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Registration of the protein with compact disk

Yuri Dmitrievich Ivanov [a], Tatyana Olegovna Pleshakova [a], Nikolay Valentinovich Krohin [a], Anna Leonidovna Kaysheva [a,*], Sergey Alexandrovich Usanov [b], Alexander Ivanovich Archakov [a]

[a] Institute of Biomedical Chemistry, Russian Academy of Medical Science, Pogodinckaya 10, Moscow 119121, Russia
[b] Institute of Bioorganic Chemistry, National Academy of Science of Belarus, Kuprevich 5/2, Minsk 220141, Belarus

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ABSTRACT

CD-based optico-acoustical biosensor (OAB) was used for detection of various types of proteins represented by bovine serum albumin (BSA), heme-containing myoglobin (Mb), monoclonal antibody against viral protein marker of hepatitis B (anti-HBsAg) and membrane-bound cytochrome P450scc (P450scc). We applied standard compact disc reader (CD-ROM) as an optical analyzer and a standard compact disc (CD) as a biochip containing immobilized protein molecules. This biosensor can translate into a digital code the changes of optical signal from the proteins and their complexes immobilized on the CD surface.

Then, the digital code is translated into an acoustic series or, in other words, into a "music of proteins". We demonstrate the use of the OAB for direct detection of proteins with different molecular weights, such as BSA, Mb, P450scc, anti-HBsAg with the concentration detection limit (DL) about 10^{-7} M. By signal amplification achieved with autometallography, a higher sensitivity level (DL$\sim 10^{-9}$ M) for the detection of myoglobin was obtained. The method of OAB-detection of proteins is cheap: it requires no special equipment like spectrometers, refractometers and other devices. Due to the fact that acoustic series of the protein complexes antigen/antibody differs from that of single proteins, the OAB-detection is of particular interest for rapid assay in yes/no data type and for home diagnostics. Combination of the OAB with a mass spectrometer allowed the detection and identification of the target proteins fished out directly onto a standard CD surface.

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1. Introduction

The development of high-performance and low-cost methods for detecting proteins and protein complexes is of primary importance to proteomics and personalized medicine.

The present micro- and nano-technological analytical approaches for detecting proteins are based on the method of biospecific fishing. It provides for the binding and concentration of protein partners on the biosensor chip surface with immobilized ligand molecules. Various technologies, such as ELISA, SPR-array, AFM-fishing, nanowire array, etc., are based on this general principle (Zhang et al., 2009; Gowan et al., 2007, Yu et al., 2006; Ivanov et al., 2006; Archakov et al., 2009; Zhen et al., 2005). The use of modern spectrometers, refractometers, atomic-force microscopes, precise pico-femtovoltmeters and some other devices as detectors is rather expensive. So, to reduce the cost of the analyzer it is necessary to adapt cheap CD-ROM or DVD-ROM readers for the detection of proteins (Pallapa et al., 2010, Potyrailo et al., 2006, Erickson and Ligler, 2008). Standard compact discs with molecular ligand probes immobilized on their surfaces may be used as chips.

At present, two major approaches are being developed for this field of science. The first approach is to modify CD-ROM or DVD-ROM readers for the development of special compact disks and registration of the protein–protein interactions. For this purpose, the CD-ROM readers were modified to the laser-based detectors that register proteins on the CD surface (Alexandre et al., 2002). Specially designed biochips — bioCDs — were produced on the silicon wafers made of dielectrics with gold ridges deposited on the compact disc surface (Varma et al., 2004). The data were analyzed with commercially available analytical readers like abioscope, designed for the detection of allergens. These easily produced commercial devices provide for friendly reliable low-cost allergy diagnosis promoting personalized medication (Erickson and Ligler, 2008).

We used the second approach that is based on the usage of a standard CD-ROM and standard compact discs with immobilized molecular probes for detecting proteins on the CD surface (La Clair and Burkart, 2003, 2006; Yu et al., 2008).

* Corresponding author. Tel.: +79 1583 8091.
E-mail address: kaysheva@gmail.com (A.L. Kaysheva).

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The second approach ensures the detection of proteins with DL$\sim 10^{-12}$ M (La Clairn and Burkart, 2003) as was demonstrated for the biotin/streptavidin pair (Kd$=10^{-14}$ M) (Sano and Cantor, 1990), with biotin immobilized on the CD surface. The DL$\sim 10^{-6}$ was reported for the mannoside/concanavalin pair (Kd$=10^{-4}$ M) (La Clair and Burkart, 2003). In our studies, the CD-based approach was used for the analysis of the laser-light scattering data obtained with the protein complexes on the CD surface; the result was given in digital form. At the same time, a number of subsequent studies showed that the detection limit achieved with standard CD-player (laser beam $\lambda=780$ nm) is insufficient for the registration of unlabeled biomolecules (with a size of less than 10 nm) using standard software (Li Yu. et al., 2008). Thus, the detection of proteins with the CD-ROM biosensor was carried out with autometallography for the signal amplification of the labeled proteins captured with gold nanoparticles. After that, a reductive precipitation of silver on the gold particles with the increase of their size up to 200 nm was carried out. Authors have obtained the DL$\sim 10^{-8}$ M for the biotin/streptavidin pair and the DL$\sim 10^{-9}$ M for Ig/anti-Ig pair. The data were analyzed with standard software and the number of errors was presented in graphic form.

