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Portable optical biosensors for the determination of biologically active and toxic compounds

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1. Introduction

This study was undertaken on the initiative of Yu M Evdokimov (Engelhardt Institute of Molecular Biology, EIMB RAS) and was performed jointly by the EIMB RAS, the Institute of Spectroscopy RAN (ISAN), and the Institute of Biochemistry, University of Munster, Germany. At its final stage, the study was supported by Bioanalytical Technologies Ltd. (BAT).

In 2003, the world scientific community observed the 50th anniversary of a highly important event, the discovery of the molecular structure of DNA by J Watson and F Crick. The objective of the present report is to illustrate how results of basic research are transformed into new technologies and instruments.

The work addresses the needs of health care and has the purpose of developing methods for highly sensitive (at the genetically important level) determination of biologically active and toxic compounds (genotoxicants) in liquids. Biologically active and toxic compounds (BATC) comprise antibiotics, antitumor drugs and other pharmaceutical products, heavy metals, proteins, biologically active additives, dioxins, pesticides, poisonous substances, depressants, narcotics, etc. The fluids under study include blood, blood plasma, urine, water, various physiological and technological solutions, etc.

Rapid detection and quantitation of biologically active substances in the said fluids are necessary for establishing accurate medical diagnosis and/or managerial decision-making because they serve as markers characterizing a clinical condition or technological process, the quality of a raw material and finished articles, the quality of foodstuffs, environmental pollution with genotoxic agents, etc. Hence, the main areas of potential application of optical biosensors are clinical diagnostics, biochemical analysis, pharmacology, biotechnology, the food industry, ecomonitoring, scientific research, and education.

Generally speaking, practically all BATC can be determined by traditional methods, such as chemical and biochemical analysis, spectrometry, and chromatography. The problem is the detection and quantitative determination of many BATC markers are expensive (hundreds of dollars) and time-consuming (up to several days) procedures, the sensitivity and selectivity of which are often insufficient for the purpose. Hence, there is the necessity to use alternative approaches, e.g., biosensors.

2. What is a biosensor?

Any biosensor is composed of two basic components [1, 2]. One is a biosensitive element (biosensing unit) on which a specific 'recognition' reaction occurs, with only selected compounds in the mixture interacting with certain characteristic elements of the biologically specific surface of the biosensing unit (e.g., as a result of incorporation of the compounds into these elements). The other component transforms the signal generated in this reaction and is functionally related to the concentration of a given compound. In other words, a biosensor is a combined device containing two closely connected transducers, one biochemical, one physical.

The current literature describes a large number of devices in which enzymes, nucleic acids, cell receptors, antibodies, and even intact cells are combined with electrochemical, optical, piezoelectric, and thermometric transformers. The advantages of such biosensors are high sensitivity and specificity of analyses; no need for sample pretreatment, special working areas, and highly skilled personnel; and the possibility of rapid on-site measurements. A drawback is that the above biosensors are not universally applicable.

3. Liquid-crystalline DNA molecules as biosensing units

In an attempt to overcome this limitation, Yu M Evdokimov and his coworkers at EIMB RAS proposed constructing a biosensing unit that involves double-stranded nucleic acids [3, 4]. Why?

1. The double-stranded DNA (dsDNA) is unique in that its reaction to various groups of compounds is highly specific and has different outcomes. For example, some compounds become inserted (intercalated) between nitrogen bases or into the space between the strands while others split strands, cross-link them at different angles making bonds of varying rigidity, attach themselves to the DNA molecule by breaking off its nitrogen bases or otherwise, etc. Thus, the double-stranded DNA molecule has a well-developed and variably specific biosurface and is potentially one of the most universal biosensing units.

2. Basic studies performed at EIMB RAS on physicochemical and biological properties of a DNA state characteristic of the heads of certain virus particles, spermatozoa, and protozoan chromosomes have shown that this state of DNA molecules is a liquid crystal state, in which the mobility of molecules coexists with their spatial order. Both macroscopic liquid-crystalline phases of DNA and microscopic particles of these phases can exist.

