Biol. Stud. 2024; 18(2): 21–32 • doi: https://doi.org/10.30970/sbi.1802.773
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 577.353

BIOPHYSICAL FEATURES OF USING A RECOMBINATION SENSOR TO DETECT LACTATE DEHYDROGENASE: SENSITIVITY MECHANISMS ANALYSIS

Oleksii Kozinetz*, *Bogdan Sus*, *Olga Tsymbalyuk*, *Sergii Litvinenko
Taras Shevchenko National University of Kyiv, 64/13 Volodymyrska St., Kyiv 01601, Ukraine

Kozinetz, O., Sus, B., Tsymbalyuk, O., & Litvinenko, S. (2024). Biophysical features of using a recombination sensor to detect lactate dehydrogenase: sensitivity mechanisms analysis. *Studia Biologica*, 18(2), 21–32. doi:10.30970/sbi.1802.773

Background. Most pathologies of the human body (in particular, malignant neoplasms, myocardial hypoxia, liver diseases, etc.) are accompanied by a violation of the integrity of cells in target tissues and the release of intracellular macromolecules into the extracellular environment. Thus, an important diagnostic and prognostic indicator is the level of activity of certain enzymes in blood serum, which are normally intracellular. One of the most promising areas of modern medical electronics and biophysics is the development and optimization of enzyme screening methods in biological fluids. In this study, we aimed to investigate the biophysical characteristics of using a recombination sensor for determining lactate dehydrogenase (LDH) activity in biological fluids.

Materials and Methods. Experiments were performed on preparations of standard human blood serum. The reference determination of LDH activity was carried out photometrically based on the change (decrease) in the concentration of the reduced form of the nicotinamide adenine dinucleotide (NADH) coenzyme. The passage of the LDH reaction was experimentally recorded by measuring the photocurrent of a silicon structure with a buried barrier under light irradiation from the region of strong absorption (λ = 532 nm).

Results. The biophysical features of the device were studied. The detection of lactate dehydrogenase becomes possible due to the transfer of a hydrogen ion from NADH to pyruvate, as a result of which lactate and NAD^+ are formed. The effect is explained by the local electrostatic influence on the parameters of the recombination centers in the near-surface bending zone near the silicon surface, which leads to a change in the surface recombination rate.

Conclusions. Our approach can be considered as a promising way to develop a highly sensitive method for the detection of LDH. It has been experimentally shown that effective detection is possible in two changes at the surface bending of the deep barrier silicon substrate zone.

Keywords: lactate dehydrogenase, enzymatic activity, deep silicon barrier, initial band bending, photoelectrical transducer, surface recombination velocity, biomedical diagnostic

INTRODUCTION

LDH is a constitutive tetrameric enzyme that facilitates the reversible conversion of pyruvate to lactate, concurrently converting the coenzyme NADH to NAD^+ (Guo *et al.*, 2021; Khan *et al.*, 2020). The activity of LDH is vital for sustaining the glycolysis and gluconeogenesis processes. There are four LDH genes: LDHA, LDHB, LDHC, and LDHD; the former three genes have been identified in vertebrates (Valvona *et al.*, 2016). The LDHA gene codes for the muscle-type subunit (referred to as LDH-M), while the LDHB gene codes for the heart-type subunit (referred to as LDH-H). These subunits, derived from the protein products LDHA and LDHB, combine to form versions ranging from homotetramers 4M and 4H to heterotetramers with varying numbers of M and H subunits, resulting in five isomeric types labeled by numbers: LDH-1 (containing all 4H subunits), LDH-2 (containing 3H and 1M subunits), LDH-3 (containing 2H and 2M subunits), LDH-4 (containing 1H and 3M subunits), and LDH-5 (containing all 4M). Enzymes with different combinations of M and H subunits exhibit varying enzymatic activity and preferred reaction direction (LDH-5 predominantly catalyzes the conversion of pyruvate to lactate, while LDH-1 catalyzes the opposite reaction) (Du *et al.*, 2022; Kanaoka & Minami, 2023; Valvona *et al.*, 2016).

It is now beyond doubt that changes in the expression of specific LDH isozyme types, as well as an increase in total LDH activity in biological samples (fluids and tissues of the organism), are important diagnostic indicators of many diseases, including solid tumors, autoimmune diseases, cardiovascular and cerebrovascular diseases, acute conditions, blood disorders, and kidney diseases (Wu *et al.*, 2021). In particular, the expression level of the LDHA gene in tissues is proportional to the demand for intensive lactate synthesis in glycolysis. LDHA is overexpressed in many types of malignant tumors, supplying the tumor with NAD^+ through accelerated glycolysis and causing a decrease in pH to facilitate more effective invasion and evasion of immune destruction (Feng *et al.*, 2018). Additionally, increased expression of LDHB contributes to tumor progression and the proliferation of its cells by elevating the concentration of H^+ and enhancing lysosomal function more effectively. Therefore, elevated levels of LDHB expression serve as a poor prognostic indicator for patients (Brisson *et al.*, 2016).

Since LDH appears in blood serum after cell integrity disruption and necrosis, the activity of this enzyme accurately reflects the level of cell damage and necrosis. A large body of data has now been accumulated indicating that changes in LDH activity in biological fluids are an important diagnostic indicator of the human body condition. In the case of malignant tumors, the level of LDH in patients' blood plasma indicates the effectiveness of treatment (Petrelli *et al.*, 2019; Van Wilpe *et al.*, 2020; Vlasiou *et al.*, 2023).