The OAB has been developed to further increase the concentration sensitivity and facilitate the data analysis. For processing and presentation of data, not only in graphic, but also in audio-format, we developed special software (http://195.178.207.170/ibmcsoft/). Audio data presentation allows the wide user community and physicians to analyze the data.

It was demonstrated that the OAB is capable of detecting proteins with different molecular weights: the proteins fished out from the solution were registered and covalently bonded on the CD surface. The proteins were detected with the use of labels and without them. In the last case, the signal has been amplified. The identification of proteins on the compact disc surface was carried out by mass-spectrometric analysis. For this purpose, a method of combining the CD-ROM biosensor and mass-spectrometer has been developed.

The OAB was used for biospecific protein detection. Upon that, between the protein ligands immobilized on the CD surface and their partners in a sample solution the protein complexes were formed. The redox partner complexes formed between adrenodoxin (Ad) and P450scc (i) within the cytochrome P450scc-containing monooxygenase system and anti-HMb/Mb complexes (ii) were registered. The concentration detection limit for the label-free proteins was about 10^{-6} M. Due to the signal amplification by autometallography, the detection sensitivity increased up to 10^{-8} M for myoglobin, for example.

2. Materials and methods

2.1. Reagents

The following proteins were purchased from USBiological: cardiac human myoglobin (Mb, 8.1 mg/ml, catalog no. M9800-18); mouse first type anti-human myoglobin; cardiac myoglobin (anti-HMb1, 1.05 mg/ml, M9800-16 A), mouse second type anti-human myoglobin, and cardiac (anti-HMb2, 1.1 mg/ml, M9800-16). Bovine serum albumin (BSA, 1 mg/ml, 82516) was provided by Fluka, mouse anti-HBsAg (3.6 mg/ml, 5016-500) was obtained from Aldevron and trypsin (100 μg, V5280) from Promega.

The proteins of bovine P450scc and Ad have been expressed in *E.coli* by Prof. S.A. Usanov. Purification of recombinant proteins was done as described earlier (Chu and Kimura, 1973; Tarr et al., 1983). The concentrations of cytochrome P450scc and Ad were determined using the molar extinction coefficients 91 $mM^{-1}\,cm^{-1}$ at 450 nm, 11 $mM^{-1}\,cm^{-1}$ at 450 nm and 10 $mM^{-1}\,cm^{-1}$ at 414 nm (Calabro et al., 1976; Hirayama et al., 1990).

All chemicals were of analytical grade. The buffer solutions were prepared using the deionized water on Milli-Q system (Millipore, MA, USA). The following reagents were obtained from Sigma: 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), Tris–HCl, ammonium bicarbonate (NH_4HCO_3), formic acid, and trifluoroacetic acid (TFA). Photoactivated coupling was carried out using bis-[-(4-azidosalicylamido)ethyl]disulfide (BASED) from Pierce. All the other chemicals were obtained from Reakhim (Moscow, Russia).

2.2. Device for the CD-ROM registration

The CD-ROM measurements were carried out on the PLEXTOR CD/DVD devices. The CD is made of 1.2 mm thick, almost-pure polycarbonate plastic. To make it reflective, a thin layer of aluminum, and more rarely, gold, is applied. The CD data are stored as a series of tiny indentations known as pits, encoded in a spiral track molded into the top of the polycarbonate layer. The areas between pits are called the lands. Each pit is approximately 100 nm in depth and 500 nm in width and its length varies from 850 nm to 3.5 μm. The distance between tracks (the pitch) is 1.6 μm. The CD is read by focusing a 780 nm wavelength (near-infrared) semiconductor laser through the low polycarbonate layer. The change in height between the pits and lands leads to differences in the intensity of the reflected light. Measuring the change in intensity with a photodiode, it is possible to read data from the disc.

2.3. Translation of the digital data of protein analysis into an acoustic form

The CD-ROM detects and corrects errors occurring in the process of reading. The detection and correction of data reading errors was made with ReedSolomon (CIRC). The CD-frames analysis was carried out using CDIO program (v.1.00) with the three types of kodaks CIRC (C1, C2, CU). Kodak C1 registers and corrects one or two bit-sized errors. Kodak C2 corrects 4 error bits per frame; kodak CU corrects even more errors per frame. The errors of C2 occur in defects with sizes of more than 0.3–0.5 mm, and the errors of CU are caused by defects with a size of 0.5–0.9 mm. The error data are used to identify the sample.

The error data were obtained with standard "SFF Committee Information Specification for ATA Packet Interface for CD-ROMs Rev 2.6 January 22, 1996". The developed C language software in ambient ".Net OC MS Windows" makes it possible to measure the noise level and to construct the error distribution diagram which allows one to conclude about the composition of the sample.

