REGISTRATION OF ACTIVITY OF A SINGLE MOLECULE OF HORSERADISH PEROXIDASE USING A DETECTOR BASED ON A SOLID-STATE NANOPORE

Yu.D. Ivanov[1], A.N. Ableev[1], A.V. Vinogradova[1], E.D. Nevedrova[1], I.D. Shumov[1]*, V.S. Ziborov[1], A.F. Kozlov[1], I.A. Ivanova[1], N.V. Vaulin[2,3], D.V. Lebedev[2,3], A.S. Bukatin[2,3], I.S. Mukhin[2,3], E.A. Ponomarenko[1], A.I. Archakov[1]

[1]Institute of Biomedical Chemistry,
10 Pogodinskaya str., Moscow, 119121 Russia; *e-mail: shum230988@mail.ru
[2]Alferov University, 8 Khlopin str., Bldg. 3, St. Petersburg, 194021 Russia
[3]Institute for Analytical Instrumentation, 31-33 Ivan Chernykh str., St. Petersburg, 190103 Russia

This work demonstrates the use of a solid-state nanopore detector to monitor the activity of a single molecule of a model enzyme, horseradish peroxidase (HRP). This detector includes a measuring cell, which is divided into cis- and trans- chambers by a silicon nitride chip (SiN structure) with a nanopore of 5 nm in diameter. To entrap a single HRP molecule into the nanopore, an electrode had been placed into the cis-chamber; HRP solution was added into this chamber after application of a negative voltage. The reaction of the HRP substrate, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), oxidation by the enzyme molecule was performed in the presence of hydrogen peroxide. During this reaction, the functioning of a single HRP molecule, entrapped in the nanopore, was monitored by recording the time dependence of the ion current flowing through the nanopore. The approach proposed in our work is applicable for further studies of functioning of various enzymes at the level of single molecules, and this is an important step in the development of single-molecule enzymology.

Key words: nanopore detector; enzymatic activity; nanopore; single molecule enzymology

DOI: 10.18097/PBMC20247005349

INTRODUCTION

Proteomic data are much more difficult to analyze than genomic data [1]. This is due to the fact that there are no methods for protein amplification, and the level of protein expression cannot be predicted using the level of their transcription [2–4]. In this regard, an important direction in proteomics is the development and advancement of methods, which can analyze single protein molecules.

Although modern methods such as X-ray diffraction analysis (XRD) or nuclear magnetic resonance (NMR) are applicable to study the structure of single protein molecules, nevertheless they do not provide information on the functioning of a single protein molecule in real time [5].

At the same time, nanotechnological approaches based on the use of nanopore, atomic force and nanowire detectors provide the development of unique platforms for the detection of single biological macromolecules and the study of their functional properties.

One of the most popular methods applicable to study single enzyme molecules and their functioning is the atomic force microscopy (AFM) method [6–8]. The disadvantages of the AFM method are the high cost of the equipment, as well as the need to immobilize the studied biological macromolecules on the surface of a solid substrate. The latter circumstance may have a significant impact on the properties of the studied biological macromolecules.

Nanotechnological methods also include the nanowire detection method, which is used to study single viral particles [9]. In the context of studying protein molecules, this method is used mainly to investigate the interactions of these molecules with other molecules, but not to study the functioning of single protein molecules [10].

Recently, a new method for studying proteins based on nanopore detection has been proposed [11]. The basic principle of using nanopores is that when macromolecules function in a nanopore, a fluctuation in the ion current flowing through this nanopore is observed. In a nanopore detector, a partition with a nanopore separates two chambers (cis- and trans-), to which two electrodes are connected; these electrodes maintain the voltage between these chambers (Fig. 1).

During measurements by means of a nanopore detector, the ion current flowing through the nanopore is recorded. A protein molecule is usually entrapped in a nanopore, which allows monitoring its behavior by changing the ion current flowing through this nanopore [5, 12–16].