Classical methodological approaches to determining LDH activity include photometric measurement of changes in light absorption at a wavelength of 340 nm (the maximum absorption of NADH with a molar extinction coefficient of 6220 $M^{-1}cm^{-1}$) during alterations in the concentration of the reduced form of the NADH coenzyme (Vanderlinde, 1985; McNaught & Wilkinson, 1997). However, such determination of LDH activity requires laboratory personnel to strictly adhere to the physicochemical conditions under which the enzymatic reaction takes place, as well as the use of colorless samples for determination (Zhou *et al.*, 2022). Therefore, as alternative approaches to measuring

LDH activity in biological samples, colorimetric, fluorimetric, and electrochemical methods have now been developed based on determining the concentrations of NADH and NAD^+ (Vlasiou *et al.*, 2023).

Based on colorimetric approaches to measuring LDH activity, several biosensors on paper substrates have been developed. These biosensors are treated with reagents to facilitate the LDH reaction (Kannan *et al.*, 2015; Papaneophytou *et al.*, 2021). Alternatively, paper sensor substrates contain lactate, NAD^+, and iodonitrotetrazolium chloride. The latter reagent reacts with NADH, the reduced form of LDH serum, resulting in the formation of a formazan dye with a blue-violet color (λ = 570 nm) (Kannan *et al.*, 2015). Another set of reagents for the colorimetric sensor of LDH activity is a mixture of lactate, NAD^+, nitroblue tetrazolium, and phenazine methosulfate (Papaneophytou *et al.*, 2021). The advantage of such paper-based biosensors is their accessibility for household testing with the possibility of using mobile applications and smartphone cameras, while the disadvantage is their relatively low sensitivity and dependence on the storage conditions of the test strips (Zhou *et al.*, 2022a). Fluorimetric methods for determining LDH activity (and other enzymes that utilize NADH and NADPH coenzymes) are based on the ability of NADH to quench the fluorescence of quantum dots based on CdTe, CdSi, and CdS (Yang *et al.*, 2011; Zhou *et al.*, 2022b). However, these methods have low sensitivity, specificity, and lack in linear dependence of fluorescence on low concentrations of NADH.

Additionally, for determining LDH activity, electrochemical biosensors have been developed, which are based on cyclic voltammetry, differential pulse voltammetry, stripping voltammetry, alternating current voltammetry, polarimetry, square wave voltammetry, and linear sweep voltammetry methods (Atta *et al.*, 2017; Mutyala & Mathiyarasu, 2016; Zhou *et al.*, 2022a). As was reported in our previous works, the recombination photoelectric sensor can be used for real-time determination of transaminase activity in blood serum and ATPase suspension of plasma membranes (Kozinetz *et al.*, 2022; Kozinetz A *et al.*, 2023a). We also found that the application of this method is possible for recording LDH activity in human serum samples (Kozinetz *et al.*, 2023b). Therefore, in this study, we aimed to investigate the biophysical characteristics of using a recombination sensor for determining LDH activity in biological fluids.

MATERIALS AND METHODS

For all studies, a preparation of normal human blood serum (Sigma, USA) was used. LDH activity was determined in an incubation medium preheated to 25 °C with the following composition: substrate-buffer solution (mM/L): 1 NADH (stabilized with NaOH), 1.5 pyruvate, 62.5 6.25 EDTA, and Tris buffer (pH 7.4)) 2 mL, blood serum 40 μL.

To study the use of a recombination sensor for determining LDH activity, we made a series of silicon structures with deep junction barriers. These substrates had a specific resistance of 50 ohms, were *p*-type, 100-oriented. The sensor surface was illuminated with a signal LED emitting light with a wavelength of λ = 550 nm and a power of 10 mW. Additionally, the LED featured amplitude modulation at a frequency of f = 976 Hz providing an alternating photocurrent signal in low-level injection mode. For additional parametric illumination, we utilized another blue LED with a wavelength of λ = 470 nm and a power of 50 mW. During a 1-second interval, the power of this LED increased linearly to its maximum, and then decreased linearly to zero. This experimental setup ensured a quasi-static mode of additional constant illumination with a high level of carrier injection.

An amplifier with a selective voltmeter tuned to a frequency f was used to obtain the amplitude of the photocurrent.

Under in vitro conditions, the dissociation of NADH coenzyme molecules from the active center of LDH leads to the acquisition of a net positive charge of approximately $1.77 \cdot 10^{17}$ units by the mixture in the sensor cell. Consequently, in the final phase of the reaction, a transition is made from a net negative charge to a net positive charge. It can be inferred that throughout the reaction, there is an increase in positive charge within the system of reagents that comes into contact with the sensor surface.

The reference method was also used to estimate LDH activity – the kinetics of photometrically determined decrease in NADH concentration when the reaction of NADH oxidation takes place and pyruvate is converted to lactate. The samples were incubated for 1 min at 25 °C, then the extinction of the solution was recorded (at a wavelength of 340 nm) using a spectrophotometer SPECORD M 40 (Germany) every 1 min for 3 min. For each sample, the average value of the change in extinction in 1 min was calculated. Data were processed using the Origin2018 program. Consequently, it was established that LDH activity in the blood plasma consists of 2.58 ± 0.09 μkat/L (154.9 ± 5.4 units/L) for our experiments, with a sample size of n = 9.