The studies revealed different spatial forms of these phases obtained by the 'phase exclusion' method. They included cholesteric liquid crystals of DNA (lcDNA) formed upon the addition of polyetheleneglycol to an aqueous-salted solution of DNA, in which case it was energetically more favorable for free dsDNA molecules to form anisotropic phases rather than to remain isolated in the solution (Fig. 1).

Each layer of the liquid-crystalline DNA phase thus formed is composed of identically oriented molecules spaced at fixed distances, with the direction of orientation changing along the length of the helix. Packaging density in a 0.5 μm liquid crystal drop is rather high (over 10^4 DNA molecules). Due to these properties of the cholesteric liquid-crystalline phase of DNA, its optical activity is unusually high (dozens and hundreds of times that of isolated DNA molecules). It is manifest as an abnormal optical signal of circular dichroism (CD) detected as the difference of absorption of circularly polarized light with opposite directions of rotation of the polarization vector recorded within the UV absorption band of DNA constituent elements, i.e., nitrogen bases (chromophores), at the 270 nm wavelength.

3. It has been shown that transition to a liquid crystal state does not destroy the ability of the main structural components of DNA to 'recognize' chemically or biologically active compounds, that is, to form DNA/BATC complexes with

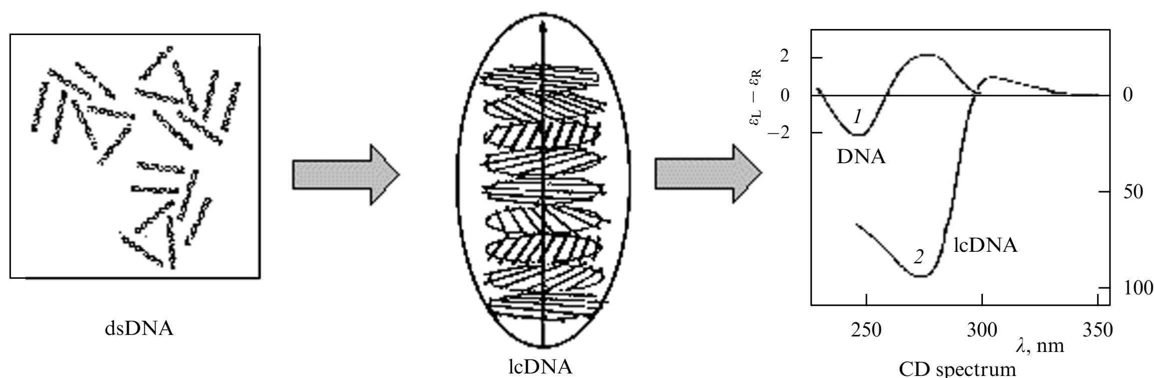

Figure 1. Schematic representation of the formation of the cholesteric liquid-crystalline phase of DNA and the appearance of an abnormal CD signal.

specific properties. For example, interactions of colored antibiotics or antitumor compounds with nitrogen bases result in the appearance of an additional anomalous circular dichroism in the absorption band of these products. This confirms that nitrogen bases of DNA may serve as sensitive elements, i.e., biosensors.

4. It was also shown that DNA molecules 'squeezed' into the spatial structure of particles in the liquid-crystalline phase are actually building blocks between which additional sensitive elements can be inserted, with their properties changing in response to the presence of various compounds in the medium ('molecular design' technology).

5. CD spectral characteristics provide a practically important analytical criterion: a) if the DNA molecules are native and uncontaminated, then liquid crystals of such molecules are characterized by the spectrum shown in Fig. 1; b) the CD signal of a DNA/BATC complex is proportional to the concentration of biologically active and toxic compounds interacting with liquid crystals of DNA.

Thus, it has been shown that particles of the cholesteric liquid-crystalline phase of DNA function as miniature (0.5 μm) optical biosensing units whose characteristics vary in response to the action of BATC, which is readily distinguishable using a fairly simple criterion based on the magnitude, sign, and spectral maximum of an abnormal circular dichroism generated during such interaction.

4. Composition and principle of action of optical biosensors

Biosensor [5] comprises lcDNA biosensing units, a portable dichrometer, and software-supported techniques for determination of various BATC.

