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THE FIRST APPLICATION OF SENSORY STRUCTURES BASED ON PHOTOELECTRIC TRANSDUCER FOR THE STUDY OF ENZYMATIC REACTIONS

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Background. The development of highly sensitive sensor equipment with the possibility of registering analytes in real time is a fast growing research area and a promising diagnostic biomedical technology. Currently, the standard laboratory method for determining the activities of ATPses is an indirect spectroscopic study of the concentration of inorganic phosphate formed as a result of ATP hydrolysis by these enzymes. However, there is no commercially available phosphate sensor with satisfactory parameters of sensitivity, selectivity and stability over time. The purpose of our research was the development of a photoelectric recombination sensor system for the real-time detection of biochemical markers and its testing on the example of screening ATPase activity of rat erythrocyte plasma membrane suspension preparations.

Materials and Methods. Experiments were performed on suspension preparations of plasma membranes of erythrocytes of Wistar rats. Preparations of plasma membrane suspensions obtained by Dodge method from each animal were divided into aliquots and used for the simultaneous study of ATPase activity by the reference method of Rathbun & Betlach, as well as the registration of photocurrents induced during the passage of the ATPase reaction using the photoelectric recombination multisensor system of our own design.

Results. The application of silicon sensory structures based on photoelectrical transducer principle for detecting the activity of adenosine triphosphate hydrolases on the example of total Mg^{2+},Na^{+},K^{+}-ATPases preparations of plasma membranes of rat erythrocytes has been experimentally tested. The directly measured analytic parameter is the photocurrent of the deep silicon barrier structure under illumination with high absorption coefficient. The physical features of the device operation have been examined. Detection

of such metabolites becomes possible due to reactions intermediates with their own dipole moment (inorganic phosphate, which is one of the products of ATP hydrolysis). The drastic change of photocurrent that characterizes the course of biochemical reaction was observed in real time. The effect is explained by local electrostatic influence on the parameters of recombination centers at the silicon surface that results in surface recombination velocity change. The sensor operation is qualitatively explained in the frame of Stevenson-Keyes's theory.

Conclusions. Our approach can be regarded as a promising way to elaborate technically simple and highly sensitive method for detection of quantitative behavior of enzymatic reactions. Moreover, the local modification of silicon surface allows obtaining time depending scenarios of the adsorption and thus improving the sensitivity of the sensor. These circumstances open up the possibility of elaborating the complex sensory structures with optimized parameters for certain enzymatic reactions.

Keywords: enzymatic reactions, photoelectrical transducer principle, surface recombination velocity, biomedical diagnostic technology

INTRODUCTION

The development of modern biomedical diagnostic technologies largely focuses on the creation and testing of new methods of laboratory screening of enzymatic and metabolic markers of the human body physiological state (Gallagher *et al.*, 2021; Formica *et al.*, 2021; DeBerardinis & Keshari, 2022). Optimally, such studies are performed in real time, are non-invasive or minimally invasive, highly sensitive and accurate, and are easy to apply. Enzymatic activity is characterized by the rate of conversion of substrates into some product during a biochemical reaction (measured in mole of a product per unit of time).

Thus, in the last decade, the methods for screening biological markers of organs and systems of the body have been widely considered. The main goals of researchers in this case are determination of normal metabolic patterns (taking into account population, age and gender characteristics) and various pathophysiological conditions (Formica *et al.*, 2021; DeBerardinis & Keshari, 2022). In particular, extracellular adenosine triphosphate (ATP) is a messenger molecule that acts as a neurotransmitter in synaptic contacts, and in the microcirculatory tract – a paracrine regulator with a broad spectrum of action (Sookoian & Pirola, 2015). The concentration of ATP in human plasma is normally about 1 μmol/L (Gorman *et al.*, 2007; Zarębska *et al.*, 2019; Zhu *et al.*, 2019) whereas in some pathologies these parameters can vary significantly (Lader *et al.*, 2000).

The suspension of plasma membranes (PM) of erythrocytes is a modeling object for laboratory researches. Erythrocyte PM is the subject of biomedical research as a marker of the general condition of the human body and aging processes (Pandey & Rizvi, 2010; Maurya *et al.*, 2015; De Souza Gonçalves *et al.*, 2020; Turpin *et al.*, 2021). ATP hydrolases (ATPase) of erythrocytes are a model system for biochemical screening of the state of the general level of energy metabolism in the body, the efficiency of oxygen and nutrients transport, as well as maintaining pH homeostasis of blood (Rodrigo *et al.*, 2007; Ajima *et al.*, 2017). It is known that ATPase activity in erythrocyte PM is significantly reduced under various pathologies (Rodrigo *et al.*, 2007; Namazi *et al.*, 2015; Vultaggio-Poma *et al.*, 2020; Turpin *et al.*, 2021). It should be noted that the erythrocyte PM ATPase activity is also sensitive to the concentration of ATP in blood plasma (Konukoglu *et al.*, 2001; Tsymbalyuk *et al.*, 2020).