RESULTS AND DISCUSSION

The studies by S. Litvinenko *et al*. (2015) and O. Kozinetz *et al*. (2022, 2023a, 2023b, 2024) introduce a theoretical model for calculating the amplitude of alternating photocurrent based on the effective charge absorbed on the sensor structure's surface. This model facilitates the establishment of a correlation between photocurrent amplitude, recombination rate, and surface band bending. Additionally, the study explores the mechanisms of effective charge formation near the surface. When the sensor surface (SiO_x/Si) interacts with an analyte solution containing molecules that possess inherent dipole moments, specific adsorption of ions with distinct charges and the formation of a Helmholtz layer become feasible. As the distance from the surface increases, the analyte tends to be neutrally charged on average. Schematically, the operating principle of the recombination sensor is illustrated in **Fig. 1**. Specific adsorption alters band bending and carrier concentrations, thereby changing the rate of surface recombination. Silicon structures with deep barriers enable optimal detection of these changes through variations in photocurrent when the surface is strongly illuminated. In our series of experiments, identical silicon substrates were utilized, with strict adherence to the order, application conditions, and reagent combinations. It is worth noting that we observed both increases and decreases in the photocurrent amplitude. This trend persisted even when additional constant illumination was applied to further influence the surface recombination parameters. Taking into account the constant magnitude of the effective positive charge due to the transfer of a hydride ion from NADH to the C_2 atom of pyruvate, which results in the production of lactate and NAD^+, it can be assumed that an increase or decrease in photocurrent amplitude depends on the initial band bending Y_{s1}. Let us consider this assumption in more detail. The surface of the silicon sensor is always covered with a non-stoichiometric oxide SiO_x with a thickness of 10–40 Å.

The initial band bending is a parameter that may depend on the embedded ions in the SiO_x film and the capture of surface states at the phase boundaries. If the initial band bending, Y_{s1}, is close to zero, then in the case of specific adsorption of positive charge, the surface region transitions to the regime of intrinsic conductivity or depletion, Y_{s2}, and the photocurrent amplitude i_{ph} decreases (**Fig. 1*A*,*C***).

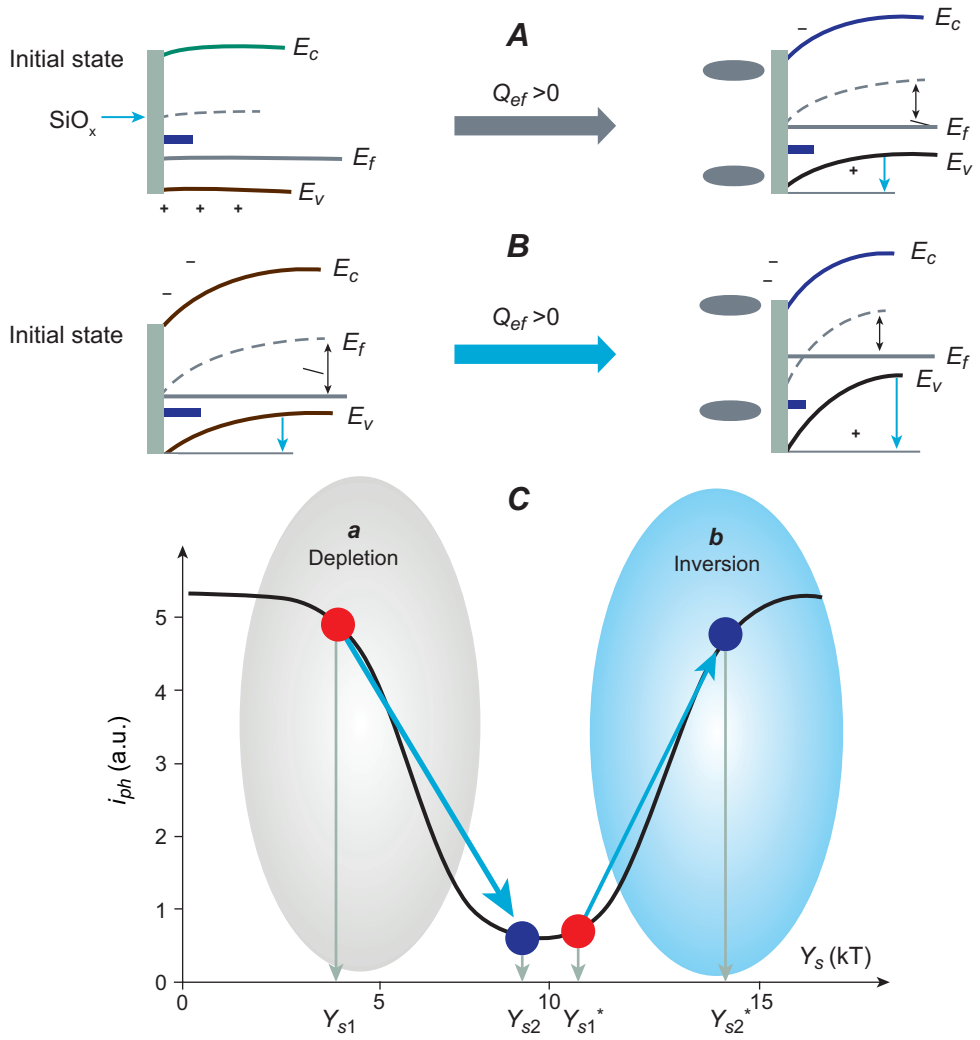

Fig. 1. Simplified band bending diagrams, illustrating positive charge adsorption processes for the surface transitions to the regime of intrinsic conductivity or depletion (***A***); simplified band bending diagrams, illustrating positive charge desorption processes for the surface transitions to the inversion regime (***B***); dependency of photo-current amplitude i_{ph} on surface band bending Y_s, depicting sensor performance in the indicated regions (band bending change from Y_{s1} to Y_{s2} or from Y_{s1}^* to Y_{s2}^*) (***C***)

If the initial band bending corresponds to the regime of intrinsic conductivity Y_{s1}^*, then in the case of specific adsorption of positive charge, the surface region transitions to the inversion regime, Y_{s2}^*, and the photocurrent amplitude i_{ph} may increase through the transition (**Fig. 1*B*,*C***).

