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On regularities in the spontaneous formation of structural hierarchies in chiral systems of nonliving and living matter

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Abstract. We review a general regularity concerned with the spontaneous formation of alternating-sign L–D hierarchies of chiral structures that are initially nonequilibrium due to homochirality. Mechanical, hydrodynamic, macromolecular, and liquid-crystal systems, as well as proteins and nucleic acids, are considered. Biomacromolecule chirality is related to the presence of an asymmetric carbon atom and, further, to the formation of helical and superhelical intra- and supramolecular structures. Chirality is a physical vehicle that generates stratification and folding in biological macromolecular systems. The hierarchies of alternating-sign chiral structures underlie the formation of a periodic molecular-biological system of cells.

Keywords: chirality, nucleic acids, proteins, hierarchy of structures, folding, molecular machines

1. Introduction

Nearly 30 years ago, *Physics–Uspekhi* journal published a remarkable article by V I Gol'danskii and V V Kuz'min under the title "Spontaneous breaking of mirror symmetry in nature and the origin of life" [1]. The article began as follows: "Over 100 hundred years have elapsed since Louis Pasteur drew the 'demarcation line' between living and nonliving, i.e., discovered mirror asymmetry of organisms. It seems rather a long time for practically any research area to be exhausted. However, the fact that living organisms use only one of the two mirror isomers of such molecules as amino acids and sugars but do not use the other... remains an intriguing enigma." The article contained a detailed analysis of physical aspects of deracemization of Earth's biosphere that continue to be relevant up to now. In past years, the functions of the main chiral compounds in this 'right, left living world' have been clarified, and specific biochemical features of mirror asymmetry of biomacromolecules have become better understood; however, the physical nature of this asymmetry parentage remains to be elucidated [2].

The authors of the present paper wondered not about *how chiral dissymmetry appeared and fitted* into living systems but about *why it appeared* at all. The paper is designed to give an insight into the physical role of chirality in structure formation processes in molecular and molecular-biological systems.

The most general construction principles of living matter, such as discreteness, departure from equilibrium, nonlinearity, synergism, hierarchism, machinery, combination of phase states, conjugation of quantum and macroscopic scales and processes, and systematism, are physical in essence, whereas the universal 'chemical' instrumentarium makes it possible to effectively realize most diverse life forms characteristic of Earth's biosphere. Importantly, these systematic properties of living matter are related to the notion of symmetry.

The ideas of symmetry are of fundamental significance for considering physical regularities at different organizational levels of matter. There are observable symmetries of macroscopic levels, but the most general latent symmetries constitute the primary cause rather than manifestations of general physical regularities [3]. It is equally natural to regard the ideas of symmetry and its breaking as a fundamental factor in the consideration of physical grounds of living system structuring, origin, and evolution. Symmetry characteristics of system's orderliness at one of the levels predetermine its scale and symmetry properties at the next hierarchical level of evolutionary development and biological organization.

When considering the problem of structural and functional hierarchies, one encounters the concrete implementation of a system's principle in nature, namely using the beneficial structural features or spontaneous processes in

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inanimate nature and their conversion into suitable functions. Specifically, the universal hierarchization of natural systems is a functionally meaningful attribute of all biological communities, because it makes possible combining differently scaled processes in time and space, as well as conjugating them in accordance with concrete real-life situations. The core of the concept being developed in this article is the statement that the formation of molecular-biological hierarchies is directly related to the chirality phenomenon.

2. Structural destinations of chirality

Schrödinger's insightful remark in his book *What Is Life...* [4] to the effect that biological structures and molecules are 'aperiodic crystals' actually implies that they ensure a high degree of ordering in manifestations of biological phenomena, and this exempts them from the totality of known 'trivial' symmetries intrinsic in periodic crystals of an abiotic nature. Indeed, animate nature 'inventively' departed from the realm of periodic crystals of an inanimate nature and thereby emerged from the control of rigorous rules and structural limitations specified in the studies by Curie and other crystallographers for point symmetries. To ensure regularity, nature reserved for biological molecules only chiral dualism from the world of symmetry to enable them creating regular vertical hierarchical constructions combining the structural stiffness and lability needed for the work of molecular machines, e.g., in the process of biosynthesis and cellular motility mechanisms. Periodic structures usually present in protein molecules are α-helices and their combinations, as well as β-structures, all of them being chiral entities representing the topological type of periodicity, as opposed to the geometric one.

To consider molecular-biological systems, we shall adopt the classical definition of *chirality* as a property of molecules or objects to be incompatible with their mirror images upon participating in any combination of movements and rotations in a three-dimensional space [1]. An enantiomer [1] (enantiomorph) exhibits neither between-side nor axial symmetry. We shall also use a more general L/D classification of chirality signs oriented toward macro- and supramolecular structures, in contrast to the S/R nomenclature encompassing concrete chemical groups. The chirality signs of an asymmetric sp^3-carbon, used in this paper, initially related to the rotation of the light polarization plane, as well as the direction of spirals, both two- and three-dimensional, are actually conventional but make up an established consistent system.

The homochirality of monomers as basic components of informationally determinate biopolymers, proteins, and nucleic acids (L-amino acids and D-carbohydrates) is usually perceived as an evolutionary oddity, a sort of 'payment' for the unique ability of carbon to form a large number of organic and inorganic compounds, harmonically integrating structures and functions of living systems into a whole. In the heterochiral outer space, the homochirality of L-amino acids present in proteins ensures stereospecificity of complementary interactions and reduces to a minimum the amount of information necessary for unambiguous encoding of amino acid sequences by nucleic acids. To recall, the requirement of homochirality of protein building blocks and nucleic acids does not rule out the extensive participation of D-amino acids and L-sugars in the systems of regulation, reception, and protection [5]. Thus, certain bacteria (cholera vibrio and hay bacillus) synthesize D-amino acids that serve as binders in the plasma membrane, regulate the activity of enzymes responsible for cell wall reinforcement, and prevent the degradation of cell walls against the antagonistic action of enzymes-hydrolyses that affect L-amino acids alone. D-amino acids have until recently been regarded as occurring only in bacteria and fungi. Today, however, they are known to perform as well various functions in eukaryotes. By way of example, D-serine is a neurotransmitter in the mammalian brain, while D-aspartic acid participates in the regulation of embryogenesis and modulates hormonal secretion in the neuroendocrine tissues of mammals. Large amounts of D-amino acids are present in regulatory oligopeptides.