Currently, several methodological approaches are used in laboratory research to determine the enzymatic activities of ATPase. Firstly, it is an indirect spectroscopic method for determining inorganic phosphate (P_i) as a product of ATP hydrolysis reaction. In this way, the total Mg^{2+}-dependent activity can be measured using a calibration curve (Tsymbalyuk *et al.*, 2020). However, the disadvantages of this method are a long duration of measurement, and inability to observe the kinetics of the process (Vultaggio-Poma *et al.*, 2020). Secondly, several ion-selective sensors sensitive to P_i have been developed, which, however, have low selectivity and are sensitivity dependent on physicochemical conditions (Engblom, 1998). Thirdly, enzyme biosensors and multisensors are a large group of tools for measuring the concentration of P_i in liquids. Thus, several dozen amperometric, conductometric and potentiometric enzyme sensors have been developed, the operation of which is based on the use of enzymes (Engblom, 1998; Hargrove & Nieto, 2011; Ray *et al.*, 2020).

Thus, in the current medical and biological laboratory research there are a number of issues: 1) optimization of ATPase activities estimation with the possibility of its dynamic measurement, 2) development of highly sensitive sensors for measuring P_i in biological fluids.

Recently, a novel type of chemical electronic sensor was developed (Kozinetz *et al.*, 2017, 2021) that corresponds to the category of well-known semiconductor devices based on a different physical principle with possible advantages. The operation of the sensor structures is based on the effect of controlled and reproducible change of recombination characteristics of the semiconductor surface interface due to adsorption. The directly measured analytic parameter is the photocurrent of the deep silicon barrier structure under illumination with high absorption coefficient. Structures of this type combine high sensitivity to analytes containing molecules with their own or induced dipole moment, conformity to the electronic tongue approach, and the relative simplicity of technical implementation. Therefore, the aim of our study was to demonstrate the ability of the photoelectric recombination sensor to detect biochemical markers in real time, to test the possibility of screening of ATPase activity.

MATERIALS AND METHODS

Firstly, we selected all necessary reagents and checked the enzymatic activity by an independent method. The detailed description of preparative processes is given below.

The blood of Wistar rats of the vivarium population of the Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine was used for research (the number of animals used in the experiments was 7). Rats were fed on a standard diet and kept under standard conditions: temperature 20±2 °C, relative humidity 50–70 %, light regime – light: darkness = 12:12 hours.

All manipulations with animals were carried out in accordance with the International Convention on Animal Welfare and the Law of Ukraine „On Protection of Animals from Cruelty" (Minutes of the meeting of the Commission on Bioethics of NSC „Institute of Biology and Medicine" No 3 of May 2, 2019). Killing of animals was carried out by dislocation of cervical vertebrae under etheric anesthesia.

Preparation of erythrocytes. Blood with anticoagulant (heparin, 5000 IU/mL) in the ratio blood: heparin = 9:1 was centrifuged at 300 rpm for 10 minutes. Plasma was carefully pipetted and stored at 4 °C for no more than 24 hours for use in comparative experiments and further used to obtain preparations of erythrocyte PM to study the specific reactions of ATP hydrolysis.

Preparations of erythrocyte plasma membrane. The preparations of suspension of erythrocyte plasma membrane were obtained by slightly modified method of Dodge (Tsymbalyuk *et al.*, 2020). Erythrocytes were washed thrice with a cooled solution of the following composition: NaCl 145 mM, Tris-HCl 20 mM (pH 7.6 at 20 °C), each time precipitating the cells by centrifugation at 3,000 rpm for 10 min. Erythrocyte membranes were obtained by hypoosmotic hemolysis in the following solution: EDTA 10 mM, Tris-HCl 10 mM (pH 7.6 at 20 °C). For this purpose, one volume unit of washed erythrocytes was thoroughly mixed with 20 volume units of cooled (4 °C) hemolytic medium and kept at the same temperature for 15 min. The hemolysate was centrifuged at 18,000 rpm for 15 min. The precipitate (membranes) was washed thrice with 20 volume units of 10 mM Tris-HCl buffer (pH 7.6 at 20 °C). The preparations of plasma membranes were further kept for 12–24 h at 4 °C until used in the experiments to study the enzymatic activity of Mg^{2+}, Na^+, K^+-ATPase, basal Mg^{2+}-ATPase and Na^+, K^+-ATPase. Protein concentration in the preparations of rat erythrocyte plasma membrane was determined by Lowry's method (Lowry *et al.*,1951).

Then, aliquots of plasma membrane preparations from each animal were separated and used for simultaneous determination of ATP-ase activity according to the method of W. Rathbun et V. Betlach (Rathbun & Betlach, 1969) and registration of photocurrents (n = 7).

Determining total ATPase activity. Total Mg^{2+}, Na^+, K^+-ATPase activity was determined at 37 °C in the fraction of erythrocyte plasma membranes in the standard incubation medium (volume of 0.4 mL) of the following composition (in mM): 1 ATP, 3 $MgCl_2$, 125 NaCl, 25 KCl, 1 EGTA, 20 Hepes-Tris-buffer (pH 7.4), 1 NaN_3 (inhibitor of mitochondria ATPase) (Tsymbalyuk *et al.*, 2020) with the addition of 0.1 μM thapsigargin (selective inhibitor of Ca^{2+}, Mg^{2+}-ATPase of endo(sarco)plasmatic reticulum) and 0.1 % digitonin (factor of plasma membrane perforation). The amount of plasma membrane fraction protein in the final volume of the sample was 20–30 μg, the incubation lasted 4 min.