Let us qualitatively analyze the sensitivity regions of the sensor structure. **Fig. 2** shows the results of theoretical calculations of the photo-current amplitudes i_{ph} as a function of surface band bending Y_s. These curves correspond to different light absorption coefficients in silicon in the range of 10^3–$5{\cdot}10^6$ cm^{-1}. Typical parameters of the structure were used for the calculations: the concentration of majority carriers $p_0 = 10^{15}$ cm^{-3}

(n_0 = 10^4 cm^{-3}), diffusion length l = 200 μm, thickness of the wafer d = 250 μm, electron diffusion coefficient D_n = 40 cm^2/s, capture coefficients of carriers at the surface level $c_n = c_p = 10^{-9}$ cm^{-2}, energy position of the recombination level relative to the middle of the forbidden band E_{ti} = 3 kT, concentration of surface states $N_t = 10^{13}$ cm^{-2}.The dependence between surface and volume concentrations can be calculated as: $n_s = n_0 \exp(Y_s/kT)$ and $p_s = p_0 \exp(-Y_s/kT)$. The recombination rate at the interface through a simple local center within the framework of Stevenson's theory is expressed by:

$$S(Y_s) = \frac{c_p c_n N_t (p_0 + n_0)}{c_n \left(n_s(Y_s) + n_i \exp\left(\frac{E_{ti}}{kT}\right)\right) + c_p \left(p_s(Y_s) + n_i \exp\left(\frac{E_{ti}}{kT}\right)\right)}. \quad (1)$$

It should be noted that the dependence of the surface recombination rate on the band bending (1) reaches a maximum in the case where concentration values n_s and p_s are close to one another. It also has intervals of a recession for Y_s corresponding to the depletion or inversion regimes. Naturally, the shape of this curve mainly determines the dependence of the photocurrent amplitude on a band bending Y_s. Let us analyze the influence of other factors on the photocurrent amplitude.

The parameter of the wavelength of modulated illumination λ determines the absorption magnitude α(λ). Therefore, the absorption magnitude α(λ), can be experimentally selected by simply choosing the signal light-emitting diode. If the frequency f is less than 2–3 kHz (Kozinetz *et al.*, 2023) and the intensity of the signal illumination is determined by law

$$i(t) \cong A(1 + \sin 2\pi f t) \quad (2)$$

the photocurrent through the sensor structure expresses as

$$i(t) \cong i_{ph} A(1 + \sin(2\pi f t + \varphi)) = A \frac{1 + \frac{S(Y_s)}{\alpha(\lambda) D}}{S(Y_s)\frac{l}{D} sh\left(\frac{d}{l}\right) + ch\left(\frac{d}{l}\right)} (1 + \sin(2\pi f t + \varphi)). \quad (3)$$

The phase shift φ in (3) between instantaneous values of the intensity and the photocurrent depends on the frequency and the diffusion length in a complex way.

As can be noted from **Fig. 2**, the most significant changes in the photocurrent amplitude and correspondingly the highest sensitivity $\Delta i_{ph}/\Delta Y_s$ can occur in the region of the left arm (depletion or intrinsic conductivity regime) or the right arm (inversion). Between these regions, there exists a range of Y_s values for which the photocurrent amplitude remains unchanged, and detection becomes impossible, i.e., $\Delta i_{ph}/\Delta Y_s = 0$. Our theoretical analysis shows that as the absorption coefficient α(λ) increases (correspondingly 1/α(λ) decreases – photons are absorbed closer to the surface), the range of Y_s values that form a kind of "blind zone" for the sensor decreases. It should be noted that simultaneously with the increase in α(λ), there is also an increase in the minimum value of i_{ph}. From the perspective of reducing the range of the "blind zone" Y_s, it is optimal to use light with a sufficiently high absorption coefficient (on the order of α(λ) ~ $5 \cdot 10^5$ cm^{-1} λ ~ 600 nm).

In **Figures 3*A*** and ***B***, typical experimental dependencies of the photocurrent on time are depicted for cases where the transfer of a hydride ion from NADH to pyruvate occurs on the sensor surface. The LDH enzyme was added at time t = 600 s (red arrow). In the region of t > 600 s after the reaction activation, we observed two scenarios of signal

change: 1) a decrease in the photocurrent amplitude from 600 to 400 a.u. for the case in **Fig. 3*A*** and 2) an increase in the photocurrent from 300 to 600 a.u. for the case in **Fig. 3*B***. The first scenario can be qualitatively described using the diagrams in **Fig. 1*A***, and the second one accordingly using the diagrams in **Fig. 1*B***. It is worth emphasizing that in both cases, the sensor provides the necessary qualitative detection of LDH activity.