Visualization of the CD-surface defects was carried out in graphic form (a function of an absolute error value on the sector) and in acoustic form (audio). The number of errors in sector E(n) is defined as

$$E(n) = C_1 + C_2 \times 10 + CU \times 100$$

$$F(n) = A_1 \times (127 \times E(n)/E_{max})(1 + (A_1 \times (127 \times (E(n)/E_{max})) + A_0 \quad (1)$$

where E_{max} is the maximum number of errors in all sectors of the reading zone; and A_0, A_1 and A_2 are the regression coefficients.

For acoustic reproduction and melody storage, we used a MIDI-interface software, whereby each acoustic signal with particular frequency was assigned a value from 0 to 127 (the higher the value, the higher the frequency of sound—F(n)). With the registration of large quantity of errors (E) occurring in the screening of the CD surface with immobilized protein complexes,

the sound series in high-frequency area is generated, while with the registration of small quantity of errors on the CD surface with immobilized protein probes but without protein complexes, the sound series in the low-frequency region is commonly detected.

2.4. Modification of the CD-surface

For the CD-surface modification we used protocol similar to Aslan et al. (2005). The polycarbonate surface of working sectors of the CD was hydrolyzed in 2 M aqueous NaOH solution for 30 min and rinsed with deionized water. Then, the surface of working sectors of the CD-chip was modified by a spacer with NH_2-terminal groups, according to our patent (Archakov et al., 2008). For this purpose, the hydrolyzed CD-surface was sylanized with 10% v/v solution of APTES in the mixture of ethanol:water (1:4) for 30 min at 37 °C and 80% humidity. The solution of hydrolysis was applied into working zones of the CD in the form of track of 14 drops in 1 μl placed on the same radius. Sylanized CD-surface was rinsed several times with ethanol and water to remove the unbound APTES. Then, the prepared CDs were used for covalent biospecific fishing out of proteins.

2.5. The covalent capture of protein onto the CD-surface

The covalent capture of proteins was performed as follows: a protein was fished out from solution onto the CD surface and captured there using covalent photo-cross-linking. The proteins were covalently captured in three working zones (2 mm × 14 mm) on the CD-chip surface. Photo-cross-linking protocol for protein covalent capturing was adopted from Pierce instructions (Hermanson, 2008). For this purpose, the samples were prepared by diluting the stock solution in 50 mM PBS buffer (pH 7.4) to the final protein concentrations ranging from 10^{-4} to 10^{-9} M. 40 μl of analyte solution and 5 μl of DTBPA (0.1 mM stock-solution in DMSO) were added to the working zones of modified CD. The solution of analyte was applied into working zones of the CD in the form of track of 14 drops in 3 μl placed on the same radius. The CD surface was treated with UV radiation (1.5 mW UV-lamp, 10 cm, $\lambda \sim 300$ nm) for 20 min. After the incubation, the CD was washed in water for 90 min, dried and scanned by CD-ROM. The dependence of the error level signal on the sector was registered.

2.6. The label-free biospecific fishing out of proteins Ad/P450scc and anti-HMb/Mb complexes

In these experiments, the solution of analyte (solution of P450scc or Mb) was added to the working zones of the CD surface with immobilized probe proteins (Ad and anti-HMb1, respectively) according to the procedure described in Section 2.4. The samples of analyte were prepared by diluting stock solution in 50 mM PBS buffer (pH 7.4) to the final protein concentrations ranging from 10^{-4} to 10^{-9} M. The solution of analyte was applied into the working zones of the CD in the form of track of 14 drops in 3 μl, placed on the same radius. The sample was incubated for 90 min at RT and 80% humidity. During incubation, the complexes have been formed on the CD-surface between the immobilized probes and proteins from analyte solution. After incubation, the CDs were washed in water for 90 min to remove the unbound protein, dried and scanned by CD-ROM. The dependence of the error signal level on the sector was registered.

2.7. Biospecific fishing for myoglobin revelation using gold-based autometallography

In this series of experiments we used the CDs with anti-HMb1 immobilized in the working zones and prepared according to Section 2.5. After the biospecific capture of Mb out of 2×10^{-8} M solution (Section 2.6.), the solution of anti-HMb2 conjugated with Au was applied into the control and working zones of the CD-disk. The anti-HMb2 and anti-HMb1 formed complexes with different Mb epitopes. The concentration of solution of anti-HMb2 conjugated with Au label (Oliver, 1994) in PBS buffer, pH 7.4, was 1 μM. The solution of anti-HMb2–Au was applied into the working and control zones of the CD in the form of track of 14 drops in 3 μl, placed on the same radius. The solution of anti-HMb2–Au was incubated on the CD surface for 1 h at RT and 80% humidity. For the silver enhancement reaction we used a reagent kit consisting of the silver salt (silver acetate) and reducing agent (hydroquinone), according to Yeh et al. (2009). Just prepared silver solution, containing 0.05 M of silver acetate and 0.5 M of hydroquinone, was applied into the working and control zones of the CD (14 drops in 3 μl) and kept for 10 min in darkness. Then the surface was thoroughly washed with water.

3. Results and discussion

3.1. The CD-ROM registration of covalently-bound proteins and their MS identification

3.1.1. The CD-ROM registration of covalently-bound proteins

In this study, the CD was used as a chip with preassigned geometry zones for the covalent binding of proteins and the CD-ROM was used as an optical reader.