In other words, the concept of 'chiral purity of the biosphere' remains as valid and actual as before, but should be interpreted in broader terms in relation to systems, structures, and functions of different levels.

In previous publications, we formulated two general rules concerning structure formation in homochiral systems [6, 7]:

• A homochiral system having a store of free energy can develop within a single hierarchical level, while retaining both the type of symmetry and the sign of predominant chirality ('right' — D or 'left' — L twist).

• An evolving homochiral system tending toward spontaneous formation of a succession of new hierarchical levels has an alternating chirality sign of de-novo structures as it passes bifurcation points.

The formation of alternating-sign chiral hierarchy is related to the initial storage of free energy (due to chiral dissymmetry) and its dissipation during the spontaneous formation of L – D sign-alternating structural hierarchies — enantiomorphs with the varying symmetry type. In the 'chemical' tradition, racemization of homochiral substances is associated with their spontaneous isomerization, i.e., formation of mirror enantiomers. In this case, the type of chirality (symmetry) is conserved. Chiral molecular structures formed in animate and inanimate nature contain structural components in the form of initial enantiomers with identical chirality signs. It will be shown that in this case not only the symmetry scale and (of course) type but also the chirality sign of the resulting structure change. We believe that this principle underlines the hierarchical structure formation in the model and biological macromolecules considered in the following sections.

For convenience of data presentation and understanding, we take advantage of traditional notations for chirality signs D and L in cases of both central and spiral symmetry. Problems related to free energy storage in homochiral systems and justification of the correlation of chirality signs in the hierarchy of structures with different symmetries will be considered in the context of the discussion of the materials obtained.

The regularity to be discussed will be illustrated as exemplified by several inanimate natural systems, including biomimetic ones and molecular-biological systems, such as proteins and nucleic acids.

3. Structure formation in homochiral systems

In the course of evolution, unrelated events of spontaneous self-organization in inanimate nature have come to the level of a systematic principle in biological objects. We think that

[1] An enantiomer consists of a pair of stereoisomers, mirror reflections of each other being incompatible in space.

the chirality of molecules and macroscopic objects is a fundamental structure-forming factor in inanimate and animate nature [7–9]. To demonstrate the general tendency of chiral structure formation, we shall consider a few examples from mechanics and physical chemistry.

The first rule concerning inanimate nature is illustrated by the example of a chiral 'seed' [10]. Polyisocyanates synthesized from achiral monomers spontaneously form right- and left-handed helical polymer chains in equal proportions, but the introduction of a small amount of a monomer's chiral analog into the medium produces a large excess of one of the enantiomorphs over the racemate,[2] i.e., chiral polarization.

Another example of the strong influence of slight chiral imbalance is the separation of amino acid enantiomers in an equilibrium heterogeneous system consisting of liquid and solid phases [10]. Small (a few percent) chiral polarization can give rise to significant chiral polarization of the solution due to the difference in relative solubility of enantiomers in different phases (in this case, the racemate sinks into the sorbent).

These examples confirm the possibility of developing systems asymmetric in terms of the number of enantiomers formed during deracemization of one of the phases. In this case, the sign of the dominating chirality remains unaltered, and the degree of predominance of one of the chiral components can be preserved and even increased.

Let us consider examples where the chirality sign is switched.

Mechanics. *A twisted cord.* Twisting elastic threads provides the clearest demonstration of our concept through an example from the realm of mechanics. Twisting a long cord in a certain direction produces reversed helical windings. Stress increasing causes the formation of windings of the same direction and greater amplitude; these are 'second mode' windings. A further increase in tension following the appearance of the second mode gives rise to pin-like protrusions and a double superhelical after folding. A helix with one chirality sign forms pins and superhelices of the opposite sign. As the system moves to the next level of structural hierarchy, its scale and symmetry change, which results in emerging new degrees of freedom over which the energy is redistributed; as a consequence, elastic stress decreases and the system lowers its free energy level.

Hydrodynamics. *A Kármán vortex street* is a repeating chain of oppositely swirling vortices observed when a fluid or a gas flows around extended cylindrical bodies (or other linearly lengthened and poorly streamlined profiles) with the longitudinal axis perpendicular to the direction of motion of a continuous medium [11]. If the medium is homogeneous, an alternating-sign chiral structure develops in a single layer on approximately one scale, certainly obeying the law of conservation of angular momentum.

Hetons are two-layer composite vortices with zero total intensity arising in a medium in the presence of seawater shear layers differing in temperature. Heton-like vortices swirl in opposite directions; they are of different scales, bound by the law of conservation of angular momentum, conjugated by mass and energy flows, and linked in movements [12]. In addition, there are intrathermoclinic vortices or lenses described as vortex spots in the framework of the three-layer model. In this case, we are dealing with a stratified system of differently scaled and directed vortices.

[2] Racemate is an equimolar mixture of two enantiomers.

Thermoconvective cells. Spiral convective microstructures can emerge on the surface of a rather thin liquid layer undergoing cooling and evaporating. They were first described by E P Khizhnyak using an infrared technique [13]. In these cells, unlike Benar cells, thermal convection occurs not only in vertical planes but has a rotational character as well. When interconnected funnel-like microstreams swirl, e.g., counterclockwise, the entire surface layer starts rotating in the opposite direction, again in accordance with the law of conservation of angular momentum, in the framework of which two processes (counter-propagating, chiral, different-scale) are coupled.