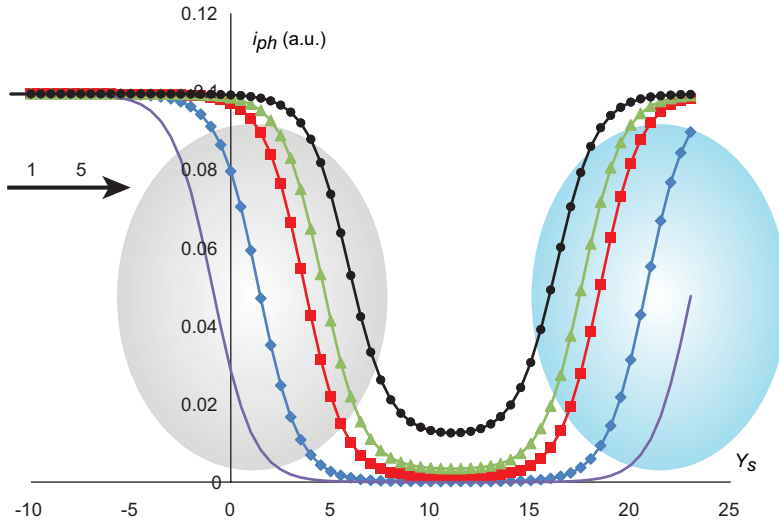

Fig. 2. Theoretical dependencies of the photocurrent amplitude i_{ph} on the surface band bending Y_s for different values of the absorption ratio α(λ) in silicon: curve 1 corresponds to α(λ) = 10^3 cm^{-1}, curve 2 – 10^4 cm^{-1}, curve 3 – 10^5 cm^{-1}, curve 4 – 10^6 cm^{-1}, curve 5 – 3 10^6 cm^{-1}, the black arrow indicates an increase in the absorption ratio in silicon. The left arms (depletion or intrinsic conductivity regime) are marked in gray. The right arms (inversion) are marked in blue

It is more challenging, from the perspective of surface physics, to analyze the impact of applying additional constant illumination. The imposition of constant illumination periodically reduces the band bending and consequently affects the filling of the surface states system. We assume that such an approach will allow the real-time detection of certain features of changes in the effective charge in the Helmholtz layer, as these factors determine the rate of recombination at the interface.

For the case of transition to depletion mode, the oscillation amplitude decreases, as shown in **Fig. 3*A*** ($\Delta 1 > \Delta 2$). This provides additional information about the reaction and determines the LDH activity. If Q_{ef} near the surface increases for t > 600 s, such inequality indicates complex processes in the interface (Kozinetz *et al.*, 2024). Indeed, let us assume that as a result of adsorption acceptor-type levels emerge at the SiO_x/Si boundary. As the light intensity gradually increases, the energy position of these levels changes according to the displacement of the band edges on the surface (the Fermi level in the bulk remains unchanged). As a result, the centers become negatively charged. This effect will obviously counteract the increase in band bending. Consequently, the values of $\Delta 2$ decrease. For t > 850 s the amplitude of oscillations clearly demonstrates the tendency for saturation. Another factor may be, for example, the appearance of new adhesion levels and recombination channels at the interface during adsorption. Moreover, the direct electrostatic influence of the positive charge Q_{ef} on the ratio of capture coefficients is possible.