Nanopore detectors can be subdivided into two classes: detectors based on biological nanopores and detectors based on solid-state nanopores. Biological nanopores are formed on the basis of biological structures, namely, on the basis of biological membranes, into which protein molecules forming nanopores are incorporated. Although detectors

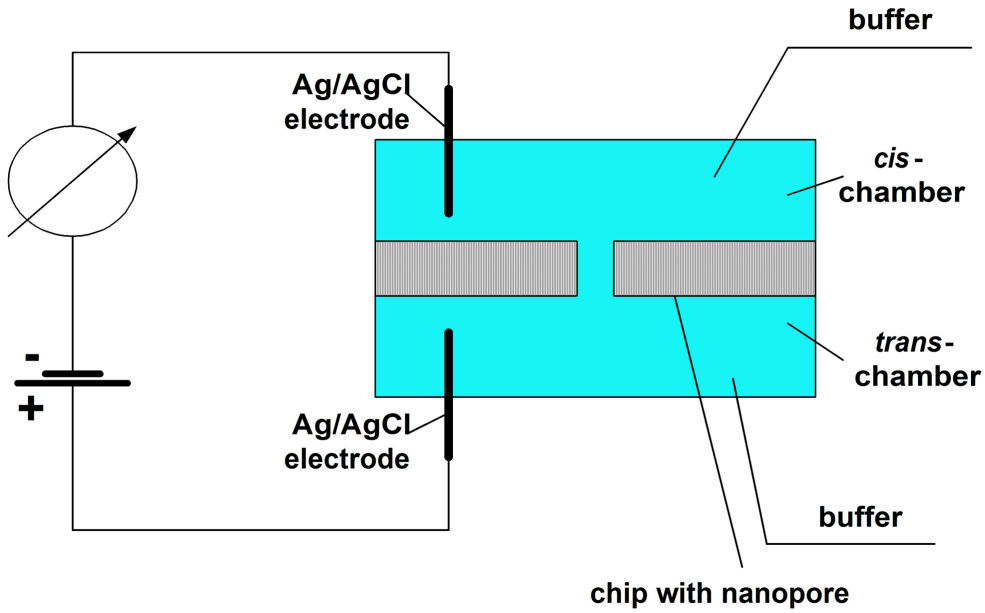

Figure 1. General scheme of the nanopore detector based on a solid-state nanopore.

based on biological structures have high sensitivity, their disadvantages include instability and sensitivity to changes in experimental conditions, such as pH, temperature, and a number of other factors. Solid-state nanopores are more stable with respect to these factors and, therefore, are more preferable for use in nanopore detectors.

Solid-state nanopores have great potential for studying the behavior of single molecules. This is due to their high sensitivity, allowing the detection of biomolecules even at the subnanomolar level [17]. Such high sensitivity of the solid-state nanopore is explained by the fact that its size (diameter) is in the same range as the size of the studied single biological macromolecules [18, 19]. Structures based on silicon (Si, SiO_2, Si_3N_4), glass, graphene, metal oxides (Al_2O_3, HfO_2, TiO_2), polymers, such as polyethylene terephthalate (PET, lavsan), polyimides, and polycarbonate are often used as nanopore structures. Composite membranes (MoS_2/SiN_x; Si_3N_4/SiO_2; graphene/Al_2O_3) are also used. The variety of materials suitable for the fabrication of solid-state nanopores allows preparing membranes with various physicochemical properties [17, 20–23].

Solid-state nanopores can be fabricated by various methods, including electron beam drilling (EBD), controlled dielectric breakdown (SDB), and creation of a nanopore by a focused ion beam (FIB) [22, 24–26].

One of the interesting and important enzymes for the development of medicine and biotechnology is horseradish peroxidase (HRP; EC: 1.11.1.7). Interest in HRP is also due to the fact that this enzyme is a model protein widely used in the study of peroxidase enzymes, which play an important role in various biochemical processes [27]. The HRP enzyme catalyzes the oxidation reactions of various organic and inorganic molecules in the presence of hydrogen peroxide (H_2O_2) [28]. Its molecular mass of 40–44 kDa [29, 30] corresponds to a molecule size of about 5 nm [31]. Previously, the functioning of the HRP molecule was studied using a nanopore detector by entrapping it in a nanopore, but the size of this nanopore was quite large and significantly exceeded the size of the HRP molecule [32, 33].

In this work, a nanopore detector was constructed in which a single HRP molecule was entrapped on the side of the cis-chamber to which a negative voltage was applied. A solid-state nanopore based on silicon nitride (Si_3N_4, hereinafter referred to as SiN) was used as a nanopore. The functioning of a single HRP enzyme molecule in the presence of its substrate 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) in the presence of H_2O_2 was carried out using this method of voltage application to the measuring cell.