Physicochemical systems demonstrating the formation of sign-alternating chiral levels. In the cases under consideration, we are dealing with L/D enantiomers of molecules containing a single asymmetric carbon atom that has four different substitutes. As far as enantiomorphs are concerned, either right- or left-handed helices and superhelices will be implied. Cited below are papers demonstrating the formation of chirally stratified structures.

Reference [14] illustrated the influence of the stereogenetic center (obtained from the tartaric acid of a dumbbell-like substitute) on the chirality of the structure formed by the sequence of synthesized aromatic oligoamides. This complex proved to be long-lived and stable enough kinetically, even if it underwent gradual transformation with time into a complex of two single helices with two substitutes and left–right reversal of the chirality sign. The system passed from the kinetically slowed left-handed complex to the equilibrium right-handed state.

Reference [15] revealed the self-assembly of twisted ribbons made from a widely used achiral cationic surface-active compound (SAC) with L- or D-tartrate as a chiral counterion in water and some organic solvents. Ribbons from achiral cationic SAC and L-tartrate proved to be right-handed, and those from achiral cationic SAC and D-tartrate left-handed. Ribbons from a racemic mixture remained untwisted.

Symmetrically substituted achiral and chiral derivatives of hexa-peri-hexabenzocoronene form columnar mesophases. The authors of study [16] developed and synthesized a series of new amphiphilic derivatives of hexa-peri-hexabenzocoronene forming helical carbon nanotubes. Left- and right-handed enantiomers are assembled into right-handed and left-handed helices, respectively (Fig. 1)

A similar system with a metaphase was examined by S V Stovbun and colleagues, who observed the formation

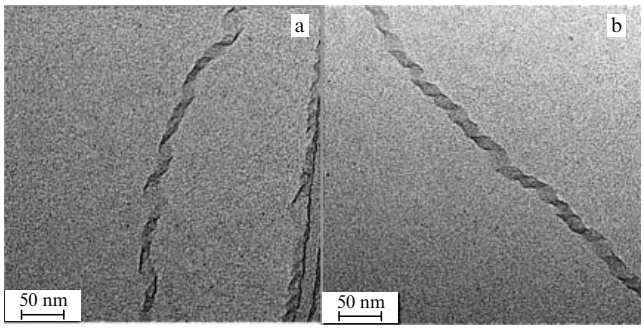

Figure 1. (a) Right-hand twisted helix from left-handed enantiomers of hexa-peri-hexabenzocoronene derivatives, and (b) left-handed helix from right-handed enantiomers [16].

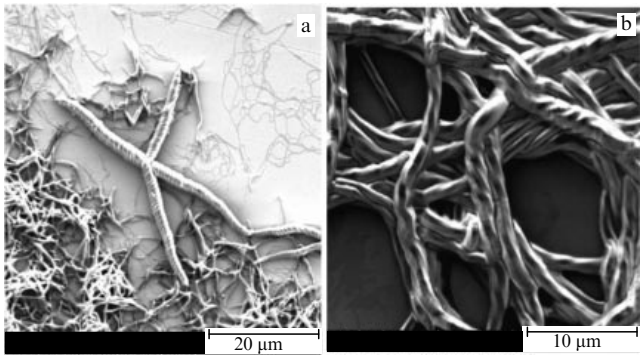

Figure 2. (a) Right-handed helix from left-handed enantiomers of synthetic tris(3(3′-carbamoilamino)-2,2′,bipyridil)benzene-1,3,5-tricarbon amide derivatives, and (b) left-handed helix from right-handed enantiomers[18].

of characteristically stiff helical structures (so-called strings) in homochiral solutions of trifluoroacetylated amino alcohols (TFAAA) in cyclohexane, cumene, and other solvents [17]. Due to dipole–dipole, van der Waals, and chirally moderated stereospecific interactions, the dextro-isomer of one of the amino alcohols formed a left-handed chiral string that, in turn, twisted into a right-handed superhelix.

It was shown in Ref. [18] that synthetic nonamphiphilic derivatives of tris(3(3′-carbamoilamino)-2,2′,bipyridil)-benzene-1,3,5-tricarbon amide containing three peripheral chiral 6HC[(R) or (8)-2-methyl butylthio]-tetrathiofulvalenes can be assembled into twisted fibers. This compound can be dissolved into a high concentration in dioxane on heating, but it slowly precipitates as the solution cools to room temperature. Analysis of the precipitate by optical or scanning microscopy allows fiber formation to be detected, with left-handed enantiomers assembling into a right-handed helix, and right-handed enantiomers into a left-handed one (Fig. 2).

Interestingly, modeling this system by molecular dynamics methods yields an opposite result, i.e., the left-handed helix formed from left-handed enantiomers is more stable than the right-handed one. Moreover, results of modeling indicate that three left-handed helical filaments from left-handed enantiomers must fit into the left-handed superhelix. In this case, the criterion for establishing the facts is experiment. It appears that the consideration of such systems in the molecular dynamics approach can be inadequate due to the underestimation of certain system's components or factors. Importantly, molecular dynamics methods disregard the entropy factor essential for the verification of our concept.

Superamphiphiles consisting of isocyanopeptides (hydrophilic block) and polystyrol (hydrophobic block) can be self-assembled in water into rod-like micelles, vesicles, and helical aggregates [19]. Micellar rods having a polystyrol core, and the surface formed from right-handed helical polyisocyanides are the first to appear. They then are assembled into left-handed superhelical fibers (Fig. 3).

Reference [20] demonstrates the formation of polyacetylene helical structures after the attachment of chiral amines. The addition of left-handed (L/S) and right-handed (D/R) enantiomers results in producing the right-handed and left-handed helices, respectively (Fig. 4).