As described above, the covalent protein fishing is the process of fishing out of the protein molecules from the analyte solution onto a small chip surface for their concentration and subsequent covalent immobilization in a preassigned surface zone (Archakov et al., 2007, 2009). Here, we have effectuated the covalent capture of proteins onto the chip surface into the zones (2 mm × 14 mm) for the protein detection using CD-ROM. Thus, the efficiency of covalent immobilization of the protein molecules (Section 2.4) was assessed by recording the number of errors occurring upon the immobilization of these proteins.

To determine the location of the working sectors on a disc within the read-in program, we have first identified the beginnings (start) and the ends (finish) of the working and control zones. For this purpose, the protein with Cy5 dye was applied onto a disc in the targeted zones. This allowed us to visually control the location of these proteins. The errors in these sectors were registered using the read-in program elaborated in the course of this work.

After that, in the same working sectors of four different CDs, the four types of proteins with different physical and chemical properties and weight—Mb (Mw 18 kDa), BSA (Mw 50 kDa), P450scc (Mw 58 kDa), and anti-HBsAg (Mw 150 kDa) were fished out from the solutions of different concentrations.

The obtained dependencies of the total number of errors are as follows: for Mb—$E(\text{errors}) = 6 \times 10^3 \times \text{LnC} + 8 \times 10^4$, for BSA—$E(\text{errors}) = 2 \times 10^4 \times \text{LnC} + 2 \times 10^5$, for P450scc—$E(\text{errors}) = 10^6 \times \text{LnC} + 2 \times 10^7$, for anti-HBsAg—$E(\text{errors}) = 3 \times 10^3 \times \text{LnC} + 5 \times 10^4$. These dependencies are shown in Fig. 1.

As seen from Fig. 1, the increase in the number of errors depending on concentration is observed for all types of proteins. However, the nature of this relationship is different. Fig. 2 shows the dependence of concentration limit of protein detection using CD-based biosensor.

As seen from Fig. 2, the detection limit (DL) for BSA (10^{-5} M) is higher than that for the other proteins: Mb (10^{-7} M), P450scc (10^{-7} M). The analysis of proteins' pI shows that for BSA the value of $\text{pI} \leq 5.0$ ($\text{pI} = 4.8$) while for the other proteins $\text{pI} \geq 5.5$: Mb ($\text{pI} = 7.4$), P450scc ($\text{pI} = 5.5$). That is, the efficiency of covalent

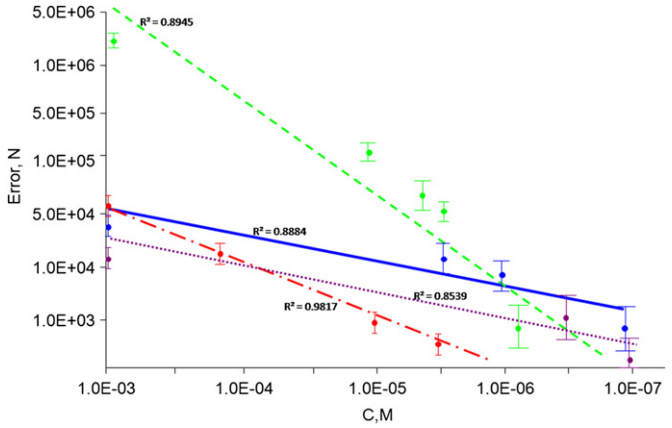

Fig. 1. The dependencies of the total number of errors on the concentrations of proteins with different weights (Mb 18 kDa, BSA 50 kDa, P450scc 58 kDa, anti-HBsAg 150 kDa). The experimental conditions were as follows: stock solution of protein (10^{-3}–10^{-7} M) in 50 mM PBS, pH 7.4, containing 0.04 M NHS and 0,04 M EDC with the volume of 28 ml was applied on the CD surface. After the incubation of the CD-chip in this solution, the chip was washed with water for 90 min and dried, whereupon the CD-ROM analysis was performed.

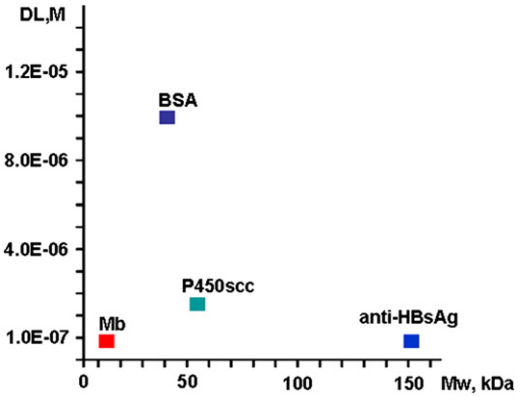

Fig. 2. The detection limit for different proteins obtained with the CD-ROM. The experimental conditions were the same as shown in Fig. 1.

capture depends on pI value of the protein. More effective immobilization on the CD surface is observed for positively charged proteins.

The representation and coding of protein properties may be different: they may be presented as figures, or color gamut, or as acoustic signal (as shown on the slide). In our study, the registered dependence of the increased level of error signal on the CD sector was represented as the dependence of the acoustic signal frequency on the sector. For this purpose we represented the acoustic signal frequency as a function of the number of errors.

