UDC: 543.556, 577.15, 621.314 https://doi.org/10.15407/biotech17.02.018

DEVELOPMENT OF CREATININE-SENSITIVE BIOSENSOR BASED ON IMMOBILIZED CREATININE DEIMINASE

V.A. BAKHMAT[1, 2], *O.O. SOLDATKIN*[1, 3], *V.M. ARKHYPOVA*[1], *S.V. DZYADEVYCH*

[1]Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine, Kyiv
[2]Taras Shevchenko National University of Kyiv, Ukraine
[3]National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"

E-mail: veronikab2406@gmail.com

Received 2024/03/29
Revised 2024/04/11
Accepted 2024/04/30

Creatinine, the end product of protein breakdown in animals and humans bodies, is synthesized within muscle tissue and subsequently released into the bloodstream. Its concentration in serum serves as a pivotal indicator of renal function due to its facile measurement as a byproduct of muscular metabolism, primarily eliminated by the kidneys through glomerular filtration [1]. Creatinine is the most widely used functional biomarker of kidney health. Beyond its role in steady-state and chronic kidney disease evaluation, creatinine holds significance as a primary criterion in defining acute kidney injury [2].

Various chemical methods are commonly used to assess patients' conditions by measuring creatinine levels. These methods have inherent limitations, including insufficient sensitivity and low specificity [3]. Additionally, instrumental techniques are utilized for creatinine determination, such as high-performance liquid chromatography, ion chromatography, capillary zone electrophoresis, and others [4–6]. Nevertheless, all these methods are characterized by significant complexities, resource requirements, and incapacity to conduct real-time creatinine analysis.

There is considerable interest in the development of biosensor-based express methods for creatinine determination. Biosensors characterize their high sensitivity and selectivity; speed of analysis; ease of use; possibility of miniaturization and high level of integration; low cost of analysis and instrument.

Aim. This work was aimed at developing a new design of an enzyme biosensor for highly sensitive determination of creatinine.

Methods. The electrochemical transducers consist of two identical pairs of gold interdigitated electrodes obtained by gold sputtering onto ceramic plate. The sensitive surface of each electrode pair was approximately 1.0×1.5 mm. The transducers were connected to the measuring device "MXP-3".

The immobilization procedure was performed as follows. The enzyme solution was prepared by dissolving 20% creatinine deiminase (microbial, EC 3.5.4.21, with activity of 36 U/mg, from Sigma-Aldrich, Japan) in 20 mM phosphate buffer solution (PBS), pH 6.5, containing 10% bovine serum albumin (BSA) and 10% glycerol. The mixture for reference membrane was prepared by the same procedure using BSA instead of enzymes. The solutions were mixed with 1% aqueous solution of glutaraldehyde (GA) in 1:1 ratio and were deposited on the electrodes. Then the membranes were dried in open air at room temperature. Before starting the experiments, the electrodes with membranes were washed out excess of unbound components used PBS.

Measurements were carried out at room temperature in an open cell filled with 5 mM PBS, pH 7.35, with constant stirring. The required substrate concentration in the cell was obtained by adding the aliquots of substrates stock solutions in the cell with PBS.

Non-specific changes in the output signal associated with fluctuations of temperature, medium pH, and electrical noise, were compensated by using differential mode of measurement. At least three series of experiments were performed.

Results and Discussion. Various immobilization procedures of the bioselective element were tested. Immobilization method with 1% GA solution was chosen. The optimal conditions for biosensors operation were found. The biosensors were characterized by high signal reproducibility at determination of substrate (RSD = 6%). The linear range of determination of creatinine was 0.01–1 mM. A calibration curve for creatinine determination showed on Figure. The biosensor was characterized by a minimal detection limit of 5 μM creatinine, which is better than known analogues (20 μM and 50 μM) [7, 8]. Other biosensors with lower detection limit use complex and expensive modifications, such as nanoparticles [9].

The sensitivity of biosensor retained 83% of the initial response value after a 60 days of storage in dry condition at +4 °C that enabled us to store this biosensor about one month without essential decreasing of it sensitivity.