MATERIALS AND METHODS

The experiments were performed using 2 mM Dulbecco's phosphate buffered saline (PBS-D), pH 7.4, which was prepared using ultrapure deionized water from a ready-made salt mixture purchased from Pierce (USA). Ultrapure deionized water was obtained using a Simplicity UV system (Millipore, France).

Horseradish peroxidase (HRP) was purchased from Sigma (USA; catalog number P6782; desalted lyophilized powder; ABTS activity 1995 units/mg, RZ 3.0 [34]). The substrate (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate, ABTS; catalog number A1888) was also purchased from the same company. For the experiments, a 0.3 mM ABTS solution was prepared in PBS-D buffer. Hydrogen peroxide (0.003%; v/v) was used in 2 mM PBS-D.

Nanopore Fabrication

Since the HRP dimensions have been determined to be 4.3×4.8×5.8 nm [35], a nanopore with sizes smaller than the characteristic sizes of the HRP molecule was formed. This did not allow the HRP molecule to freely pass through the nanopore. The nanopore was formed by the EBD method in a SiN chip. The nanopore had a length of 40 nm. An image of this pore obtained by transmission electron microscopy (TEM) is shown in Figure 2. Since the pore size was smaller than the characteristic size of the HRP molecule (5.8 nm), the latter could be retained in this pore without passing through it into the opposite (trans-) chamber.

Fabrication of a Nanopore Detector

The nanopore detector included a measuring cell. The volume of this cell was divided into two (cis- and trans-) chambers. The volume of each chamber was 700 µl. A SiN chip with a nanopore served as a partition between these chambers. The diagram of the nanopore detector designed in our work is shown in Figure 3.

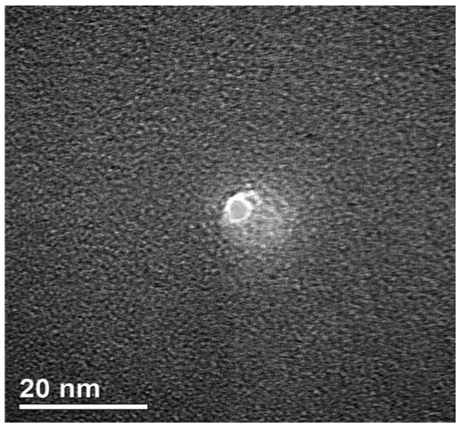

Figure 2. TEM image of a nanopore formed by the EBD method in a SiN chip.

Before the experiment, both chambers of the measuring cell were filled with ultrapure deionized water. After that, both cells were filled with PSB-D buffer. During the measurements, an electric voltage was applied between the cis- and trans-chambers using Ag/AgCl electrodes, immersing them in cells filled with the solution. The ion current passing through the nanopore was recorded in real time. After the measurements, the cell and the SiN chip were washed with ultrapure deionized water.

The detector was shielded with a Faraday cage. The current flowing through the nanopore was measured with a patch-clamp amplifier with an intrinsic noise level of 0.3 fA in a frequency band of 1000 Hz. The voltage applied to the cell was 200 mV. The ion current flowing through the nanopore was recorded using a 16-bit analog-to-digital converter (ADC). A Butterworth filter with a frequency of 1000 Hz was used to filter the signal.

Spectrophotometric Control of HRP Activity

To confirm the activity of the studied HRP enzyme under the experimental conditions, spectrophotometric measurements were performed. The essence of these measurements was to monitor the absorption of the solution at 405 nm [36] during the oxidation reaction of ABTS in the presence of H_2O_2 in 2 mM PBS-D buffer, pH 7.4. The concentrations of HRP, ABTS, and H_2O_2 in these measurements were 10^{-9} M [36], 0.3 mM [36, 37], and 0.003% (v/v), respectively. The measurements were performed