The enzymatic reaction was initiated by the introduction of the aliquot (20 μL) of plasma membrane suspension (8 °C) to the incubation medium, and terminated by the introduction of 1 mL of 20 % trichloroacetic acid (pH 4.3 at 8 °C) to the incubation mixture.

The incubation medium, having a similar composition but lacking fragments of plasma membranes, served as control for non-enzymatic hydrolysis of ATP. Aqueous solution of the membranes served as control for the amount of endogenous P_i in the membrane preparation. Thus, total ATPase activity was calculated as the difference between the amount of P_i, formed in the incubation medium with and without plasma membranes. The allowance for the amount of endogenous P_i in the membrane preparation was estimated. The amount of P_i reaction product was determined by the method of W. Rathbun et V. Betlach (Rathbun & Betlach, 1969).

Determining Mg^{2+}-ATPase activity. Basal Mg^{2+}-ATPase activity was determined in the incubation medium, used for total ATPase activity, but in the presence of 1 mM ouabain (selective inhibitor of Na^+, K^+-ATPase).

Estimating Na^+, K^+-ATPase activity. Ouabain-sensitive Na^+, K^+-ATPase activity was calculated as the difference between the amount of total Mg^{2+}, Na^+, K^+-ATPase and basal (ouabain-insensitive) Mg^{2+}-ATPase activity.

The reagents ATP, ouabain and thapsigargin produced by Sigma (USA) were used in the research; other chemical reagents were qualified as "chemically clean".

Samples were checked for their belonging to normally distributed general populations using the Shapiro-Wilk's test by means of Origin2018 program. The results were presented as mean ± standard error of mean, n – number of experiments.

Thus, we determined the total Mg^{2+}-dependent, ouabain-sensitive, and ouabain-insensitive ATPase activity as follows: (1.64±0.05) μmol P_i /(mg of protein×h), (0.85±0.06) μmol P_i/(mg of protein×h) and (0.79±0.05) μmol P_i/(mg of protein×h) (n = 7); therefore, the activity of Na^+, K^+-ATPase for rat erythrocyte plasma membrane was 51.8 % on average compared with the total ATPase activity, which served as 100%. The values of total Mg^{2+}-dependent ATPase activity and ouabain-sensitive Na^+, K^+-ATPase of erythrocytes obtained by us are consistent with the data of other researchers (Ohnishi *et al.*, 1982; Kumthekar & Katyare, 1991; Orlov *et al.*, 1991; Tsymbalyuk *et al.*, 2020).

Sensor structure based on the photoelectric principle of transformation. Theoretical approach to the sensor platform elaboration. The principle of the sensor operation is based on recharging the recombination centers in the interface due to adsorption and recombination dependent photocurrent measurement. This process can be accompanied by the change of band bending and capture cross sections at the interface. The changes of the center's concentration or energy levels in the band-gap are also possible. In other words, the principal condition that allows effective detection is mostly electrostatic influence of adsorbed molecules on the recombination parameters at the interface. However, the direct recognition of biological molecules (for example, the PM suspension) should be difficult due to the absence of particles with their own or induced dipole moment.

The proposed work investigates specific biochemical reactions involving intermediate enzymes. The products of such reactions can exhibit electrostatic effects on the recombinational parameters of our sensor (as the products usually contain acids or molecules with their own dipole moment). Such intermediates can characterize the activity of ATPases-hydrolases in PM suspension. It should be noted that reactions involving enzymes are widely studied using other approaches (like potentiometric, amperometric, conductometric sensors or field-effect transistors). The choice of application depends on the specific task.

A *p*-type silicon wafer (100), 20–50 Ohm·cm ($p = 10^{15}$ cm^{-3}) with natural oxide SiO_x was chosen to create the barrier structure. On the sensor surface, the modified areas were formed. These films were obtained by treatment with the solution containing a metal component Au, Gd, Ag, Tb. The study of interaction of transition metal salts with silicon oxide shows that metal adsorption occurs under certain conditions (Mori *et al.*, 2015). According to (Mori *et al.*, 2015), for some metals, namely Fe(III), Ni(II), and Zn(II) on silicon wafers the main adsorption species is the dissolved neutral hydroxide complex.

We expected that metal particles should locally change the function of SiO_x and, consequently, the initial bending. Another possibility is the formation of structural defects (involving states distribution, the energy of levels, the capture cross sections and concentration of surface states). The density of structural defects is expected to be influenced by the size of inserted ion. Moreover, different relief can change the adsorption efficiency. Modification of silicon wafer allows us to obtain the different scenarios of adsorption and analyze the electronic tongue approaches. Really, as each interface provides its own behavior, then registration of related photocurrents can increase the selectivity. Currently, projective techniques based on PCA are used for statistical processing of signals.