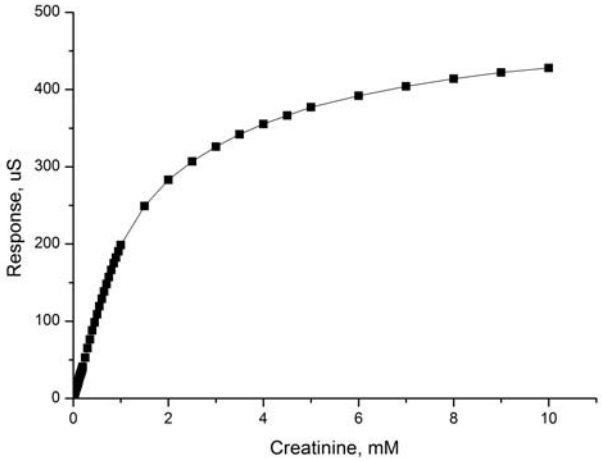

Fig. **Calibration curve of the biosensor based on creatinine deiminase. Measurements were carried out in 5 mM PBS, pH 7.35**

Conclusions. The new construction of enzyme biosensor based on creatinine deiminase was developed for the creatinine determination. The production and measurement conditions of proposed biosensor were optimized. The biosensor was characterized by high reproducibility of responses and showed high storage stability. In future the developed biosensor can be used for express evaluation of the creatinine in biological samples.

Key words: biosensor, creatinine, creatinine deiminase, creatinine enzymatic assay.

Authors' contribution. VAB performed experiments, data analysis and presentation, OOS was aiming for data analysis, VMA advised on work with the enzyme and preparation of bioselective membranes, SVD managed the project, developed a plan of experiments.

Funding source. The work was particularly supported by the National Research Foundation of Ukraine in the framework of the competition of projects for research and development "Science for security and sustainable development of Ukraine" (Project 2021.01/0010).

REFERENCES

1. *Narayanan S., Appleton H. D.* Creatinine: a review. Clinical chemistry, 1980, 26(8):1119–1126. https://doi.org/10.1093/clinchem/26.8.1119
2. *Kashani K., Rosner M.H., Ostermann M.* Creatinine: from physiology to clinical application. European journal of internal medicine, 2020, 72: 9–14. https://doi.org/10.1016/j.ejim.2019.10.025
3. *Zakalskiy A., Stasyuk N., Gonchar M.*, Creatinine deiminase: characterization, using in enzymatic creatinine assay, and production of the enzyme. *Curr. Protein Pept. Sci.*, 2019, 20(5): 465–470. https://doi.org/10.2174/1389203720666181114111731
4. *Durán I., Espinosa-Mansilla A., Rodriguez Gómes M.J.* Determination of methotrexate, several pteridines, and creatinine in human urine, previous oxidation with potassium permanganate, using

HPLC with photometric and fluorimetric serial detection. *Anal. Biochem.*, 2005, 346(2): 201–209. https://doi.org/10.1016/j.ab.2005.07.038
5. *Yokoyama Y., Tsuji S., Sato H.* Simultaneous determination of creatinine, creatine, and UV-absorbing amino acids using dualmode gradient low-capacitycation-exchange chromatography. *J. Chromatogr. A.*, 2005, 1085(1): 110–116. https://doi.org/10.1016/j.chroma.2005.01.030
6. *Zinellu A., Caria M.A., Tavera C., Sotgia S., Chessa R., Deiana, L., Carru C.* Plasma creatinine and creatine quantification by capillary electrophoresis diode array detector. *Anal. Biochem.*, 2005, 342(2): 186–193. https://doi.org/10.1016/j.ab.2005.01.045
7. *Marchenko S., Kucherenko I., Soldatkin O., Soldatkin,* A. Potentiometric biosensor system based on recombinant urease and creatinine deiminase for urea and creatinine determination in blood dialysate and serum. *Electroanalysis*, 2015, 27(7): 1699–1706. https://doi.org/10.1002/elan.201400664
8. *Busono, P.* (2015). Development of amperometric biosensor for creatinine detection. In 7th WACBE World Congress on Bioengineering 2015: 6th to 8th July, 2015, Singapore. *Springer, Cham*. 2015, 52: 134–137. https://doi.org/10.1007/978-3-319-19452-3_36
9. *Stasyuk N., Zakalskiy A.,. Nogala W, Gawinkowski S., Ratajczyk T., Bonarowska M., Demkiv O., Zakalska O., Gonchar M.* A reagentless amperometric biosensor for creatinine assay based ons recombinant creatinine deiminase and N-methylhydantoin-sensitive CoCu nanocomposite. *Sensors Actuators B,* 2023, 393: 134276. https://doi.org/10.1016/j.snb.2023.134276