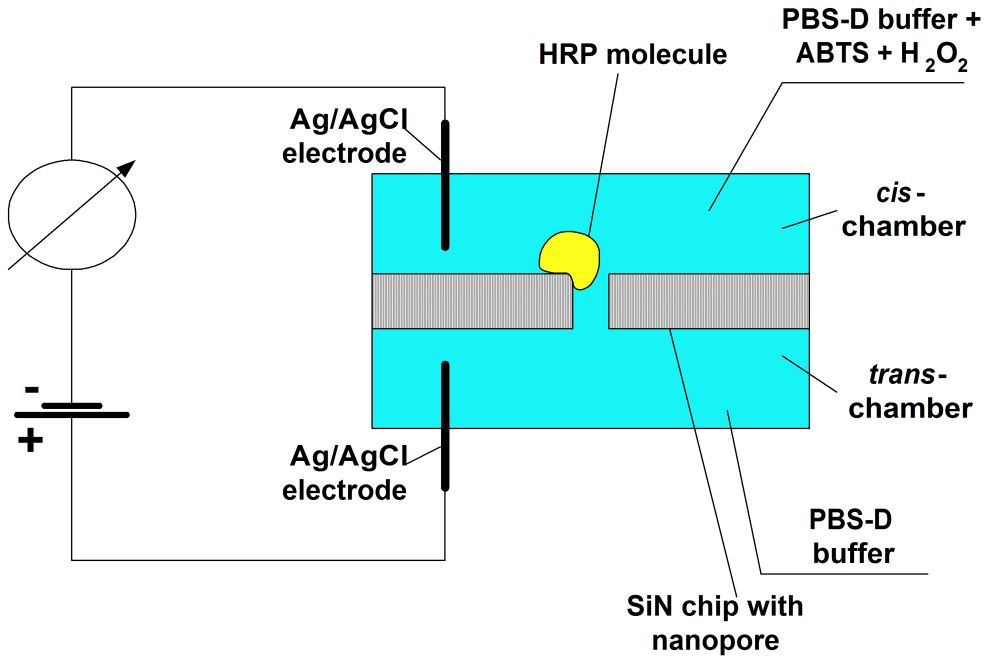

Figure 3. Schematic diagram of the nanopore detector based on a solid-state nanopore formed by the EBD method in a SiN chip, used in the experiments with HRP.

in a quartz cuvette with an optical path length of 1 cm on an Agilent 8453 spectrophotometer (Agilent Deutschland GmbH, Germany). The measurements were performed in 3 technical replicates.

RESULTS

Control Experiments with a Nanopore Detector

First, control experiments were carried out with 2 mM PBS-D without enzyme. The buffer was added to the cis- and trans-chambers; the voltage between the electrodes was 200 mV. After that, a 0.3 mM solution of ABTS in PBS-D was added to the cis-chamber, and this did not change the pore conductivity (Fig. 4).

It should be noted that addition of 0.003% H_2O_2 to PBS-D in the cis-chamber under the same conditions also did not change the pore conductivity (Fig. 5).

Then the ABTS solution was replaced with a solution containing 0.3 mM ABTS and 0.003% H_2O_2 in the cis-chamber. In this case, no changes in the pore conductivity were observed either (Fig. 6).

Registration of HRP Activity Using a Nanopore

Initially, 2 mM PBS-D without the enzyme was first added to the cis- and trans-chambers of the cell. Then, during the working experiments, the HRP enzyme was added to the cis-chamber, to which a negative voltage was applied, so that its final concentration in the chamber was 10^{-8} M, as shown in Figure 7, and the signal decreased, indicating a slight closure of the pore.

After partial closure of the nanopore by the HRP molecule was observed, the ABTS substrate solution was added to the cell to its final concentration in the cis-chamber of 0.3 mM. After that, H_2O_2 solution was introduced into the cell so that at the time point of 200 s, the cell contained 0.3 mM ABTS and 0.003% H_2O_2. The presence of H_2O_2 triggered the reaction of substrate oxidation (ABTS). During this reaction, fluctuations in the value of the ion current flowing through the nanopore were observed with the appearance of peaks on the recorded dependence of the ion current, starting from the time point of 450 s. In this case, the value of the ion current was approximately -250 pA. The amplitude of the observed fluctuations in the ion current reached ~150 pA.

Spectrophotometric Confirmation of HRP Activity

In order to confirm the HRP activity under the conditions of the nanopore detector experiment at enzyme, ABTS substrate and hydrogen peroxide concentrations of 10^{-9} M, 0.3 mM, and 0.003% (v/v), respectively, we carried out spectrophotometric measurements as described in the "MATERIALS AND METHODS" section. The resulting time dependence of the solution absorption is shown in Figure 8.