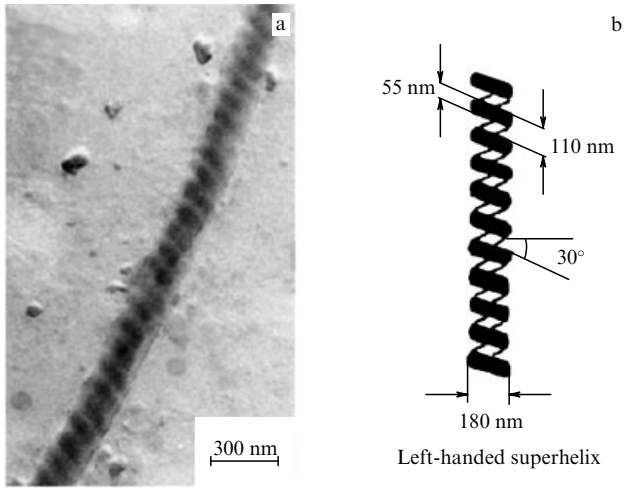

Figure 3. Left-handed superhelix from right-handed helices of polyisocyanide: (a) TEM image, and (b) schematic representation [19].

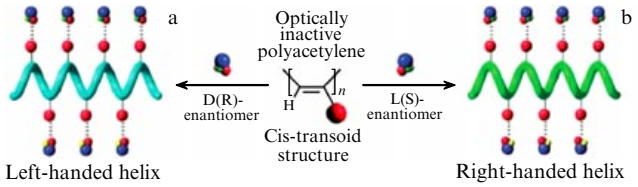

Figure 4. (Color online.) Helix formation from linear derivatives of polyacetylene upon attachment of chiral amines: (a) formation of a left-handed helix upon attachment of D(R) enantiomers, and (b) formation of a right-handed helix upon attachment of L(S) enantiomers [20].

In 1945, the very first case of the formation of helical complexes from small molecules of amphiphilic carboxylic acids with a long alkyl chain ending in an (8)sec-butyl group was reported [21]. This early publication did not mention the twist direction of individual filaments in aqueous dispersions of lithium 12-hydroxystearate, but later authors [22] showed that D-enantiomers of lithium 12-hydroxystearate assemble into right-handed filaments, and L-enantiomers into left-handed ones. In this case, as in some others, we cannot explain the cause of the failure to comply with the rules we formulated for changes of chirality signs. Nevertheless, such rare exceptions do not contradict the common pattern.

Let us turn now to 2D helical structures. In mono- and bilayers of certain amphiphiles having at least one chiral center, such as modified phospholipids, helicoidal structures are known to form under compression if some enantiomer (L or D) is present in excess. In such a case, the chirality of the helix is always rigidly connected with that of the enantiomer and undergoes sign reversal upon a change of chirality sign of the enantiomer [23].

A similar effect was observed in the formation of right-handed helical structures by L-phospholipids at the phase interface in Ref. [24], the authors of which reported the formation of a chiral structural hierarchy, but took no notice concerning the change of chirality sign.

The formation of different-scale sign-alternating chiral structures can be followed up in cholesteric liquid crystals composed of chiral molecules making up a set of 2D planar structures. Molecules in each layer are oriented largely along

the director that rotates about the cholesteric axis upon transition to the next layer, thus giving rise to a helix with the sign opposite to the chirality sign of the molecules. The cause of director rotation is rooted in stereospecific constraints imposed by the shape of the molecules. The cholesterol molecule has a roughly planar structure, with methyl groups CH_3 located both above and beneath the molecular plane [25]. Levo-cholesterol (with respect to asymmetric carbon) determines the dextrorotation of the director [26].

The cholesteric phase of DNA is also associated with the right-to-left change of chirality sign upon transition to a higher organizational level [27, 28]. The cholesteric phase exhibits a standard organization of parallel layers of DNA molecules, each rotating through a small angle with respect to the previous one. It was shown in experiment that right-handed DNA forms layers that, in turn, give rise to the left-handed helix.

The above examples illustrate the rule for changing the chirality sign upon transition to the next hierarchical level. Many of them concerning structural hierarchies were borrowed from the detailed review [29]. We chose those examples where it was possible to distinctly identify the sign of chirality for each structural hierarchy level. However, the said review contains many references to studies lacking data on the signs of individual levels. They could not be analyzed in the present paper. We noticed very rare exceptional cases of levels with repeating chirality signs, which suggests the involvement of certain third participants in such 'chiral metamorphoses', although the general rule is fulfilled.

This pattern appears to be the sole symmetric invariant known thus far that meets the conjugation of prebiological and biological systems in the end-to-end self-organizing flow [7]. This phenomenon, exemplified by mechanical constructions along with hydrodynamic, polymeric, liquid-crystalline, and surface-active systems has come as a matter of course into animate nature in the form of the principle of hierarchical formation of discrete structures making up the backbone (or more exactly flexible skeleton) of biological macromolecules. It is conjectured that the above properties of abiotic systems created a prerequisite for the systematic character of the saltatory [3] development of the biosphere during prebiological and biological evolution [30].

4. Periodic molecular-biological system of alternating-sign chiral structures with an achiral core

We do not consider here various hypothetical mechanisms of the origin of precursors to living cells presented in Ref. [31] and discussed before in our publications [32, 33]. Suffice it to emphasize in the context of this review the acquisition of two fundamental asymmetries (dissymmetry and antisymmetry) in the course of prebiological evolution: *cellular ionic asymmetry*, which predetermined the thermodynamic disequilibrium of primary cells, and *molecular chiral asymmetry*, intrinsic in primary protein and nucleic acid molecules, i.e., information-determinate structures [30]. This asymmetry also includes *thermodynamic disequilibrium*, which will be discussed in Section 6.

Chiral asymmetry is more widespread in molecular-biological systems than is considered to be the case. Apart from left-handed amino acids in proteins, right-handed carbohydrates (ribose and deoxyribose) in nucleic acids, and all other right-handed carbohydrates involved in basal metabolism, various lipids just as well represent homochiral classes of substances in organisms of different taxonomical ranks [2]. Chiral molecules provide a basis for the formation of bigger chiral molecular and supramolecular structures possessing the distinguished degrees of freedom needed for molecular machines to form and function: from enzymatic machinery to cytoskeleton integrating activities of the entire cell.