Using the translation of the number of the errors E(n) detected by CD-ROM in n sector, into a frequency of acoustic signal F(n), one can obtain the dependence of the acoustic signal frequency on the sector. In personal computer, this frequency is reproduced in the form of melody, i. e. optical–acoustic signal.

As an example, Fig. 3 represents two dependencies of the error levels E(n) obtained upon covalent capture of Ad and P450scc at the same concentration 20 mM in 50 mM KP buffer, pH 7.4.

The experimental conditions were as follows: the incubation solution containing 50 mM KP buffer, pH 7.4, 0.04 M NHS and 0.04 M EDC was applied onto the control and working zone of the CD surface. After the incubation, the CD-chip was washed with water for 90 min, dried and the CD-ROM analysis was conducted.

The proper acoustic melodies are represented on the web site—http://195.178.207.170/ibmcsoft/. In addition to the functions of E(n), there have been recorded acoustic signals for P450scc and Ad. Upon reproduction of the acoustic series using MIDI-interface for P450scc, the optical–acoustic signal associated with the last protein was represented by higher frequencies compared to Ad—which correlates with a greater number of errors accounting for one sector.

3.1.2. Combination of the CD with MS-analysis

Since the CD-ROM analysis is based on the registration of inhomogenuities on the surface, the question arises whether we observe the reliably detected proteins rather than the inhomogeneities-associated artifacts. To improve the reliability of detection, the CD-ROM registration can be combined with mass-spectrometry. The possibility to perform MALDI-MS-analysis of objects on the gold-modified disc surface was demonstrated (Varma et al., 2004). In our work, mass-spectrometric identification of proteins fished out onto the disc surface was carried out. Mass spectra of Mb, BSA, P450scc and anti-HBsAg proteins are shown in Fig. 4A–D.

3.2. Registration of biospecific Ad/P450scc protein complexes using the label-free CD-ROM

Based on the above-mentioned principles of detection, we have created the optico-acoustical biosensor designed for biospecific registration of specific protein–protein interactions. The creation of the OAB involved several steps. At first, a biochip for the CD-based biosensor was made by means of covalent immobilization of proteins probes on the CD surface (Section 2.5).

Modification of the CD by probe-immobilized proteins led to the appearance of C1 read errors, due to the changes in optical density on the disk surface. The distribution of errors was collected and identified as a propagation of erroneous bits within a string of bits. Pouring the solution with partner proteins onto the biochip resulted in the formation of protein complexes—which in turn provided the increase of read errors, i. e. C2 errors. The number of protein complexes formed can be calculated from CU by subtracting C1 from C2 errors' distribution. Then the distribution of CU from the sector was presented as an acoustic signal on a PC.

The proteins of mitochondrial cytochrome P450scc-containing hydroxylase system were selected as representative proteins. Mitochondrial cytochrome P450scc is a key enzyme of the cytochrome P450 hydroxylase system. The system plays a key role in the catalysis of cholesterol-into-pregnenolone conversion reaction. It involves water-soluble protein adrenodoxin-reductase (AdR), adrenodoxin (Ad) and membrane-bound cytochrome P450scc (P450scc) (Kussmann and Roepstorff, 2000). The system operates by sequential two-electron transfer from AdR to P450scc through the intermediate protein Ad and generates the formation of both the binary and ternary protein complexes (Ivanov et al., 2001). In this study the registration of Ad/P450scc complexes was carried out. Then, the biochip with Ad, immobilized in 4 different zones (2×14 mm each), was made and transferred to the CD-ROM of a PC; subsequent registration of read errors in the zone with immobilized Ad demonstrated the dependence of the error level on the sector. This was accompanied by the acoustic signals corresponding to this dependence. Then the four zones of this biochip were incubated in P450scc-containing solutions of various concentrations (0 μM—control zone, 5 μM, 10 μM and 20 μM—working zones). The corresponding error distributions for these zones are represented in Fig. 5.

It can be seen that the increase of P450scc concentration in the incubation media led to the increase in the amount of captured