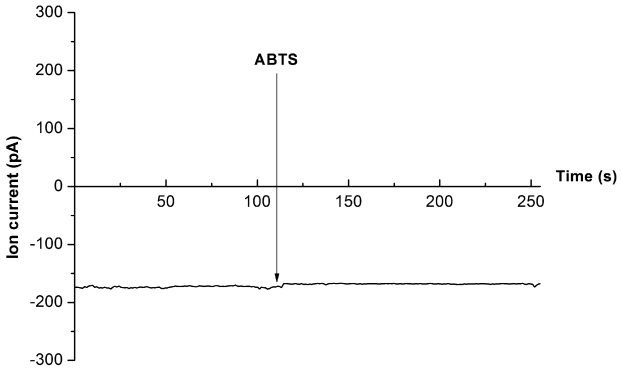

Figure 4. Time dependence of the ion current flowing through the nanopore in the control experiment aimed at determining the effect of ABTS addition. The arrow shows the time of adding the ABTS solution to the cis-chamber of the measuring cell. The final concentration of ABTS in the measuring cell was 0.3 mM.

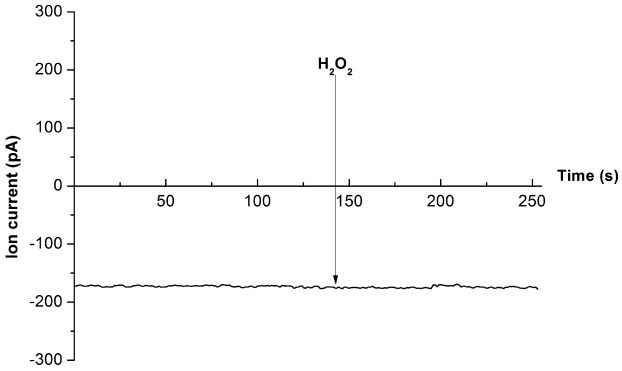

Figure 5. Time dependence of the ion current flowing through the nanopore in the control experiment aimed at determining the effect of the H_2O_2 addition. The arrow shows the time of adding the H_2O_2 solution to the cis-chamber of the measuring cell. The final concentration of H_2O_2 in the measuring cell was 0.003% (v/v).

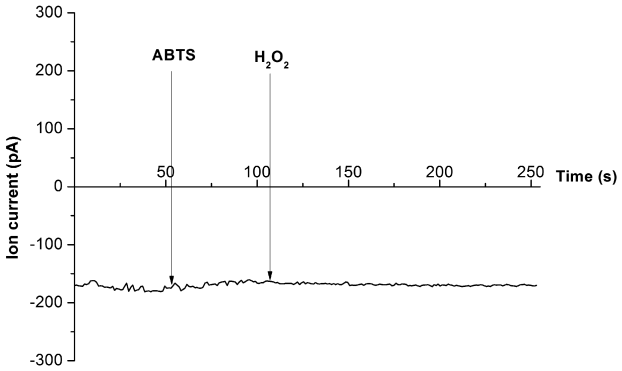

Figure 6. Time dependence of the ion current flowing through the nanopore in the control experiment without the enzyme. The arrow shows the time of adding the H_2O_2 solution to the cis-chamber of the measuring cell. The final concentrations of ABTS and H_2O_2 in the measuring cell were 0.3 mM and 0.003% (v/v), respectively.