Earlier, we put forward and substantiated the concept of natural regularity of chiral concordances in intra- and supramolecular structures, biosynthesis pathways, and metabolism networks. It was shown that *the system of chiral concordances of molecular structures can be represented in the form of a* '*Periodic Table*' (Fig. 5). Sign-alternating hierarchies of chiral structures were identified for the first time as chiral invariants in the successions from the 'lower' asymmetric carbon atom in sp^3-hybridization up to superhelices and supramolecular structures [7]. Chirality sign alternation, D–L–D–L, upon transition to a higher level of DNA structural and functional organization was documented for the most widespread B-form. Specifically, it was shown that deoxyribose molecules linked by phosphodiester bonds are D-isomers. Most nucleotides containing them occur in the left-handed gosh conformation [34], which allows complementary base-pairs to connect to one another using hydrogen bonds and thereby form a right-handed DNA double helix representing the next organizational level. Subsequent superspiralization, characteristic of semiflexible polymer DNA chains, manifests itself as the right-handed double helix levofolding observed in prokaryotes.

Worthy of special note is the fact that we consider the linear sequence of deoxyribose molecules rather than that of nucleotides (as traditionally accepted) to be the primary structure of DNA, thus facilitating a comparison between similar structural levels of DNA and proteins in terms of chirality signs. According to this line of reasoning, the sequence in which the complete double-helix structure in B-conformation forms in the cell is unessential; what is important relates to the fully developed and functionally active structural hierarchy.

The sequence of sign alternation in the structural–functional hierarchy of proteins resembles that in DNA, but it starts from the left-handed enantiomer: L–D–L–D. Proteins are known to be linear polymers made from L-amino acid residues [35]. D-amino acid residues occasionally present in peptides are not actually coded to enable their participation in matrix protein synthesis and are incorporated into a polymeric chain by special enzymes or during spontaneous racemization. Polypeptide chains making up the secondary structure of proteins are folded into right-handed α-helices or wrinkled β-sheets. Other regular structures do occur, but rarely. The tertiary structure of proteins is constituted by interacting α-helices. Practically in all cases of visually observable close intramolecular overlap between α-helices, they show a plainly apparent tendency toward winding into a left-handed superhelix (Fig. 6). The quaternary structure of proteins is represented by supramolecular structures formed largely from right-handed folding of left-handed superhelices.

Figure 5 shows a half-period shift between DNA and protein columns of the table owing to the fact that protein

[3] Saltatory: jump-like.

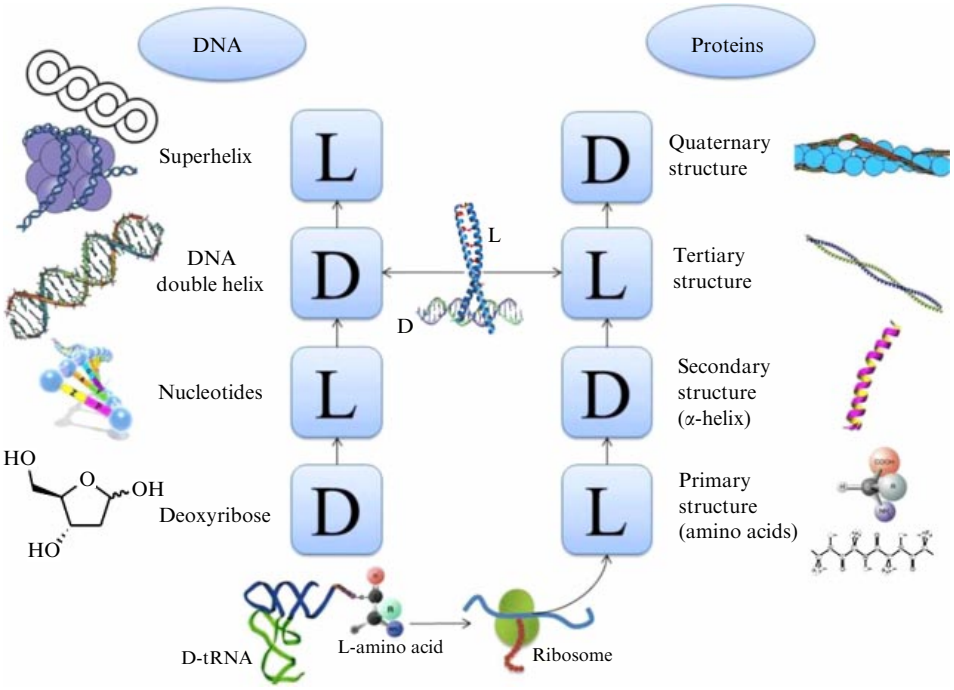

Figure 5. (Color online.) 'Periodic Table' of alternating-sign hierarchies of chiral (helical) structures, from primary to quaternary, for DNA (left column) and proteins (right column): L is the left-handed configuration of an enantiomer or helix, and D is the right-handed configuration. The achiral central block of molecular-biological structures is composed of unlike chiral units with a central symmetry axis.

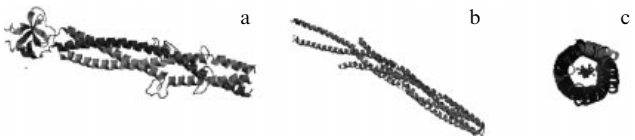

Figure 6. Protein structures containing superhelices: (a) PDB ID: 1AA0 (based on three α-helices), (b) PDB ID: 1QU7 (based on four α-helices), and (c) PDB ID: 1MZ9 (based on five α-helices).

hierarchy starts from L-amino acids, while nucleotide one from deoxyribose (a D-carbohydrate).