The biosensing unit is a molecular construction based on liquid-crystalline dispersion of nucleic acids or their complexes. It can be either fluid or imbedded into the polymer matrix of a carrier in the form of a gel or film [6]. Each type of such a molecular construction is intended for the determination of a certain class of BATC. Within one class, biosensing units of a given type are used in combination with a battery of techniques with which individual BATC are specifically assayed. By now, biosensing units for the determination of more than 15 classes of compounds (over 50 BATC) have been constructed by molecular engineering.

Sample preparation. A BATC-containing liquid to be analyzed is mixed with a polymer that is neutral in relation to DNA. Simultaneously, liquid crystals of DNA are formed of linear double-stranded fragments of low molecular mass in an aqueous-salted solution of the same polymer. The analyzed liquid solution in the polymer is mixed with the DNA solution in the polymer (i.e., liquid-crystalline DNA dispersion) to obtain the desired sample.

Versions of the biosensing unit have been worked out in which lcDNA are immobilized in special hydrogels or films with nanopores that are optically neutral and maintain a long service life of the DNA liquid crystals.

Determination of BATC. In order to obtain a CD signal, a beam of circularly polarized light with oppositely directed polarization vectors is passed through the absorption cell, hydrogel, or film containing the analyzed sample and placed in the dichrometer. The optical absorption signal A is recorded at *two* wavelengths, one in the clDNA absorption region at 270 nm (reference signal) and the other in the BATC absorption region.

The BATC concentration is then determined from the ratio of these two signals using the previously calibrated dichrometer and an analytic curve describing the dependence of the CD signal on the BATC concentration.

5. Composition and construction of a polyfunctional dichrometer

The dichrometer [5, 7] comprises a source of broad-band light radiation (xenon lamp), a monochromator cutting out the narrow-band light flow of a given wavelength, a polarizer (Glahn prism), a modulator transforming the linearly polarized light flow into a circularly polarized one with a periodically changing direction of the polarization vector rotation, a cell for the sample to be analyzed, a photomultiplier, and a personal computer (Fig. 2).

The light flow passing through a test sample exhibiting abnormal circular dichroism undergoes modulation of intensity. This results in the appearance of an electrical signal at the output of the photomultiplier that has a variable constituent ΔA at the polarization modulation frequency proportional to the CD signal value. From the photomultiplier, the signal then enters the digital tracking system and is fed to the computer following amplification, filtration, and conversion to a digital code. The microcontrloller-based interface card serves to ensure the necessary interaction of all units of the device, recording and primary processing of the CD signal, data transfer to the computer, and testing the parameters of all systems of the dichrometer.

The device is operated by a software package that controls different working regimes of the biosensor and supports a library of techniques for the determination of an individual

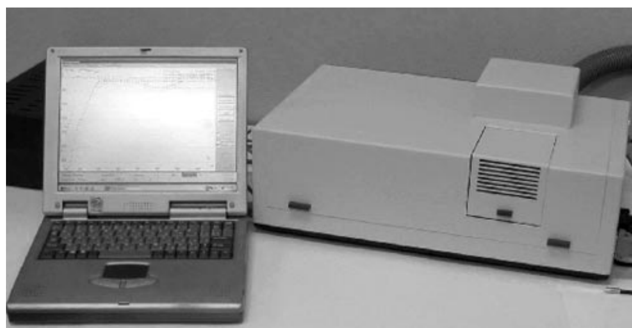

Figure 2. Portable CDS-2 dichrometer for high-sensitivity measurements within an extended spectral range (200 – 750 nm).

BATC. This enables the user to choose the desired substance from the menu, perform a sequence of actions prescribed by the technique, and obtain the result in the form of the concentration of the relevant BATC in the sample.

The use of the two-wavelength technique implies the possibility of rapid switching. For this, the monochromator contains an original galvanometric driver of a positional type for turning and setting the diffraction grating in a broad spectral range with a high accuracy ($\sim 10^{-4}$) and operating speed (0.2 s).