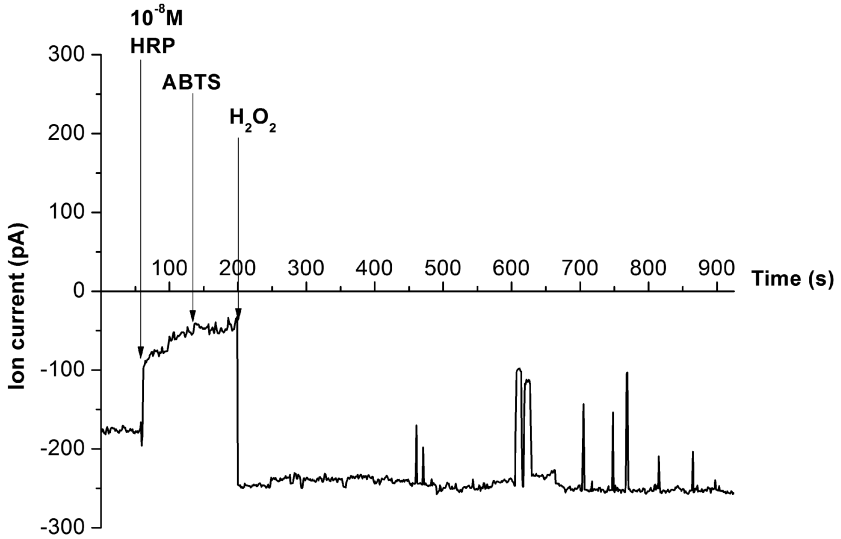

Figure 7. Time dependence of the ion current flowing through the nanopore. The arrows show the time points of adding the HRP, ABTS, and H_2O_2 solutions to the cis-chamber of the measuring cell. The final concentrations of HRP, ABTS, and H_2O_2 in the measuring cell were 10^{-8} M, 0.3 mM, and 0.003% (v/v), respectively.

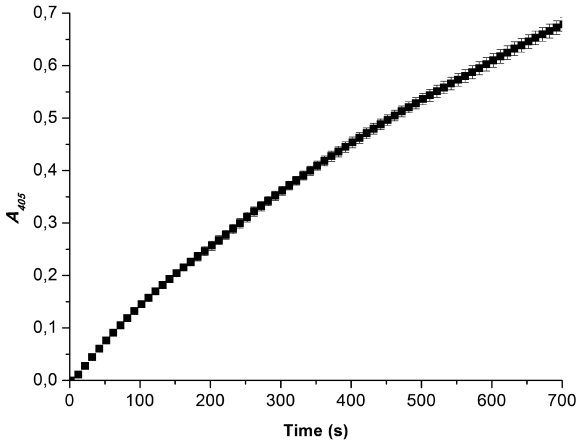

Figure 8. Time dependence of absorption of a solution (A_{405}) containing HRP, ABTS, and H_2O_2 monitored at 405 nm. The final concentrations of HRP, ABTS, and H_2O_2 in the cuvette were 10^{-9} M, 0.3 mM, and 0.003% (v/v), respectively. The optical path length of the cuvette was 1 cm. The measurements were performed in 2 mM PBS-D (pH 7.4).

The A_{405}(t) dependence shown in Figure 8 confirms that the studied HRP enzyme retained its functional activity throughout the entire observation time, corresponding to that in the nanopore detector experiment (~700 s).

DISCUSSION

In this work, we have investigated the possibility of registration of the catalytic activity of a single HRP molecule entrapped in a nanopore from the cis-chamber side. The nanopore diameter of ~5 nm allowed retaining the HRP molecule within the nanopore, preventing it from penetrating from the cis-chamber to the trans-chamber. After addition of a HRP solution to the cis-chamber, one of the HRP molecules approached the nanopore and blocked its lumen, as evidenced by the change in the ion current flowing through the nanopore in the period from 60 s to 130 s. Then, after adding the substrate solution, a H_2O_2 solution was added to the cis-chamber, thus initiating the enzymatic reaction of substrate oxidation. Figure 7 shows the signal fluctuations observed in the working experiments. In blank experiments (in the absence of HRP), ABTS or H_2O_2 did not affect the conductivity of the nanopore. Thus, we demonstrated the possibility of monitoring the functioning of a single HRP enzyme molecule after its insertion into a nanopore from the cis-chamber side of the measuring cell of the nanopore detector.

CONCLUSIONS

In this work, the possibility of recording the functioning of a single HRP molecule in real time in a SiN nanopore with a diameter of about 5 nm was experimentally confirmed. The studies were carried out under conditions when the enzyme was added to the cis-chamber. During the functioning of HRP, ABTS was oxidized in the presence of hydrogen peroxide. Fluctuations in the ion current flowing through the nanopore, the lumen of which was blocked by the HRP molecule, were observed. It should be noted that in the control experiments (in the absence of HRP), such fluctuations in the ion current were not observed. The enzyme activity under the experimental conditions was confirmed by the spectrophotometry method. The approach we have proposed in this work may be applicable for further studies of the functioning of various enzymes at the single-molecule level, which is important for the development of single-molecule enzymology.