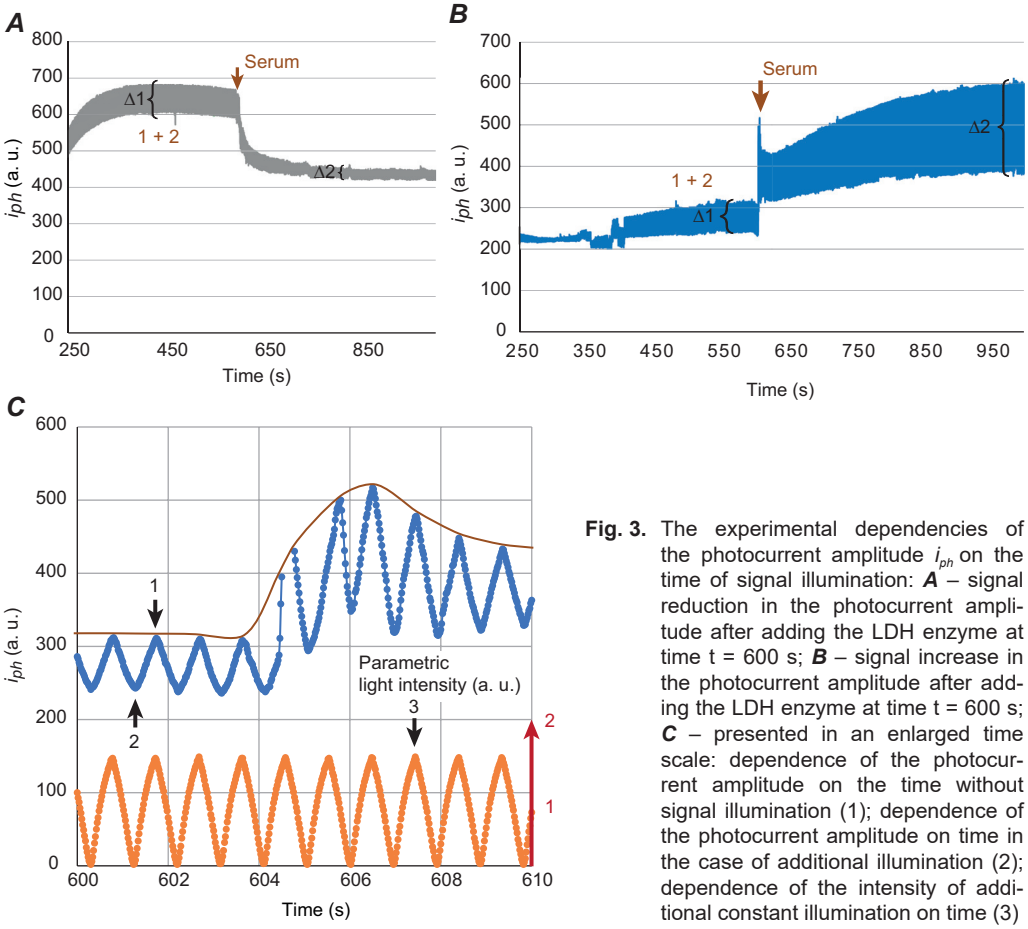

Fig. 3. The experimental dependencies of the photocurrent amplitude i_{ph} on the time of signal illumination: ***A*** – signal reduction in the photocurrent amplitude after adding the LDH enzyme at time t = 600 s; ***B*** – signal increase in the photocurrent amplitude after adding the LDH enzyme at time t = 600 s; ***C*** – presented in an enlarged time scale: dependence of the photocurrent amplitude on the time without signal illumination (1); dependence of the photocurrent amplitude on time in the case of additional illumination (2); dependence of the intensity of additional constant illumination on time (3)

For the case of transitioning to the inversion mode in **Fig. 3*B*** ($\Delta 1 < \Delta 2$), the oscillation amplitude increases after the reagents are combined. It is noteworthy that during the time intervals from 650 to 900 s, the amplitude $\Delta 2$ gradually increases and stabilizes. If the effective positive charges Q_{ef} in the Helmholtz layer near the surface increase, Y_{s2} will also gradually increase in the region of the right arm. For $t > 900$ s the amplitudes of oscillation demonstrate the tendency for saturation. The imposition of constant illumination, in a sense, is equivalent to applying a direct voltage to the floating junction. In other words, from the inequality $Y_{s1} < Y_{s2}$, it follows that $\Delta 1 < \Delta 2$. Unlike the previous case, changes in the parameters of the adhesion levels at the SiO_x/Si interface are not observed. The **Fig. 3*C*** shows that quasi constant regime of parametric illumination is maintained.

The imposition of constant illumination thus provides additional information for determining the activity of LDH; however, the analysis of sensitivity mechanisms is more complex and ambiguous.

CONCLUSIONS

We have demonstrated and theoretically analyzed the biophysical properties of employing a recombination sensor for the determination of LDH activity in biological

fluids. Our findings indicate that the directly measured analytical parameter is the photocurrent of the deep silicon barrier structure under modulated illumination. Our analysis emphasizes the necessity of a high absorption coefficient to establish the connection between surface processes and photocurrent through the p/n-junction.

The experimental study highlights the pivotal role of LDH catalysis in enabling the transfer of a hydride ion from NADH to pyruvate's C_2 atom, ultimately yielding lactate and NAD^+. The observed transition in charge states of the reactants, from negative to positive, underscores the predictability of this phenomenon through an understanding of reagent dissociation constants. These alterations directly impact the parameters of recombination centers at the interface, underscoring the intricate relationship between LDH activity and surface processes. This comprehensive analysis sheds light on the underlying biophysical mechanisms governing LDH function and offers valuable insights for the development of advanced sensor technologies in biomedical applications. The operation of the sensor is qualitatively explained within the framework of Stevenson's theory and the formation of Helmholtz layers in polar analytes. Utilizing theoretical approximations, the study demonstrated that effective detection is achievable in two regions of surface band bending change. Experimental demonstrations indicate that detection outcomes may result in either a decrease or increase in photocurrent amplitude, corresponding to these regions. Significantly, the selection of parameters for variable sensor illumination allows for partial control of sensitivity in the working regions of band bending change. A more intricate factor in sensitivity analysis is the influence of carrier capture on adhesion centers and potential changes in capture cross-sections or energy positions of recombination centers due to adsorption. The application of additional illumination aids in improving sensitivity in working regions of band bending and enhances the probability of accurate detection.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Science and Education of Ukraine, Grant 0122U001697 "The creation of a multi-sensor system based on a highly efficient recombination transducer for general analyzes and screening metabolites" and by "Ultrasmall nanohybrides for advanced theranostics (UNAT) project" Grant agreement ID: 101008159, Funded under EXCELLENT SCIENCE – Marie Skłodowska-Curie Actions.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

Human Rights. This article does not contain any studies with human subjects performed by any of the authors.

Animal Studies. This article does not contain any studies with laboratory animals.

AUTHOR CONTRIBUTIONS

Conceptualization, [K.O.; L.S.; O.T.; S.B.]; methodology, [K.O.; L.S.; O.T.; S.B.]; research, [K.O.; L.S.; O.T.; S.B.]; resources, [K.O., L.S, O.T., S.B.]; data processing, [K.O., L.S, O.T., S.B.]; writing – preparation of the original project, [L.S.]; writing – review and editing, [K.O.; L.S.; O.T.; S.B.]; visualization, [K.O.] supervision, [L.S.]; project management, [L.S.; K.O.]; funding search, [–].

All authors have read and agreed to the published version of the manuscript.