The development of the portable apparatus included optimization of the spectral resolving power of the monochromator and reduction to a minimum of the number of its optical elements for improved light transmittance. This allowed us to simplify the design of the dichrometer as a whole, reduce its dimensions, ensure mobility, and make it easy to maintain and operate. In particular, a maximum luminous flux was obtained using a light source of relatively low power which obviated the need of cooling the device with water or gaseous nitrogen. The detecting capacity of the new dichrometer $\Delta A/A$ is 2 – 3 times higher than that of commercial devices, while its dimensions and weight are 3 and 5 – 7 times smaller, respectively.

6. Characteristics of the dichrometer and optical biosensor

In what follows, characteristics of the proposed device are outlined. In Tables 1 and 2, figures in parentheses (right column) are factors indicating the degree of improvement of a given parameter over the respective one of the prototype apparatus (J-810 Spectropolarimeter, Jasco, Japan).

Checking the analytical possibilities of the dichrometer in EIMB RAS by determining various compounds in blood plasma (from heavy metal salts to antibiotics and proteins of an antitumour drug) has demonstrated the high efficiency of the optical biosensor comprising a liquid-crystalline biosensing unit and a portable dichrometer.

Table 1. Characteristics of the dichrometer.

Spectral range	250 (200) – 750 nm	
Spectral resolution	3 nm	
Time of wavelength tuning between the extreme points of the spectral range	0.5 s	(6)
Sample temperature control range	12 – 80 °C	(+)
Dimensions of the spectral block	500 × 330 × 170 mm	(2)
Weight of the spectral block	14 kg	(6)
Minimal detectable CD signal ($\Delta A/A$)	3×10^{-7}	(3)

Table 2. Characteristics of the optical biosensor.

BATC detection limits	$10^{-7} – 10^{-14}$ mol l^{-1}	(> 100)
Detection time	10 – 20 min/sample	(> 100)
Exploitation expenditures	1.5 USD/h	(~ 5)
Cost of determination	0.4 USD/sample	(> 10)

Advantages of the proposed optical biosensor include direct detection and quantitation of practically any BATC, determination of a large number of different substances with a single type of biosensing units, high sensitivity and selectivity, quick and low-cost analyses, compactness and portability, no need for cooling water or gaseous nitrogen, and no special training of operating personnel.

The created biosensor *has no analogs* in terms of the principle of action. The novelty of the joint development of two RAS institutions (EIMB and ISAN) has been confirmed by Russian, US, and German patents. The device was awarded gold medals at the International Innovation Exhibition 'Eureka-Brussels-2001' and by the Presidium of the Russian Academy of Sciences (2002), Grand-Prix of the Competition of Russian innovative projects (2003).

7. Ongoing research and development

Biosensing units of different types (to detect a specified class of BATC) and the portable dichrometer are used in combination with special techniques that imply a sequence of actions guaranteeing high-quality results of measurements. Presently, EIMB RAS has developed or continues developing techniques for the determination of the following BATC: colored antitumor drugs of the anthracycline and anthraquinone groups, blood heparin, total proteins, amino acids and metabolites of physiologically important amino acids, ascorbic acid, and chitosan cleavage compounds. In addition, a method is being developed to identify water unsuitable for drinking.

The proposed techniques serve to form a library of detectable compounds and the corresponding biosensing units.

Four portable dichrometers manufactured at ISAN are presently exploited in laboratories of RAS and the Russian Academy of Medical Sciences for the development of new types of biosensing units and BATC assays with the objective of extending the range of application of the biosensor. The first pilot lot of 10 apparatuses for research purposes were manufactured at the Experimental Plant of Scientific Instrumentation, RAS (Chernogolovka, Moscow Region).

At present, EIMB RAS, ISAN, and BAT are jointly engaged in the following research and development projects aimed at constructing optical biosensors:

— development of new biosensing units with the use of liquid-crystalline DNA and new BATC assays, including those for the analysis of multicomponent substances;

— further improvement of the polyfunctional dichrometer, its automation and miniaturization using MEOM technologies;

— development of specialized compact versions of the dichrometer;

— development of new apparatuses combining a dichrometer and Fourier spectrometer, a dichrometer and fluorimeter, a chromatograph and dichrometer, etc.

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