The simplified expression for photocurrent through the sensory structure for light with high absorption coefficient is (Kozinetz *et al.*, 2017)

$$i_{ph}(S) \cong \frac{1+\frac{S}{\alpha(\lambda)D}}{S\frac{l}{D}sh\left(\frac{d}{l}\right)+ch\left(\frac{d}{l}\right)}, \quad (1)$$

where D is the diffusion coefficient of electrons, $\alpha(\lambda) \sim 10^5$ cm^{-1}, for λ = 532 nm ($1/\alpha(\lambda) \sim 0.07$ μm), S is the surface recombination velocity, d is the silicon wafer thickness. The conventional analysis can be performed in the framework of Stevenson–Case theory (Kozinetz *et al.*, 2017; Kozinetz *et al.*, 2021) for a simple single recombination level in the band gap. The dependence of the recombination velocity on the band bending can be expressed as

$$S(Y_s) = \frac{c_p c_n N_t (p_0 + n_0)}{c_n(n_s(Y_s)+n_1)+c_p(p_s(Y_s)+p_1)}, \quad (2)$$

where n_0 and p_0 are the concentrations of equilibrium carriers in the volume; $n_1 = n_i\, exp\,(E_{ti}/\mathrm{kT})$, $p_1 = n_i\, exp\,(-E_{ti}/\mathrm{kT})$ – carrier concentrations, when the Fermi level on the surface coincides with the energy of the recombination level E_{ti} (from the middle of the band gap); $c_n = \sigma_n v$, $c_p = \sigma_p v$ – capture cross sections, v – thermal velocity; n_i – intrinsic concentration, N_t - concentration of recombination centers. The functional relationship between surface and bulk concentrations can be given as $n_s = n_0\, exp\,(Y_s/\mathrm{kT})$ and $p_s = p_0\, exp\,(-Y_s/\mathrm{kT})$, the doping level of silicon wafer defined by $\mathrm{kT}\ \ln(p_0/n_i)$ factor. The parameters of the model are shown in **Fig. 1.**

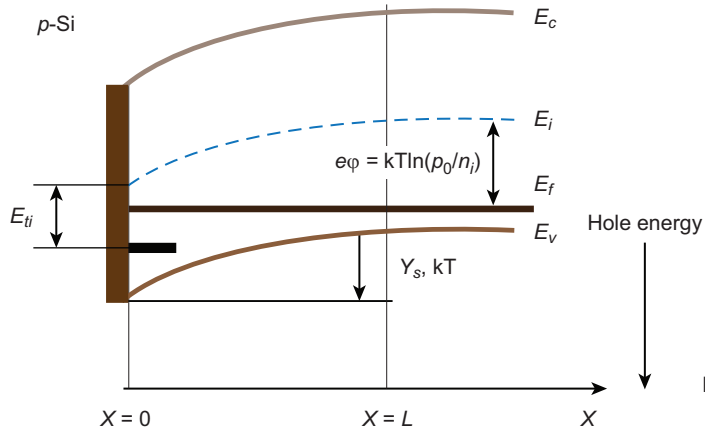

Fig. 1 A simplified band diagram of the sensory structure

Figure 2 shows the functional dependences of the photocurrent on the surface bending Y for different parameters of the recombination centers, which are calculated with (1) and (2). The functional dependences of the photocurrent on the surface bending Y for different parameters of the recombination centers were calculated with (1) and (2). Therefore, the change in the initial band bending causes a change of the photocurrent through the sensor. Schematically, the adsorption process is shown here by an arrow that connects the initial (contact from the air) and final (contact with the analyte) state of the surface. If the local electric field of the adsorbed molecules affects only the band bending, the operation of the recombination sensor is described by the same curves as

shown above. If the ratios of the cross-sections of the capturer the energy position of the level change as well, the transfer between different two curves is possible.

Moreover, the farther the recombination level E_t (at c_n/c_p = 1 condition) is from the middle of bandgap, the lower minimum value of photocurrent can be observed in practice (curves 2 and 4). Changing the c_n/c_p ratio causes a shift in the minimum of the photocurrent curve along the axis of the band bending (Kozinetz *et al.*, 2017; Kozinetz *et al.*, 2021) (so for a *p*-type, the condition $c_n n_s = c_p p_s$ will be satisfied for smaller Y in the case of $c_n / c_p > 1$ and larger Y in the case of $c_n/c_p < 1$). It is clearly illustrated by curves 1, 2 and 3. Certainly, these parameters cannot be measured in a direct way but they allow constructing a fairly complete and adequate theory of the sensor. Thus, the start and end points can now lie on different photocurrent curves (Kozinetz *et al.*, 2021). These calculations also show that if the interfaces of the individual sensors (placed on the same Si substrate) have modified recombination parameters, the corresponding changes in photocurrents during adsorption will significantly depend on the type of such modification. This circumstance opens up the possibility of elaborating complex sensory structures whose parameters are optimized for specific enzymatic reactions.