The '*Periodic Table*' of primary-to-quaternary chiral structures of DNA and proteins taken together (Fig. 5) *represents an achiral invariant making up the symmetrical core of molecular-biological structures* with a central axis of symmetry. It should be clearly understood that we mean in our discussion no more than *a tendency* toward chirality sign alternation in the hierarchical structure of macromolecules, rather than a rigorous rule. Variance can be due, for example, to stereochemical constraints and the disregarded involvement of chiral molecules or structures (including a solvent) in structure-forming processes. Equally essential are enthalpic and entropic factors.

An abnormality in the protein secondary structure is exemplified by the presence of a polyproline helix stabilized by van der Waals forces. It occurs in two variants: right-handed poly(Pro)I and left-handed poly(Pro)II helices twisted differently due to cis- and trans-isomeries, respectively. Two left-handed polyproline helices form the right-handed superhelix. A similar structure is inherent in collagen, where three left-handed poly(Pro)II helices with interchain hydrogen bonds give rise to a right-handed collagen superhelix [35].

5. L vs. D

The fundamental principles of molecular biology include the well-known concepts of complementary intermolecular interactions accounting for their selectivity. These concepts find wide application in model calculations using the molecular dynamics method, e.g., to choose specific interactions of biologically active compounds with enzyme receptors or active centers. Direct physical interactions between contacting groups are mostly taken into consideration, whereas specific structural properties of an entire macromolecule are disregarded. This may account for the frequent failure of detailed calculations or the discrepancy between theory and experiment.

The fundamental question of why an enzyme needs a large hierarchically complicated globule has remained unresolved for many decades. The most popular answer is an enzyme constitutes a molecular machine. We are of the opinion that not only an enzyme but also any other molecular machine processing matter, energy, or information has a 'large body' with a chirally determined hierarchical structure designed for two purposes: to maintain operation of the machine as a whole, and to enable systematic recognition of chiral partner molecules.

The concept of a 'Periodic Table' of molecular-biological structures illustrated in Fig. 5 appears to reflect a most important property of intermolecular concordances, namely that molecules of the same type tend to interact with a single class of chiral structures, either L or D, and different-type macromolecules within complementary pairs of chiral enantiomers differing in sign. Each macromolecule participating in intermolecular interactions has the dominant chirality sign of the higher-level intramolecular structure directly involved in the interaction. Interacting molecules of the same type (protein–protein, DNA–RNA, tRNA–mRNA, ribozymes)

usually have identical chirality signs (L – L or D – D), while different-type molecules (DNA–protein, tRNA–amino acids, enzyme–substrate) have different signs (D – L or L – D).

For example, DNA and RNA interact between themselves at different structural levels with the same sign of chirality, while the tertiary left-handed helical structure of proteolytic enzymes with the primary left-handed helical structures of substrates-proteins [36]. The interaction of chiral structures in functionally different DNA-protein systems is exemplified by a 'leucine lightning bolt'. Intramolecular covering of two parallel right-handed α-helices at the level of protein tertiary structure results in the formation of a left-handed zipper dimer interacting with right-handed DNA, also at the tertiary structure level [35, 36].

One of the main physical characteristics of tRNA molecules selecting specific cytoplasmic amino acids, transferring them into ribosomes, and ensuring their direct participation in peptide bond synthesis is their chiral L-selectivity as regards amino acids [37]. Each amino acid interacts with its own tRNA molecule. The system does anti-entropic work, i.e., a necessary L-amino acid is sampled from the cytoplasm, where its right-handed counterpart can be present, too. ATP molecules serve as energy sources for this process. In other words, right-handed tRNA molecules and left-handed amino acids interact in selection and transport processes.

In this way, a left-handed homochiral polypeptide chain (protein primary structure) selected in energy-consuming synthesis from a mixture of L/D amino acids and turned into a regular sequence by a ribosome obtains free energy. It eventually becomes an active one-dimensional medium with a distributed resource of free energy (three-dimensional in the case of globule formation) [38]. To recall, homochirality of protein polypeptide chains has never been regarded in the literature as a source of free energy for structural rearrangements. In the case of the biomimetic chiral structures discussed in Section 3, the free energy of entropic constituents comes in during artificial formation of the initial homochirality of the system's elements.

On the whole, it can be assumed that hierarchies of alternating-sign chiral structures contribute to the formation of the periodic molecular-biological system in a living cell. The central block of this system, consisting of four structural levels of proteins and DNA, forms a closed achiral invariant. Here, the realm of molecular biology ends and a window to the world of cytology opens up.

It is paramount from the biological standpoint that chiral concordances naturally develop in supramacromolecular structures integrating the activities of the entire cell. Therefore, Nature furnished the cytoskeleton with 'two hands' in the form of parallel thread-like structures with two chirality signs. On the cellular scale, they are proteins folded into right-handed helices of fibrillar actin and left-handed tubulin microtubes (Fig. 7) [36]. Even a superficial look demonstrates that a branched network of left-twisted microtubules interacts with a system of right-handed DNA double helices in chromosomes, while the right-handed helices of actin microfilaments interact with lipid systems having negative (L) chirality signs in bacterial and eukaryotic cells. According to Fisher's classification, both bacteria and eukaryotes use L-phospholipids. Cholesterol and its derivatives rank among L-enantiomers as well [39].

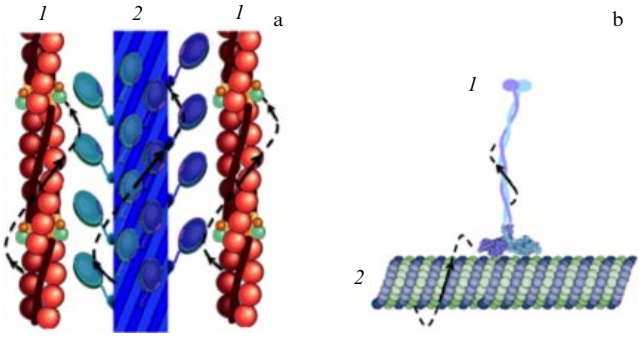

Figure 7. (Color online.) (a) Schematic of interaction between a right-handed actin fibril (1) and a right-handed myosin fibril (2); (b) schematic of interaction between a kinesin left-handed helix (1) and a tubulin left-handed microtube (2). Dashed arrows show twist direction.