REFERENCES

Atta, N. F., Abdel Gawad, S. A., El-Ads, E. H., El-Gohary, A. R. M., & Galal, A. (2017). A new strategy for NADH sensing using ionic liquid crystals-carbon nanotubes/nano-magnetite composite platform. *Sensors and Actuators B: Chemical*, 251, 65–73. doi:10.1016/j.snb.2017.05.026
Crossref ● Google Scholar

Brisson, L., Bański, P., Sboarina, M., Dethier, C., Danhier, P., Fontenille, M.-J., Van Hée, V. F., Vazeille, T., Tardy, M., Falces, J., Bouzin, C., Porporato, P. E., Frédérick, R., Michiels, C., Copetti, T., & Sonveaux, P. (2016). Lactate dehydrogenase B controls lysosome activity and autophagy in cancer. *Cancer Cell*, 30(3), 418–431. doi:10.1016/j.ccell.2016.08.005
Crossref ● PubMed ● Google Scholar

Du, M., Yu, T., Zhan, Q., Li, H., Zou, Y., Geng, M., Meng, T., & Xie, Z. (2022). Development of a novel lactate dehydrogenase A inhibitor with potent antitumor activity and immune activation. *Cancer Science*, 113(9), 2974–2985. doi:10.1111/cas.15468
Crossref ● PubMed ● PMC ● Google Scholar

Feng, Y., Xiong, Y., Qiao, T., Li, X., Jia, L., & Han, Y. (2018). Lactate dehydrogenase A: a key player in carcinogenesis and potential target in cancer therapy. *Cancer Medicine*, 7(12), 6124–6136. doi:10.1002/cam4.1820
Crossref ● PubMed ● PMC ● Google Scholar

Guo, Q., Zhu, Z., Wang, J., Huang, W., Zhang, C., Zeng, J., Zhao, H., Qi, T., Zhou, W., Zhang, T., Zhang, C., & Xiao, F. (2021). Preparation, stability and commutability of candidate reference materials for lactate dehydrogenase (LDH). *Clinical Biochemistry*, 91, 45–51. doi:10.1016/j.clinbiochem.2021.02.002
Crossref ● PubMed ● Google Scholar

Kanaoka, K., & Minami, S. (2023). Elevation of serum lactate dehydrogenase during methylprednisolone pulse therapy as a predictor of high mortality in acute respiratory failure: a single-center, retrospective study. *SAGE Open Medicine*, 11, 20503121231195993. doi:10.1177/20503121231195993
Crossref ● PubMed ● PMC ● Google Scholar

Kannan, B., Jahanshahi-Anbuhi, S., Pelton, R. H., Li, Y., Filipe, C. D., & Brennan, J. D. (2015). Printed paper sensors for serum lactate dehydrogenase using pullulan-based inks to immobilize reagents. *Analytical Chemistry*, 87(18), 9288–9293. doi:10.1021/acs.analchem.5b01923
Crossref ● PubMed ● Google Scholar

Khan, A. A., Allemailem, K. S., Alhumaydhi, F. A., Gowder, S. J. T., & Rahmani, A. H. (2020). The biochemical and clinical perspectives of lactate dehydrogenase: an enzyme of active metabolism. *Endocrine, Metabolic & Immune Disorders – Drug Targets*, 20(6), 855–868. doi:10.2174/1871530320666191230141110
Crossref ● PubMed ● Google Scholar

Kozinetz, A., Tsymbalyuk, O., & Litvinenko, S. (2023). Application of sensor structures based on a photoelectric transducer to determine the activity of aspartate and alanine aminotransferases in blood plasma. *Biomedical Physics & Engineering Express*, 9(4), 10.1088/2057-1976/acd55b. doi:10.1088/2057-1976/acd55b
Crossref ● PubMed ● Google Scholar

Kozinetz, A., Tsymbalyuk, O., & Litvinenko, S. (2022). The first application of sensory structures based on photoelectric transducer for the study of enzymatic reactions. *Studia Biologica*, 16(4), 3–18. doi:10.30970/sbi.1604.698
Crossref ● Google Scholar

Kozinetz, A., Tsymbalyuk, O., & Litvinenko, S. (2023). Application of sensor structures based on a photoelectric transducer to determin(e the activity of aspartate and alanine aminotransferases in blood plasma. *Biomedical Physics & Engineering Express*, 9(4), 045016. doi:10.1088/2057-1976/acd55b
Crossref ● PubMed ● Google Scholar

Kozinetz, A., Sus, B., Tsymbalyuk, O., & Litvinenko, S. (2024). Photovoltaic recombination sensor as system for real-time determination of lactate dehydrogenase activity. *Sensing and Bio-Sensing Research*, 43, 100620. doi:10.1016/j.sbsr.2024.100620
Crossref ● Google Scholar

Litvinenko, S. V., Kozinetz, A. V., & Skryshevsky, V. A. (2015). Concept of photovoltaic transducer on a base of modified p–n junction solar cell. *Sensors and Actuators A: Physical*, 224, 30–35. doi:10.1016/j.sna.2015.01.014
Crossref ● Google Scholar

McNaught, A. D., & Wilkinson, A. (1997). *Compendium of chemical terminology.* Oxford: Blackwell Science.
Google Scholar

Mutyala, S., & Mathiyarasu, J. (2016). A highly sensitive NADH biosensor using nitrogen doped graphene modified electrodes. *Journal of Electroanalytical Chemistry*, 775, 329–336. doi:10.1016/j.jelechem.2016.06.011
Crossref ● Google Scholar

Papaneophytou, C., Zervou, M.-E., & Theofanous, A. (2021). Optimization of a colorimetric assay to determine lactate dehydrogenase B activity using design of experiments. *SLAS Discovery*, 26(3), 383–399. doi:10.1177/2472555220956589
Crossref ● PubMed ● Google Scholar

Petrelli, F., Ardito, R., Merelli, B., Lonati, V., Cabiddu, M., Seghezzi, S., Barni, S., & Ghidini, A. (2019). Prognostic and predictive role of elevated lactate dehydrogenase in patients with melanoma treated with immunotherapy and BRAF inhibitors: a systematic review and meta-analysis. *Melanoma Research*, 29(1), 1–12. doi:10.1097/CMR.0000000000000520
Crossref ● PubMed ● Google Scholar