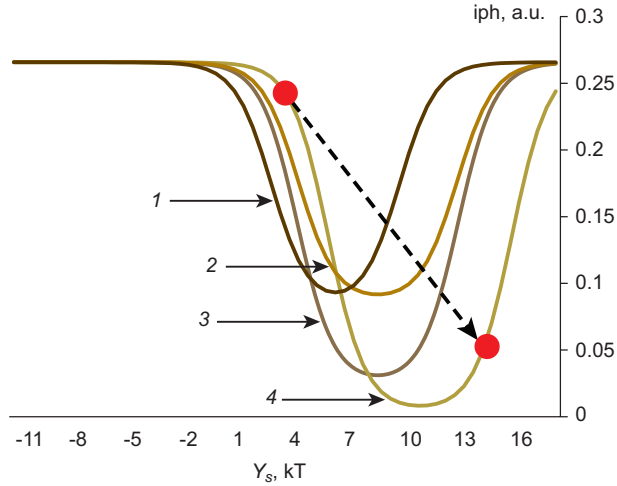

Fig. 2. Estimated dependences of the photocurrent of the sensor on the surface band bending for different interfaces:
1) E_t = 0.075 eV, c_n/c_p = 0.01;
2) E_t = 0.05 eV, c_n/c_p = 1;
3) E_t = 0.05 eV, c_n/c_p = 100;
4) E_t = 0.05 eV, c_n/c_p = 0.01.
Schematically, the adsorption process is shown here by the dashed arrow

The laser scanning equipment was created (developed and manufactured) at Taras Shevchenko Kyiv National University. To scan an object, the laser beam is deflected in two X and Y directions using acousto-optic deflectors. The latter also implement the amplitude modulation function. Focusing on the object (in this case on the sensor structure) is carried out using an optical system (collimator, lens). Control of the scanning process using a convenient user interface, as well as processing of the resulting photoelectric signal is supported by the original software. This installation was described and used in publications (Litvinenko *et al.*, 1999; Litvinenko *et al.*, 2000).

To illuminate the sensor structure and generate the photocurrent, a continuous laser with a radiation power falling on its surface of about 2 mW is used. In order to effectively amplify the current induced by the laser beam and separate the constant component from the background illumination, the laser beam is modulated by amplitude with a duty cycle of 2 (that is, the ratio of the period to the pulse length equals 2), the frequency is several kilohertz, and the modulation depth is 100%. Thus, the peak power of each pulse is 2 mW, and the length is 100 μs at a modulation frequency of 5 kHz.

RESULTS AND DISCUSSION

Mg^{2+}-dependent ATPases of PM realizes hydrolyze of ATP and the energy released as a result of this reaction (about 7.3 kcal/mol) is used for selective transport of Na^+, K^+ and Ca^{2+} ions against gradients of their concentrations, providing a fundamental property of living cells – creating the transmembrane difference in the concentrations of these ions. These systems of primary active ion transport of PM include Na^+, K^+-ATPase, which in one catalytic act injects $2K^+$ ions into the cell and pumps out $3Na^+$, and Ca^{2+}-pump providing extrusion of Ca^{2+} into the extracellular space (Veklich *et al.*, 2020). In all cases, the product of ATP hydrolysis is inorganic phosphate P_i (PO_4^{3-}). In the active center of the enzyme ATPase in the presence of molecules of ATP and water, as well as cofactor (Mg^{2+} ion), a chemical reaction of ATP cleavage of γ-phosphate residue occurs with the formation and dissociation of adenosine diphosphoric acid (ADP) and P_i.

Considering the above, it is possible to propose an alternative approach to determining the activity of Mg^{2+}-dependent ATPase activity using a platform based on a recombination converter.

Fig. 3 shows a typical example of operation of the device. The surface of the deep silicon barrier structure was scanned by a laser beam with λ = 532 nm wavelength. The round-shaped spots of the modified surface can be clearly distinguished on a 2D photoelectric map (lighter areas correspond to a higher photocurrent signal). On the surface, individual points were selected within each modified part, which allows observing the signals of photoelectric current due to the processes under study. Thus, we obtain the photocurrent kinetics for 4 points that reflects the course of the reactions. It should be noted, that the proposed approach provides one of the simplest possibilities to investigate reactions of analytes.

Firstly, a standard incubation medium was applied to the sensor surface with ATP solution. During the measurement, the sensor surface with the deposited liquids was covered with a transparent cup to prevent evaporation. All the processes were observed at 25 °C ambient temperature. During the stay of this mixture, changes in the photocurrent were observed, which slowed down after 15 minutes. In our study, we used this ATP solution and a preparation of rat erythrocyte PM suspension, so the reaction would take place in the active centers of ATPases. Thus, 3000 s after the start of the process, 75 µl of rat erythrocyte suspension preparation was applied. The corresponding kinetics of the photocurrent is presented in **Fig. 4**.

It can be seen that in the presence of a standard incubation medium and ATP, the non-monotonic response of the photocurrent is observed. Moreover, the different curves correspond to different sensor areas. Thus, it confirms the operation of the device on the principle of electronic tongue. Addition of PM suspension to the solution led to significant changes in photocurrent. Despite the significant change (decrease) of the signal, its measurement remains reliable due to a rather large dynamic range of the photocurrent of our sensory structure and the supporting set-up, which reaches up to 5 orders of magnitude. After application of the plasma fluid (PM suspension), the photocurrent changes non-monotonically and depends on the area according to the mentioned surface modification. It is possible that the latter provides additional opportunities for the characterization of analytes and chemical reactions. However, doubt remains that the change in the signal is not due to a chemical reaction, but simply to the properties of the added analyte. Similarly, we observed (the curves on **Fig. 4** before 3000 s), for example, some

changes in the photocurrent when the ATP solution is already on the surface of the sensor, but when no reaction occurs yet. Therefore, we conducted an experiment in which we added the rat erythrocyte PM suspension to the standard incubation medium, but without the presence of ATP (the other conditions are the same). The results of the experiment are shown in **Fig. 5**.