FUNDING

The work was performed within the framework of the Program for Basic Research in the Russian Federation for a long-term period (2021–2030) (No. 122030100168-2).

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or the use of animals as objects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. *Uhlen M., Ponten F.* (2005) Antibody-based proteomics for human tissue profiling. Mol. Cell. Proteomics, **4**(4), 384–393. DOI: 10.1074/mcp.R500009-MCP200
2. *Archakov A.I., Ivanov Y.D., Lisitsa A.V., Zgoda V.G.* (2007) AFM fishing nanotechnology is the way to reverse the Avogadro number in proteomics. Proteomics, **7**(1), 4–9. DOI: 10.1002/pmic.200600467
3. *Gygi S.P., Rochon Y., Franza B.R., Aebersold R* (1999) Correlation between protein and mRNA abundance in yeast. Mol. Cell. Biol., **19**(3), 1720–1730. DOI: 10.1128/MCB.19.3.1720
4. *Futcher B., Latter G.I., Monardo P., McLaughlin C.S., Garrels J.I.* (1999) A sampling of the yeast proteome. Mol. Cell. Biol., **19**(11), 7357–7368. DOI: 10.1128/MCB.19.11.7357
5. *Luo Y., Wu L., Tu J., Lu Z.* (2020) Application of solid-state nanopore in protein detection. Int. J. Mol. Sci., **21**(8), 2808. DOI: 10.3390/ijms21082808
6. *Galvanetto N., Ye Z., Marchesi A., Mortal S., Maity S., Laio A., Torre V.* (2022) Unfolding and identification of membrane proteins *in situ*. eLife, **11**, e77427. DOI: 10.7554/eLife.77427
7. *Radmacher M., Fritz M., Cleveland J.P., Walters D.A., Hansma P.K.* (1994) Imaging adhesion forces and elasticity of lysozyme adsorbed on mica with the atomic force microscope. Langmuir, **10**(10), 3809–3814. DOI: 10.1021/la00022a068
8. *Pleshakova T.O., Bukharina N.S., Archakov A.I., Ivanov Y.D.* (2018) Atomic force microscopy for protein detection and their physicochemical characterization. Int. J. Mol. Sci., **19**(4), 1142. DOI: 10.3390/ijms19041142
9. *Patolsky F., Zheng G, Hayden O., Lakadamyali M., Zhuang X., Lieber C.M.* (2004) Electrical detection of single viruses. Proc. Natl. Acad. Sci. USA, **101**(39), 14017–14022. DOI: 10.1073/pnas.0406159101
10. *Omichi M., Asano A., Tsukuda S., Takano K., Sugimoto M., Saeki A., Sakamaki D., Onoda D., Hayashi T., Seki S.* (2014) Fabrication of enzyme-degradable and size-controlled protein nanowires using single particle nano-fabrication technique. Nat. Commun., **5**(1), 3718. DOI: 10.1038/ncomms4718
11. *Ying C., Ma T., Xu L., Rahmani M.* (2022) Localized nanopore fabrication via controlled breakdown. Nanomaterials, **12**(14), 2384. DOI: 10.3390/nano12142384
12. *Sheng Y., Zhang S., Liu L., Wu H.C.* (2020) Measuring enzymatic activities with nanopores. ChemBioChem, **21**(15), 2089–2097. DOI: 10.1002/cbic.202000079
13. *Pham B., Eron S.J., Hill M.E., Li X., Fahie M.A., Hardy J.A., Chen M.* (2019) A nanopore approach for analysis of caspase-7 activity in cell lysates. Biophys. J., **117**(5), 844–855. DOI: 10.1016/j.bpj.2019.07.045
14. *Chen H., Lin Y., Long Y.T., Minteer S.D., Ying Y.L.* (2022) Nanopore-based measurement of the interaction of P450cam monooxygenase and putidaredoxin at the single-molecule level. Faraday Discussions, **233**, 295–302. DOI: 10.1039/D1FD00042J
15. *Wloka C., van Meervelt V., van Gelder D., Danda N., Jager N., Williams C.P., Maglia G.* (2017) Label-free and real-time detection of protein ubiquitination with a biological nanopore. ACS Nano, **11**(5), 4387–4394. DOI: 10.1021/acsnano.6b07760
