erg·g^{-1}·K^{-1}
Melanin, 1% H_2O	50±9	120±2	1.64±0.09	1.87	940
Melanin, 20% DEA* 10% H_2O	95±9	99±2	1.84±0.09	1.92	770
Melanosome	220±20	99±4	2.0±0.20	1.95	2.530

The data were obtained by using the equation $C = \gamma T + \beta T^3 + \delta T^5$.
*DEA = diethylamine.

Currently the nature of the observed variations is not clear.

Yet we can infer from the presented data that the changes in the coefficient γ cannot arise from changes in the phonon spectrum of the studied melanins.

Another interesting anomaly was manifested in the temperature-dependence of the heat capacity for all the melanins at 1.9 °K (see Fig. 41). The authors consider that the sharpness of the observed transition forbids ascribing it to known low-temperature transitions.

Interestingly, EPR[118] has shown that the g-factor for synthetic and natural melanins is close to two, while a paramagnetic behavior of these materials was established in the temperature range 4.2–500 °K. The authors concluded that the observed paramagnetism involves the presence of unpaired electrons associated with free radicals in the amorphous melanins.

The heat capacity above the transition point (1.9 °K) is characteristic of a material in the paramagnetic state. The authors consider that the heat anomaly at 1.9°K reflects a transition from the antiferromagnetic to the paramagnetic state.

Thus the calorimetric method at low temperatures has revealed features in the thermal properties of melanins of different types that are interesting not only for characterizing the physical properties of this pigment, but they pose the question of yet unknown functional features of these compounds. In particular, it becomes clear that the melanosomes cannot be classified as passive cellular organelles that play only the role of photoprotectors—the high density of unpaired electrons and the existence of a magnetic phase transition apparently indicate that the melanosomes play an important functional role in energy-transparent processes in the living cell.

6. CONCLUDING REMARKS

Currently we witness the onset of development of a new field in molecular biophysics—low-temperature physics of biological macromolecules ("molecular cryobiophysics"), which has already yielded a number of substantial experimental results. In this paper we have touched only on problems of the thermal properties of biomacromolecules at low temperatures.

We see from the presented material that one can use the advances of the theory of heat capacity of highly anisotropic solids (the so-called "layer" and "chain"

or "acicular" structures) for developing a theory of heat capacity of biopolymers in the solid state at low temperatures. Moreover, this review also has aimed at enhancing the attention of theoretical physicists to problems of the thermal properties of biological macromolecules at low temperatures, and thus to "firm up" the quantum-mechanical theory of the heat capacity of anisotropic materials for describing the physical features of biologically important macromolecules.

With regard to the calorimetry of biopolymers as such, we should say that the modern level of technique of calorimetric experimentation (especially considering the thermophysical characteristics of scanning differential calorimeters) quite suffices for obtaining precision data on the thermal properties of biomacromolecules over a broad range of concentrations of materials and of temperatures (from 2 °K up). We add that even now measurements can be performed on small amounts of preparations of proteins and nucleic acids. Yet we should stress that the biochemical procedures should ensure the isolation, purification, and preparation of the needed specimens (oriented moist films, supermolecular structures of biopolymers, fibers, etc.) designed for low-temperature calorimetry. In most cases the method of preparation of the specimens is decisive. As we have stressed, one should pay especial attention in performing low-temperature calorimetric measurements on biopolymers to the water/biopolymer molar ratio. The minimal amount of water needed to preserve the native structure of the macromolecules (the so-called bound, or unfreezable water) can be established by measuring the thermal effects in the region of the ice-water phase transition. Here one uses low-temperature scanning microcalorimeters of sensitivity 10^{-7} W with microgram amounts of a preparation. Attempts to calculate the entropy of proteins in the solid state (from the amino-acid composition and data on the heat capacity of the amino acids) apparently yield erroneous results precisely because they neglect the specifics of interaction of the polypeptide chains with the solvent molecules and its role in formation of the spatial structure of the protein.

A detailed study of the low-temperature heat capacity of synthetic polypeptides seems promising. On the one hand, they model the structure of the polypeptide chains of a protein and, on the other, they model the behavior of the so-called "one-dimensional" and "two-dimensional" structures. Quite likely, one can employ the character of the temperature-dependence of the heat capacity of such structures in the low-temperature region to get an estimate of the preferential conformation of the chains in proteins having an unknown spatial structure based on calorimetric data.

The discovery of low-temperature phase transitions in biomacromolecules opens up new potentialities in studying the latter. The discovery of such a transition in melanin confirms this conviction. In turn, the discovery and characterization of such phase transitions allows one to ascertain the fundamental physical properties decisive in the function of a given biopolymer. It is a highly attractive approach to devise experiments employing linear macromolecules containing in their structure hydrogen-bonded water chains that lie regularly along the long axes of the macromolecules. Of especial interest are experimental data and theoretical analysis on these systems at temperatures below 10 °K, and possibly below 1 °K.

Finally, we view it as extremely important and promising to study the low-temperature heat capacity of liquid-crystalline structures of biological origin in model liquid-crystalline systems. Such experiments at liquid-helium temperature have not yet been realized. At the same time it is quite well known that many structural formations of cells and even of organs can exist in the liquid-crystalline state. Thus, even without speaking of the cell membranes, whose liquid-crystalline structure is unquestioned, we can assuredly state that the brain essentially amounts to a complicated supermolecular complex of liquid-crystalline nature. Study of the low-temperature thermal properties of such structures can reveal completely new properties of these compounds that depend on the density and character of the packing of the ordered molecular ensembles in them and on the type of introduced defects.

Evidently "molecular cryobiophysics" cannot be based solely on methods of thermal measurements. Employment of the entire arsenal of modern physical methods for studying biopolymers in the low-temperature region (such as radiospectroscopy, neutron diffraction, nuclear spectroscopy, electrical methods, etc.) allows access to fundamental data on the properties of the most important biological macromolecules. These will be applied successfully for achieving the fundamental goal—constructing a physical theory that explains the functional properties of the macromolecules of proteins and nucleic acids.

In our country the intensive studies in the field of calorimetry of biological objects, including the study of the low-temperature thermal properties of biological macromolecules, were begun by É. L. Andronikashvili and his students as early as 1956. In writing this article, in choosing the key problems, and in discussing them, the author has constantly used the ideas, advice, and critical remarks of É. L. Andronikashvili, whose benelovent attitude toward the idea of writing such a review and repeated remarks on the expedience of publishing it have allowed the author to complete the adopted work. The author takes the opportunity to offer most sincere thanks to É. L. Andronikashvili.

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