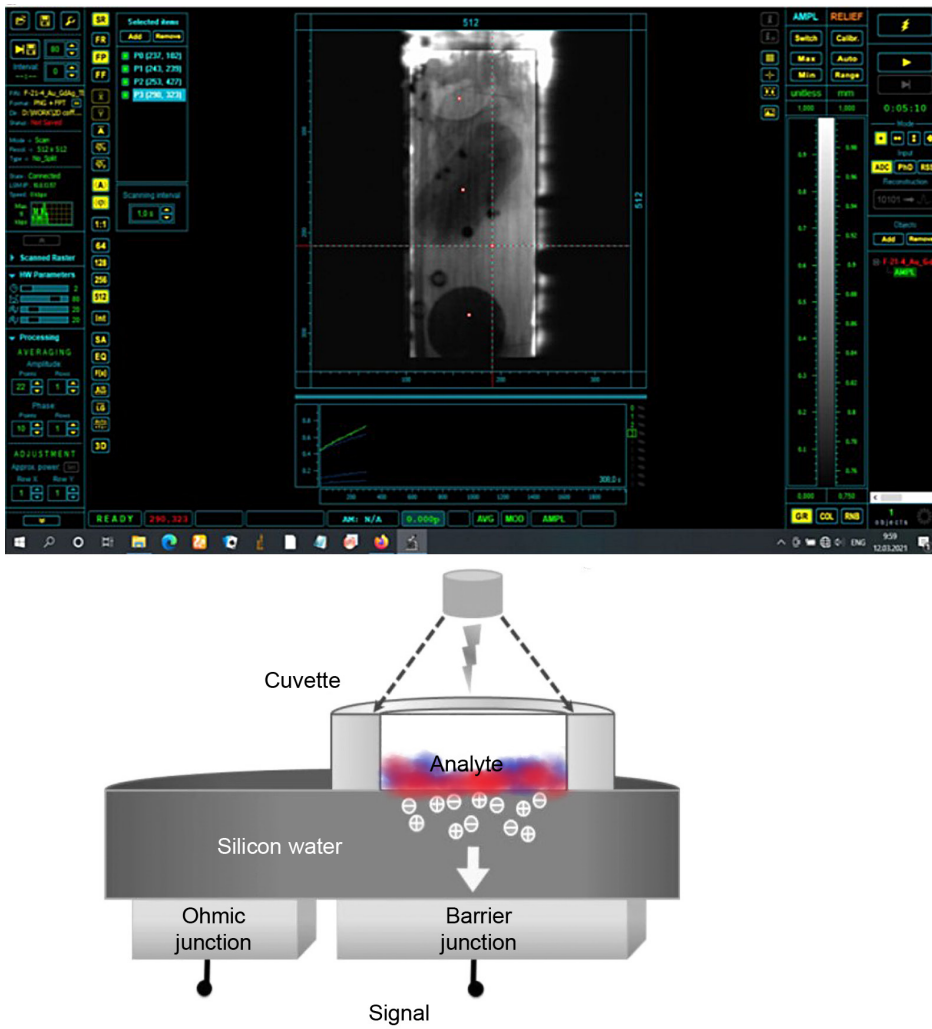

Fig. 3. The interface of our experimental set-up software with a sample of light beam induced image of the sensory structure and a schematic image of the sensory structure section

As can be seen, the addition the erythrocyte PM suspension (1200 s on the graph) induces both the sharp change in photocurrent and the nature of kinetics. It is evident that the amplitude of the photocurrent changes slightly, by 30–50 %, and the same order of magnitude remains. Thus, a comparison of the data from **Fig. 4** and **5** confirms that we have observed the course of the chemical reaction involving enzymes (**Fig. 4**), as expected before.

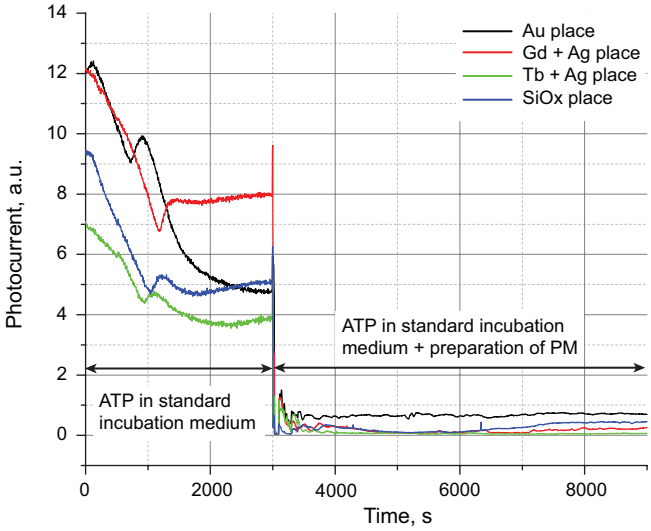

Fig. 4. Kinetics of the photocurrent in our experiment: changes in the photocurrent due to the addition of the enzyme to the reaction medium, which previously contained ATP