Valvona, C. J., Fillmore, H. L., Nunn, P. B., & Pilkington, G. J. (2016). The regulation and function of lactate dehydrogenase A: therapeutic potential in brain tumor. *Brain Pathology*, 26(1), 3–17. doi:10.1111/bpa.12299
Crossref ● PubMed ● PMC ● Google Scholar

Van Wilpe, S., Koornstra, R., Den Brok, M., De Groot, J. W., Blank, C., De Vries, J., Gerritsen, W., & Mehra, N. (2020). Lactate dehydrogenase: a marker of diminished antitumor immunity. *Oncoimmunology*, 9(1), 1731942. doi:10.1080/2162402X.2020.1731942
Crossref ● PubMed ● PMC ● Google Scholar

Vanderlinde, R. E. (1985). Measurement of total lactate dehydrogenase activity. *Annals of Clinical & Laboratory Science*, 15(1), 13–31.
Google Scholar

Vlasiou, M., Nicolaidou, V., & Papaneophytou, C. (2023). Targeting lactate dehydrogenase-B as a strategy to fight cancer: identification of potential inhibitors by *in silico* analysis and *in vitro* screening. *Pharmaceutics*, 15(10), 2411. doi:10.3390/pharmaceutics15102411
Crossref ● PubMed ● PMC ● Google Scholar

Wu, Y., Lu, C., Pan, N., Zhang, M., An, Y., Xu, M., Zhang, L., Guo, Y., & Tan, L. (2021). Serum lactate dehydrogenase activities as systems biomarkers for 48 types of human diseases. *Scientific Reports*, 11(1), 12997. doi:10.1038/s41598-021-92430-6
Crossref ● PubMed ● PMC ● Google Scholar

Yang, L., Ren, X., Meng, X., Li, H., & Tang, F. (2011). Optical analysis of lactate dehydrogenase and glucose by CdTe quantum dots and their dual simultaneous detection. *Biosensors & Bioelectronics*, 26(8), 3488–3493. doi:10.1016/j.bios.2011.01.031
Crossref ● PubMed ● Google Scholar

Zhou, Y., Qi, M., & Yang, M. (2022a). Current status and future perspectives of lactate dehydrogenase detection and medical implications: a review. *Biosensors*, 12(12), 1145. doi:10.3390/bios12121145
Crossref ● PubMed ● PMC ● Google Scholar

Zhou, Y., Qi, M., & Yang, M. (2022b). Fluorescence determination of lactate dehydrogenase activity based on silicon quantum dots. *Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy*, 268, 120697. doi:10.1016/j.saa.2021.120697
Crossref ● PubMed ● Google Scholar

БІОФІЗИЧНІ ОСОБЛИВОСТІ ВИКОРИСТАННЯ РЕКОМБІНАНТНОГО СЕНСОРА ДЛЯ ВИЯВЛЕННЯ ЛАКТАТДЕГІДРОГЕНАЗИ: АНАЛІЗ МЕХАНІЗМІВ ЧУТЛИВОСТІ

Олексій Козинець, Богдан Сусь, Ольга Цимбалюк, Сергій Литвиненко

Київський національний університет імені Тараса Шевченка
вул. Володимирська, 64/13, Київ 01601, Україна

Обґрунтування. Більшість патологій організму людини (зокрема, злоякісні новоутворення, гіпоксія міокарда, захворювання печінки тощо) супроводжуються порушенням цілісності клітин у тканинах-мішенях і вивільненням внутрішньоклітинних макромолекул у позаклітинне середовище. Отже, важливим діагностичним і прогностичним показником є рівень активності окремих ензимів у сироватці крові, які в нормі є внутрішньоклітинними. Одним із найактуальніших напрямів сучасної медичної електроніки і біофізики є розробка й оптимізація методів скринінгу ензимів у біологічних рідинах. Метою роботи було вивчити біофізичні особливості застосування рекомбінаційного сенсора для визначення активності важливого ензиму в діагностиці методами медичної біохімії – лактат дегідрогенази.

Матеріали та методи. Експерименти виконували на препаратах стандартної сироватки крові людини. Референтне визначення активності лактатдегідрогенази здійснювали фотометрично за зміною (зменшенням) концентрації відновленої форми коензиму НАДН. Експериментально здійснювали реєстрацію проходження лактатдегідрогеназної реакції за вимірюванням фотоструму кремнієвої структури з заглибленим бар'єром в умовах опромінювання світлом із ділянки сильного поглинання (λ = 532 нм).

Результати. Досліджено біофізичні особливості роботи пристрою. Виявлення лактатдегідрогенази стає можливим завдяки перенесенню іона водню від нікотинамідаденіндинуклеотиду (НАДН) до пірувату, внаслідок чого утворюються лактат і $НАД^+$. Ефект пояснюють локальним електростатичним впливом на параметри центрів рекомбінації на приповерхневому вигині зони поблизу поверхні кремнію, що призводить до зміни швидкості поверхневої рекомбінації.

Висновки. Наш підхід можна розглядати як перспективний спосіб розробки високочутливого методу виявлення лактатдегідрогенази. Експериментально доведено, що ефективна детекція можлива у двох змінах приповерхневому вигині зони кремнієвої підкладки заглибленого бар'єру.

Ключові слова: лактатдегідрогеназа, ферментативна активність, заглиблений кремнієвий бар'єр, початковий вигин зон, фотоелектричний перетворювач, поверхнева швидкість рекомбінації, біомедична діагностика

Received / Одержано	Revision / Доопрацьовано	Accepted / Прийнято	Published / Опубліковано
25 March, 2024	17 May, 2024	05 June, 2024	26 June, 2024