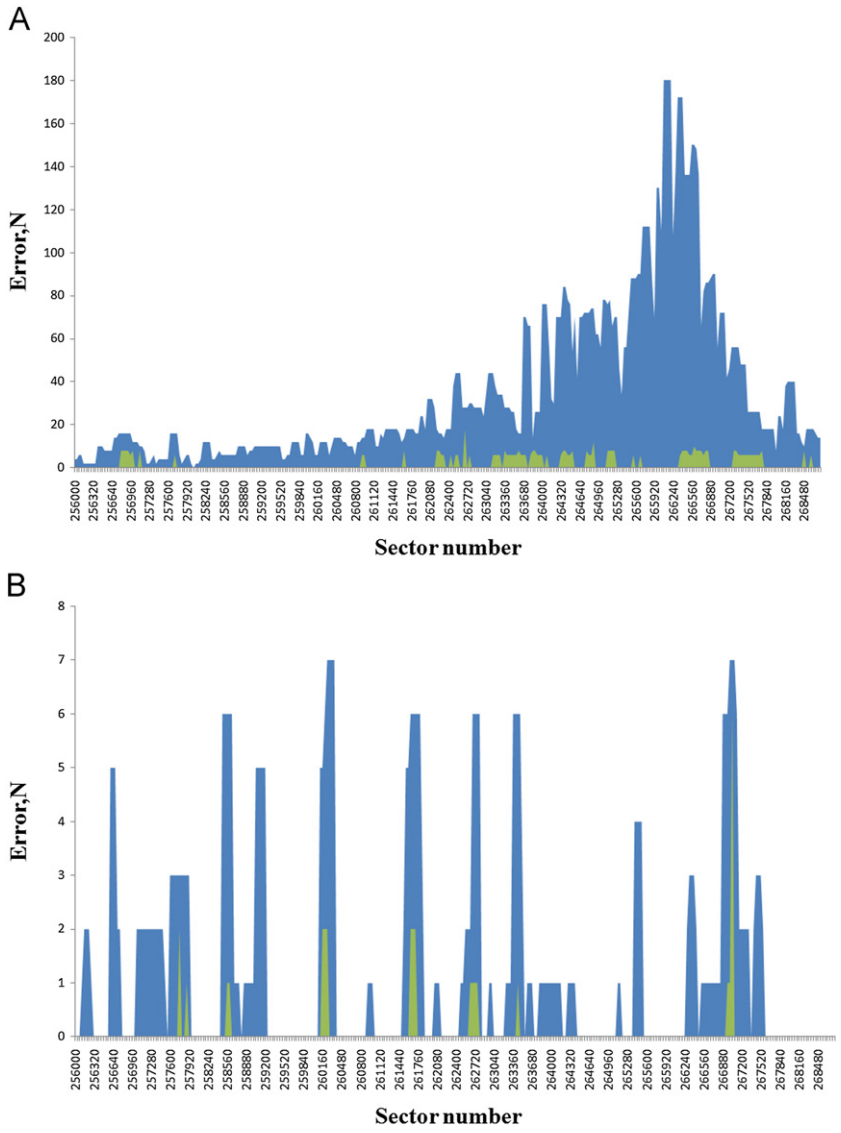

Fig. 3. The dependencies of the number of errors E(n) obtained upon covalent capture of P450scc (A) and Ad (B); the concentrations of P450scc and Ad in solution were 0 mM (control zone)—green, and 20 mM (working zone)—blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

P450scc and, accordingly, to the increase in the error level. Then the optical–acoustic signal from the biochip incubated in P450scc-containing solution at different concentrations (0 μM, 5 μM, 10 μM and 20 μM) was reproduced. This signal is represented on http://195.178.207.170/ibmcsoft/. Due to the increase in the number of error registered per sector with increasing concentration of Ad—the protein partner of P450scc, the appeared higher-frequency acoustic signal was recorded with the CD-ROM.

3.3. Registration of myoglobin with signal amplification

Optico-acoustical biosensor operating without amplification allows one to register proteins with the concentration not lower than 10^{-7} M, as shown in Section 3.1.1. For the proteins whose concentration in biological fluids is below this level, we applied the method of signal amplification. The normal myoglobin concentration in human blood is $C \sim 10^{-9}$ M, while its concentration in myocardial infarction is $C = 5 \times 10^{-8}$ M (Lange et al., 2006). Therefore, to diagnose myocardial infarction, the concentration detection limit for the analyzer should be at least 5×10^{-8} M. Silver amplification technique, autometallography, has been used to reveal the Mb level in myocardial infarction (Li Yu et al., 2008). The principle of autometallography, well-known from coloring of electrophoretic gels or tissues for electron microscopy (Matveeva et al., 2005), was illustrated by the example of silver precipitation catalyzed by gold colloidal particles bound with antibodies in immunoassay. The process of catalysis provides for the increase of the sizes of metal particles up to 200 nm ($\sim 1/4\ \lambda$), where λ is the wavelength of laser CD-ROM emission that appears to be sufficient for significant induction of the laser emission in the CD-ROM (Li Yu et al., 2008). As mentioned above, the amplification technique was applied for Mb revelation. At the first stage, Mb was registered without amplification; at the second stage—with the use of metallographic amplification. Stage I: the molecules of Mb (anti-HMb1) were covalently immobilized in the three zones on the CD surface and C1 distributions for these zones were registered. Then, the zones were incubated in Mb solution at the three concentrations: 0 M (control zone), 5×10^{-8} M (working zone 1) and 5×10^{-9} M (working zone 2). However, no difference in the error distributions in these zones was observed.