Here too, at the level of cytoskeleton and membrane structures (including the plasmatic membrane), we find chiral sign alternation associated with the interaction of macromolecules of different classes. In this way, the cellular level of structural and functional chiral periodicity forms.

The chirality of cytoskeletal elements is directly related to structural anisotropy of plant and animal cells, which, in turn, predetermines the asymmetric/chiral character of embryogenesis and tissue morphogenesis [37, 40].

6. Chirality, L- and D-enantiomers, folding, and molecular machines

To describe structural hierarchies, we took advantage of the notations 'rightness' and 'leftness' traditionally adopted in chemical nomenclature and physics. The essence of the regular pattern in question is that chirality (helicity) sign alternation at the lowest level is responsible for the change of chirality signs in structures of increasingly higher levels. A change of symmetry type is possible here. In fact, the case is 'vertical' racemization during the formation of structural hierarchies of alternating symmetries, in contrast to 'horizontal' racemization within a single symmetry type. Unlike chiral structures with opposite signs every time become 'nested'. The phenomenon being described is beyond doubt, because no exceptions have been documented in the adequately explored simple systems, examples of which we first collected and systematized. This phenomenological regularity in biomolecules characteristic of chemical polymer systems has been conserved by natural selection as the hierarchical succession of symmetries.

The following remark is in order as regards thermodynamical determination of structural hierarchies in homochiral systems. Any macroscopic homochiral system is a thermodynamically nonequilibrium system; it is either a giant fluctuation of a nonequilibrium state or a result of specific selection from equilibrium racemate by extraneous forces in an energy-consuming process. Racemization of initially homochiral matter is a dissipative process with decreasing free energy. Usual racemization of chemical compounds is associated with spontaneous chirality sign reversal in individual molecules, whereas the symmetry type (rank) remains unaltered. Such a process is essentially a 'horizontal racemization' at a single level of structural organization. However, a different dissipation mode is conceivable in principle. Heterochirality develops during the formation of bigger molecular

structures with the chirality sign opposite to the sign of the starting elements that preserve it; they can be either intramolecular regular structures or supramolecular ones. The latter case, encountered in nonliving systems and in molecular biology, actually relates to 'vertical racemization'.

In our opinion, this class of events must include the folding of primary macromolecular chains of proteins and nucleic acids into a spatially unique, functionally specific, and active configuration [41]. In 1968, C Levinthal formulated the paradox that finding the native folded state of a protein takes many orders of magnitude shorter time than it would take by a random search among all possible configurations [42]. This assertion is equally true for the folding of nucleic acids. The paradox is traditionally resolved by presuming the existence of a funnel that guides the process of polypeptide folding into the nascent conformation in complex configuration space on the potential energy surface [41]. This funnel, with a minimum free energy, is supposed to determine the folding pathway in the configuration space of a macromolecule passing through a series of local energy minima [35, 43, 44]. We believe that the above-mentioned formation of alternating-sign hierarchies of intramolecular structures constitutes a 'road map' of the folding or 'Ariadne's thread' determining the trajectory of conformational transitions into a potential well, as hypothesized by Levinthal and his successors.

From the physical point of view, the fundamental difference between living and nonliving lies in the fact that living systems make use of molecular and macroscopic machines as active elements that transform energy, matter, and information [38]. Inanimate nature lacks such machines and uses instead converters not designed to perform effective work. A machine exemplifies a device (construction) intended for energy transformation in a cyclic manner, while doing some useful work due to the presence of distinguished mechanical degrees of freedom, including quantum-mechanical (translational, rotational) ones that kinetically separate work and dissipation. Certainly, all molecular machines are isothermal due to their small size. Although ongoing discussions of the problem of interest are focused on its nanotechnological aspects, it is worth noting that the universal representation of proteins and nucleic acids as molecular machines dates back to the 1960s, when they attracted the attention of compatriot researchers, such as Yu I Khurgin, D S Chernavskii, S E Shnol', and L A Blyumenfeld, followed by V A Avetisov and A N Tikhonov [45–48]. Structural elements of molecular machines include rigid frames and lever and pivots systems. Each of these linear elements is either capable or incapable of providing force (energy) transfer inside the construction using selected degrees of freedom. A helix is a unique and the simplest nonlinear structural element of molecular machines. Stiff helices take advantage of translational and rotational degrees of freedom. However, a compressed helical structure is sensitive to the direction of translational and rotational movements. Force transfer, as well as back and forth or sideways (right and left) movements, are accompanied by symmetry breaking, by analogy with that associated with the use of a discrete one-way control device, e.g., a ratchet-and-pawl mechanism or a valve unit that, in contrast to a helix, cannot be implemented in living systems.

To realize cyclicity as a principal temporal characteristic of a machine, it must have an asymmetrical construction, while the movement along the loop requires an asymmetrical element with the property of a gate or a latch.

7. Conclusions

The tendency toward alternation of the chirality sign of intramolecular structural levels, D–L–D–L for DNA and L–D–L–D for proteins, has been traced, starting from the asymmetric carbon level in deoxyribose and amino acids. Taken together, these four-level hierarchies of alternating-sign chiral structures showing a half-period shift make up the central block of molecular-biological structures in the cell that can be regarded as a chiral invariant. Regularity in the alternation of chiral structures is inherent in inanimate nature, whence this creative feature of chiral systems appears to have come.

In furtherance of the discussion within the framework of this review, it should be emphasized that neither the initial stages nor the physical basis of deracemization of the geosphere at the time of its prebiological evolution has been conclusively established [1, 49, 50]. However, there is an opportunity to discuss the mechanisms of 'vertical' self-organization in homochiral systems accompanied by the induction of new alternating-sign symmetries and structures on an enlarged scale. Importantly, such a prebiological relay race could start from 'below', i.e., from physical and physicochemical levels of the condense state of matter, and continue up to biospheric scales.