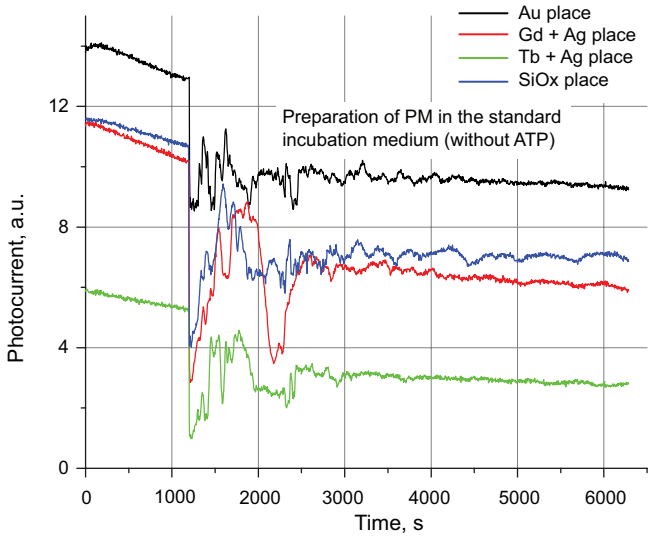

Fig. 5. The kinetics of photocurrent without the presence of ATP

In the next experiment, we changed the order of application of reagents. First, 75 μL of erythrocyte PM suspension was added to the standard incubation medium (560 μL), followed by an ATP solution (112 μL). Therefore, we expected that the reaction would take place only at the last stage. The results of the observation are shown in **Fig. 6**.

There is a slower reduction of photocurrent than in **Fig. 4**, but also very significant, with change of the signal by orders of magnitude, which leaves no doubt about the expected biochemical reaction. During these processes, photocurrent curves demonstrate interesting features of a pronounced non-monotonical form, and the curves differ from each other. It may represent some scientific and practical importance. It is likely that such differences are due to the peculiarities of the diffusion processes of the enzymatic reaction reagents.

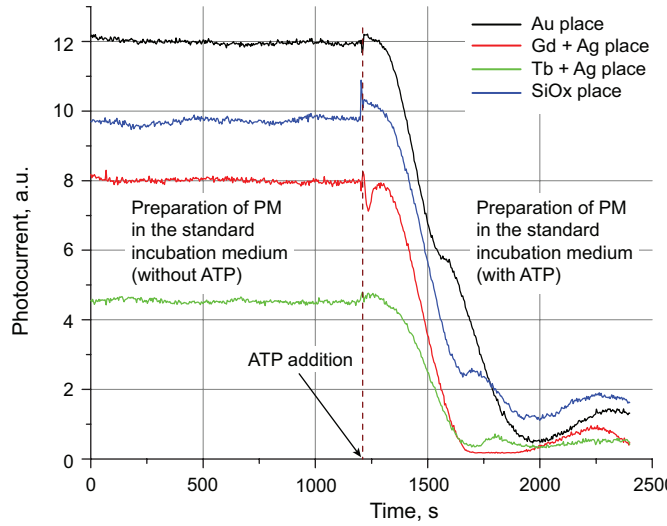

Fig. 6. The kinetics of photocurrent with change of the order of reagents application

The decrease in the magnitude of photocurrents can be qualitatively explained by the theoretical results (presented in **Fig. 2**) taking into account the individual properties of modified interfaces. The character of influence on centers of recombination reveals complex features. Thus, in the case when the PM suspension was added to a mixture of ATP and saline solution (**Fig. 4–6**) there were significant signal fluctuations. It can be attributed to balancing the concentration gradients of ATP, the components of the incubation medium (especially SDS detergent, which provides ATP access to the enzyme), and, as a consequence, a significant variation in the efficiency of the ATPase reaction. In the case where the ATP was added to the PM suspension, the reaction was limited only by the diffusion of ATP molecules to the enzyme, and the slower kinetics can be explained by the fact that ATP needs to diffuse in a more viscous medium. It should also be noted that significant changes in photocurrent in all cases under the conditions of the ATPase reaction were recorded within the first 8–10 min after induction of the reaction, which corresponds to changes in the process rate at a low (relative to the internal environment of the body) temperature (25 °C) on the surface of the sensor (Boldyrev, 1988; Esmann & Skou, 1988). Therefore, as a result of the conducted researches, the suitability of the photoelectric recombination sensor for observation of biochemical processes with the analytes of biological origin containing enzymes was proved for the first time.