Stage II: to increase the biosensor sensitivity, an autometallographic technique was employed. The solution of anti-HMb2

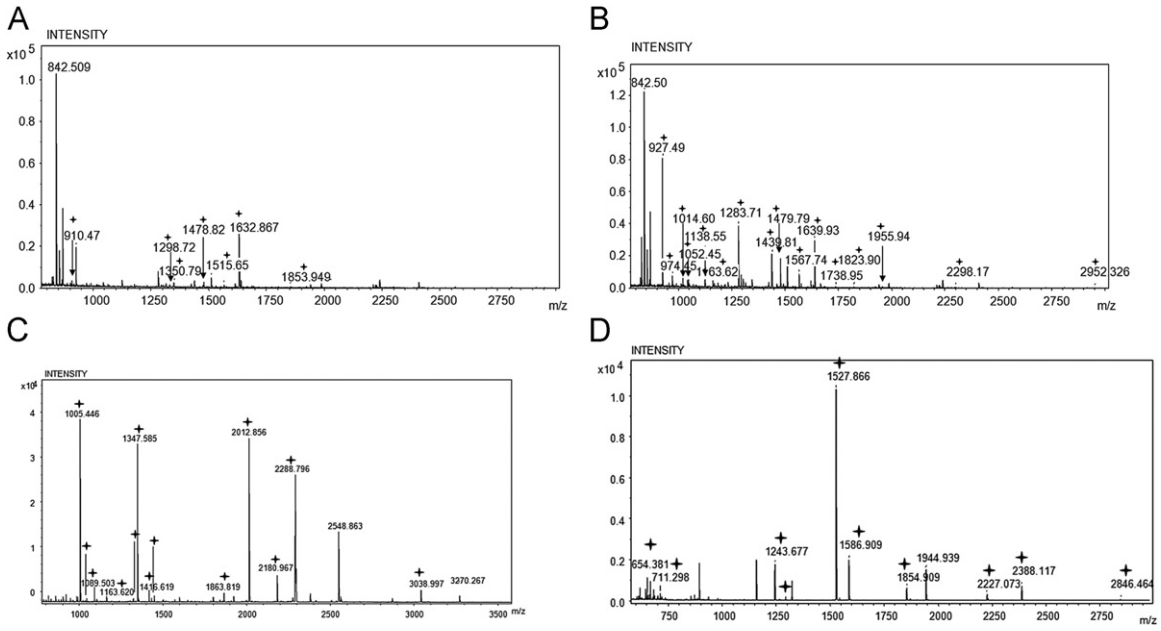

Fig. 4. Mass-spectra of the CD with immobilized zones: (A) Mb, (B) BSA, (C) P450scc and (D) anti-HBsAg whose errors were recorded. The peptides typical for these proteins are marked with asterisks. The measurements were taken on a mass spectrometer Autoflex III (Bruker). All the proteins immobilized in the working zones of the CD were reliably identified by their proteotypical peptides. Thus, the CD-ROM makes it possible to register the required protein molecules of the analyte.

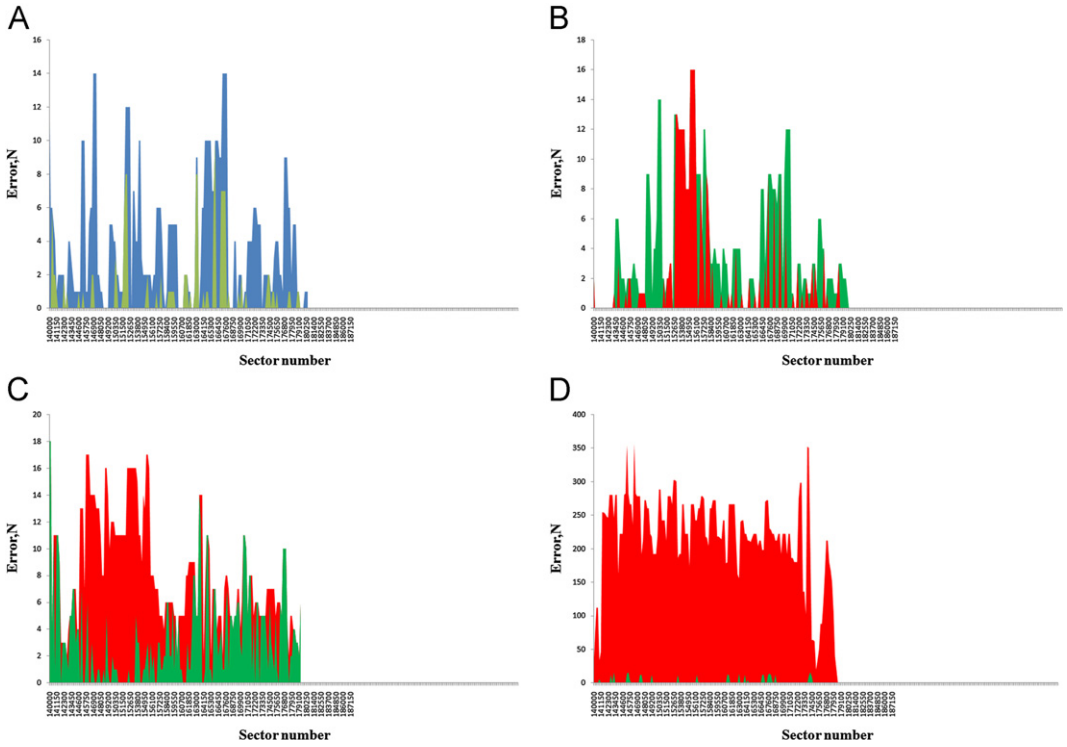

Fig. 5. The dependencies of the number of errors E(n) obtained upon covalent capture of proteins. Ad was immobilized in the four working zones (2 mm × 14 mm) on the CD surface and analyte P450scc at different concentrations—0 μM blue (A)—control, 5 μM red (B), 10 μM red (C) and 20 μM red (D) in 50 mM KP, pH 7.4 was introduced into the corresponding working zones. Nonspecific sorption of analyte P450scc on the CD surface without immobilized Ad was shown green (A–D). Ligand-to-analyte binding was registered within 90 min; then the CD was rinsed with fresh buffer for 120 min. The dependence of the level of error signal on the sector was registered. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