The essence of the regularity under consideration is as follows. If enantiomers with identical signs at a lower level of the chiral system are replaced by enantiomers of the opposite sign, the chirality sign at the next structural level also changes. Starting from the second level, the tetrahedral symmetry of the substitutes of asymmetric carbon gives way to chiral helical symmetry, and the helicity sign changes, too. At the level of tertiary, quaternary, and probably higher structures, helical and superhelical chiral structures explicitly prevail, and sign alternation occurs. A discrete stratified vertical of structures builds up, showing properties of a construction with distinguished degrees of freedom capable of functioning as a molecular machine.

It should be noted in connection with the use of L and D notations for different compounds and molecular structures that the Cahn–Ingold–Prelog rules are the same for all chemical substances (including amino acids and carbohydrates); therefore, the stereoisomer configuration is referred to as absolute. As mentioned above, notations L and D are used merely for convenience and have no meaningful sense whatsoever.

At the same time, a specific relationship between chiral characteristics of structures of different levels and symmetries is noticeable, analogous to the relationship between the molecular chirality of monomers at the lowest hierarchical level and helical organization of polymers at the next level. Let us consider circularly polarized light as a physical example. Its counterparts are right- or left-polarized photons (chiral entities in any reference frame) in quantum mechanics and dextrorotatory or levorotatory optical vectors in classical optics. Ascribing arbitrary chirality to a photon determines the chirality of the helix described by the end of the optical vector in space. The choice of chirality sign is optional but must ensure a convenient description of the system. The conditional association between chirality and its notation resembles that between a vector and its coordinate, the former being a geometric object, and the latter a tool for its description. It was reported in Ref. [51] that HIV-1 protease artificially synthesized from D-amino acids contains only left-

handed α-helices (in natural proteins, left-handed amino acids form right-handed α-helices). It was shown by numerical methods that the total energy of the left-handed α-helix obtained from D-amino acids is slightly (within the limits of error) higher than that of the right-handed α-helix from L-amino acids and roughly 10 kcal mol^{-1} lower than the total energy of the left-handed α-helix prepared from L-amino acids [52]. This means that the nomenclature used in biochemistry and molecular biology is rational from the physical standpoint.

We considered, in addition, the thermodynamic aspect of the phenomenon of interest and demonstrated, apparently for the first time, that the selection of enantiomers with identical signs of chirality from a mixture of enantiomers is due to antientropic work, which accounts for the free energy store in the resultant homochiral system. Its dissipation appears to occur in the normal fashion through racemization, i.e., the formation of mirror enantiomers. We advanced an original view that such 'horizontal' racemization may occur in parallel with 'vertical' racemization, accompanied by the formation of new structural levels with a different type of symmetry and alternating chirality sign as directly exemplified by the alternation of helical symmetry types at the level of secondary, tertiary, and quaternary structures of proteins (see Section 4 and Fig. 5). We believe that this dissipation mode can determine the trajectories of protein and amino acid molecule folding.

Authors who deal with the folding properties of macromolecules maintain that the formation of α-helices, a secondary protein structure, is due to the steric complementarity of amino acid radicals in the primary structure of polypeptide chains and to hydrogen bonding between them. Thermodynamically, it is a reduction of the enthalpic component of the free energy. These structures are, as a rule, intrinsically unstable outside a protein globule. We hypothesized that the left-handed primary structure as a whole tends to form right-handed helices and reduce the enthalpic component of the free energy store of the entire globule, with the helices being fixed in the loci where the proper neighborhood is genetically determined. Such can be the mechanism underlying the movement of the developing globule along the optimal trajectory ('Ariadne's thread') to the energy minimum. Folding behaves in a similar way in the formation of left-handed superhelices and further on.

By analogy, entropy plays an important role in intermolecular interactions by differentiating between interactions of molecules with identical or different symmetry signs (within a single molecular class or between different classes).

The regular pattern thus revealed is of an empirical character. A quantitative description of the hierarchies of chiral molecular systems with in-built chiral structures requires a special approach to the metrological characteristic of the degree of chirality as a physical quantity and the introduction of a universal chirality unit (system of units?). It would make possible the quantitative analysis of conjugate transformations of chirality in symmetry-alternating molecular systems and their thermodynamic and topological description. Attempts to provide a mathematical description of different types of chiral structures have been reported, but they do not match our systems [53]. The biological aspect of the above-described manifestations of chirality appears logical, but physical mechanisms of chiral structure stratification remain to be elucidated.

A recent paper of S K Nechaev and K Polovnikov [54] discusses theoretical approaches used to address the concept of hierarchical organization of energy landscapes with a large number of metastable states corresponding to local minima of the potential energy. The authors write: "In accordance with transition probabilities, the minima are thought to be grouped into hierarchically nested basins... ." We are of the opinion that the approach developed in Ref. [54] may have a direct bearing on the concept of chiral sign-alternating organization of hierarchical structures in biological macromolecules.

To conclude, the physical aspects of the problem of the origin and biological significance of molecular chirality have not been given proper attention in the domestic and foreign literature in recent years. Present-day concepts were considered in a recent article by G R Ivanitskii [55] and a report by A A Andronov [56] dedicated to the 90th birthday anniversary of V L Ginzburg. These studies were published in *Physics–Uspekhi* journal together with fundamental research papers by V I Gol'danskii and co-workers [1, 49]. The important ideas of these authors that chiral dissymmetry in DNA and proteins is a kinetically retarded state related to deviation from thermodynamic equilibrium found direct physical interpretation in our model. This 'linkage' accounts for the kinetic hindrance of dissipation caused by stratified structural levels of macromolecules interconnected through the variable sequence of chirality signs. Chiral discreteness of structural levels in macromolecules ensures the stability of their 3D construction. The general systematic principle that a nonequilibrium hierarchical system is more stable than its one-level 'isomer' appears to hold.

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