It should be noted that a wide group of analytical methods (potentiometric, voltammetry, amperometry, non-traditional electrochemical methods) allow investigations of ATPase reactions via the concentration of inorganic phosphate (Hargrove & Nieto, 2011; Prasad *et al.*, 2021). However, the above methods have numerous limitations. For example, potentiometric P_i sensors (although being quite easy to produce) cannot be used for several cycles of measurement, do not have high sensitivity, and require both careful temperature control and reference electrode quality. The bioelectroanalytical enzymatic sensors use other enzymes for which P_i is an inhibitor or substrate. Although such sensors are used in biomedical technologies to determine the P_i, they are expensive, complex and not stable enough. They cannot be used for dynamic studies of ATPases. Similarly, the fluorescent methods (based on periplasmic phosphate

binding protein), often used for single molecules and isolated cells, are difficult to apply, require additional equipment, pre-treatment of the biological samples (Berchmans, 2012; Assunção *et al.*, 2020). Taking into account the above, we hope that our approach can be regarded as a promising way to elaborate a technically simple and sensitive method for investigation of enzymatic reactions.

CONCLUSIONS

The example of detecting the activity of ATPases in preparations of rat erythrocyte PM suspension demonstrated the possibility of using a recombination sensor to detect biochemical markers in real time. Detection of metabolites by such sensory structure becomes possible due to the biochemical reactions involving enzymes. Intermediates of such reactions contain fragments with a pronounced dipole moment, and therefore most likely they are active in influencing the parameters of recombination centers in the interface. The mechanism of such electrostatic influence is manifested in the change of band bending and, possibly, the change of the cross sections or energy position of the recombination levels relative to the middle of the band gap. A significant decrease in the photocurrent during the enzymatic reaction can be explained by recharging the recombination centers in the interface and changing the effective recombination rate at the interface between the modified oxide-silicon films. Several channels of the analytic signal due to pattern pre-treatment of the sensory area and light addressing support operating in electronic tongue mode make it possible to identify the analytes in more detail. Amplitude and kinetic properties of photocurrent signals during the mentioned reactions allow us to conclude that the proposed sensory structure is a highly sensitive device to detect quantitative changes in reaction products. It can be used to study the peculiarities of enzymatic reactions and their kinetics in real time.

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COMPLIANCE WITH ETHICAL STANDARDS

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ПЕРШЕ ЗАСТОСУВАННЯ СЕНСОРНИХ СТРУКТУР НА ОСНОВІ ФОТОЕЛЕКТРИЧНОГО ПЕРЕТВОРЮВАЧА ДЛЯ ДОСЛІДЖЕННЯ ЕНЗИМАТИЧНИХ РЕАКЦІЙ

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Обґрунтування. Актуальним напрямом розвитку дослідницьких і діагностичних біомедичних технологій є розробка високочутливого сенсорного устаткування з можливістю реєстрації аналітів у режимі реального часу. Натепер стандартним лабораторним методом визначення активностей АТФ-аз є опосередковане спектроскопічне дослідження концентрації неорганічного фосфату, який утворився внаслідок гідролізу АТФ цими ензимами. Втім, немає комерційно доступного фосфатного сенсора з задовільними параметрами чутливості, селективності та стабільності в часі. Метою нашого дослідження була розробка фотоелектричної рекомбінаційної сенсорної системи для детектування у режимі реального часу біохімічних маркерів та її апробування на прикладі скринінгу АТФ-азної активності препаратів суспензії плазматичних мембран еритроцитів щурів.

Матеріали та Методи. Експерименти проводили на препаратах суспензії плазматичних мембран еритроцитів щурів Wistar. Отримані за методом Dodge препарати суспензії плазматичних мембран від кожної тварини розділяли на аліквоти і використовували для одночасного дослідження АТР-азних активностей референтним методом W. Rathbun & V. Betlach, а також реєстрації індукованих у ході перебігу АТФ-азної реакції фотострумів за допомогою розробленої нами фотоелектричної рекомбінаційної мультисенсорної системи.

Результати. Продемонстровано застосування кремнієвих сенсорних структур на принципі фотоелектричного перетворювача для детектування активності аденозинтрифосфатдегідрогеназ на прикладі препаратів загальної Mg^{2+},Na^{+},K^{+}-АТФ-ази плазматичних мембран еритроцитів щурів. Безпосередньо вимірюваним аналітичним параметром є фотострум глибокої кремнієвої бар'єрної структури, коли використовують освітлення з високим коефіцієнтом поглинання. Досліджено фізичні особливості роботи пристрою. Виявлення таких метаболітів стає можливим завдяки проміжним продуктам реакцій, що містять власний дипольний момент (неорганічний фосфат, який є одним із продуктів гідролізу АТФ). У реальному часі спостерігали різку зміну фотоструму, який описує перебіг біохімічної реакції. Ефект обумовлений локальним електростатичним впливом на параметри центрів рекомбінації на поверхні кремнію, що призводить до зміни швидкості поверхневої рекомбінації. Роботу датчика якісно можна пояснити в рамках теорії Стівенсона–Кієса.

Висновки. Наш підхід можна розглядати як перспективний шлях до розробки технічно простого та високочутливого методу виявлення кількісної поведінки ферментативних реакцій. Крім того, локальна модифікація поверхні кремнію дає змогу отримати залежні від часу сценарії адсорбції і таким чином підвищити чутливість сенсора. Ці обставини відкривають можливість розробки складних сенсорних структур, параметри яких оптимізовані для певних ферментативних реакцій.

Ключові слова: ферментативні реакції, принцип дії фотоелектричного перетворювача, поверхнева швидкість рекомбінації, біомедична діагностична технологія

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