conjugated with gold was added to the zones with anti-HMb1/Mb, and the complexes were formed after their washing in 50 mM KP buffer, whereupon the reductive precipitation of silver particles on gold colloidal particles took place. The dependencies of E(n) on the control and working zones (see Fig. 5) were registered, and the acoustic series corresponding to the control and working zones were generated. As seen from Fig. 6, the increase in the number of errors registered per sector corresponds both to the increase in the concentration of protein partner Mb and the appearance of the higher-frequency acoustic signals.

The acoustic series of proper sectors are represented on the website http://195.178.207.170/ibmcsoft/. Thus, the use of

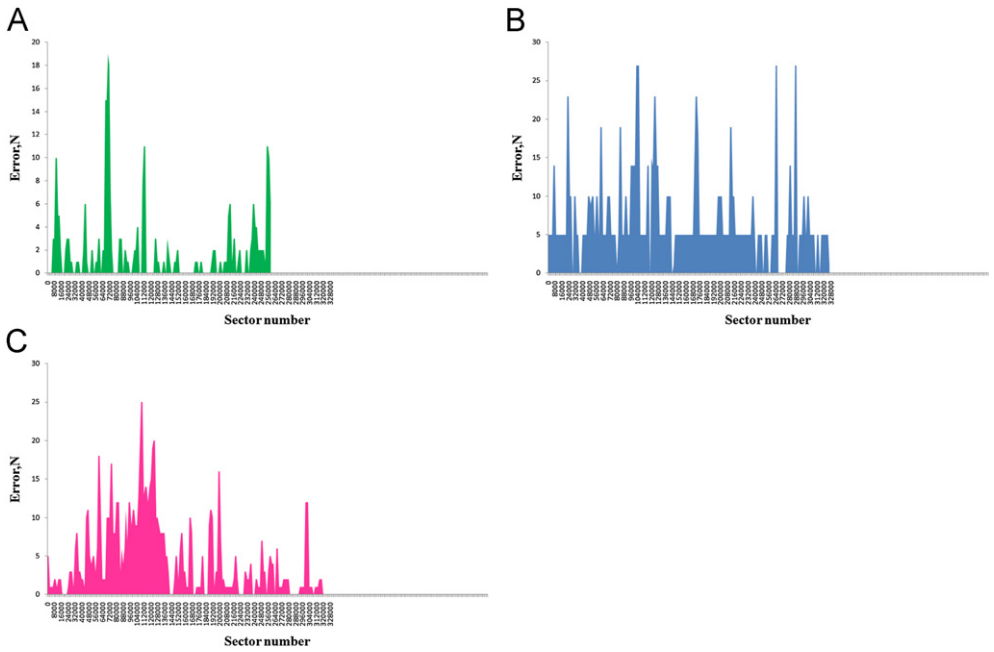

Fig. 6. The dependence of distribution of the read errors on the sector obtained with the optico-acoustical biosensor. anti-HMb1 was immobilized onto the work CD zones (2 mm × 14 mm), and Mb as analyte at different concentrations: 0 M green (A)—control, 5×10^{-9} M blue (B), purple 5×10^{-8} M (C) in 50 mM KP, pH 7.4 was introduced into the corresponding zones. Ligand to analyte binding was registered within 90 min; then the CD was rinsed with fresh buffer for 120 min. The dependence of the level of error signal on the sector was registered. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

additional autometallographic techniques for the signal amplification allowed us to increase by more than one order $\sim 5 \times 10^{-9}$ M the detection sensitivity of optico-acoustical biosensor (Fig. 6).

The concentration and time variations in silver staining provided the change of dynamic range of the concentration detection limit (Li Yu et al., 2008). Therefore, depending on conditions of reductive precipitation with silver, it is possible to achieve the lower concentration detection limit. This made it possible to develop the new devices based on the optico-acoustical biosensor for low-cost diagnosis of a number of diseases.

4. Conclusion

Thus, the CD-based optico-acoustical biosensor for detecting proteins and protein complexes was developed. We used a standard CD-ROM as the optical analyzer and compact-disc as the biochip, containing the immobilized protein molecules. The molecules immobilized on the disc surface generated C1 errors. The protein complexes, formed between the proteins immobilized on the CD surface and their protein partners, biospecifically fished out from solution were recorded as C2 errors. The CD-based biosensor and the developed software ensured the registration of the number of read errors caused by the presence of proteins and protein complexes, and their direct translation to the individual acoustic series (melody). This protein detection technology does not require the use of special and rather expensive optical analyzers (like spectrometers or refractometers). The proposed approach can be used in proteomics and in the future home medical diagnostic systems.

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