16. *Willems K., van Meervelt V., Wloka C., Maglia G.* (2017) Single-molecule nanopore enzymology. Philos. Trans. R. Soc. Lond. B Biol. Sci., **372**(1726), 20160230. DOI: 10.1098/rstb.2016.0230
17. *Lee K., Park K.B., Kim H.J., Yu J.S., Chae H., Kim H.M., Kim K.B.* (2018) Recent progress in solid-state nanopores. Adv. Mater., **30**(42), 1704680. DOI: 10.1002/adma.201704680
18. *Steinbock L.J., Krishnan S., Bulushev R.D., Borgeaud S., Blokesch M., Feletti L., Radenovic A.* (2014) Probing the size of proteins with glass nanopores. Nanoscale, **6**(23), 14380–14387. DOI: 10.1039/C4NR05001K
19. *Lee M.H., Kumar A., Park K.B., Cho S.Y., Kim H.M., Lim M.C.* (2014) A low-noise solid-state nanopore platform based on a highly insulating substrate. Sci. Rep., **4**(1), 7448. DOI: 10.1038/srep07448
20. *Deamer D., Akeson M., Branton D.* (2016) Three decades of nanopore sequencing. Nat. Biotechnol., **34**(5), 518–524. DOI: 10.1038/nbt.3423
21. *Pérez-Mitta G., Toimil-Molares M.E., Trautmann C., Marmisollé W.A., Azzaroni O.* (2019) Molecular design of solid-state nanopores: Fundamental concepts and applications. Adv. Mater., **31**(37), 1901483. DOI: 10.1002/adma.201901483
22. *Waugh M., Briggs K., Gunn D., Gibeault M., King S., Ingram Q., Jimenez A.M., Berryman S., Lomovtsev D., Andrzejewski L., Tabard-Cossa V.* (2020) Solid-state nanopore fabrication by automated controlled breakdown. Nature Protocols, **15**(1), 122–143. DOI: 10.1038/s41596-019-0255-2
23. *Tan S., Wang L., Liu H., Wu H., Liu Q.* (2016) Single nanoparticle translocation through chemically modified solid nanopore. Nanoscale Res. Lett., **11**, 1–10. DOI: 10.1186/s11671-016-1255-6
24. *Nam S.W., Rooks M.J., Kim K.B., Rossnagel S.M.* (2009) Ionic field effect transistors with sub-10 nm multiple nanopores. Nano Lett., **9**(5), 2044–2048. DOI: 10.1021/nl900309s
25. *Li J., Stein D., McMullan C., Branton D., Aziz M.J., Golovchenko J.A.* (2001) Ion-beam sculpting at nanometre length scales. Nature, **412**(6843), 166–169. DOI: 10.1038/35084037
26. *Kwok H., Briggs K., Tabard-Cossa V.* (2014) Nanopore fabrication by controlled dielectric breakdown. PloS ONE, **9**(3), e92880. DOI: 10.1371/journal.pone.0092880
27. *Krainer F.W., Glieder A.* (2015) An updated view on horseradish peroxidases: Recombinant production and biotechnological applications. Appl. Microbiol. Biotechnol., **99**(4), 1611–1625. DOI: 10.1007/s00253-014-6346-7

28. *Rogozhin V.V., Kutuzova G.D., Ugarova N.N.* (2000) Inhibition of horseradish peroxidase by *N*-ethylamide of *o*-sulfobenzoylacetic acid. Russ. J. Bioorg. Chem., **26**, 138–141. DOI: 10.1007/BF02759160
29. *Davies P.F., Rennke H.G., Cotran R.S.* (1979) Influence of molecular charge upon the endocytosis and intracellular fate of peroxidase activity in cultured arterial endothelium. J. Cell Sci., **49**(1), 69–86. DOI: 10.1242/jcs.49.1.69
30. *Welinder K.G.* (1979) Amino acid sequence studies of horseradish peroxidase. Amino and carboxyl termini, cyanogen bromide and tryptic fragments, the complete sequence, and some structural characteristics of horseradish peroxidase C. Eur. J. Biochem., **96**, 483–502. DOI: 10.1111/j.1432-1033.1979.tb13061.x
31. PyMOL. Retrieved January 16, 2024, from: https://pymol.org/
32. *Zhu L., Gu D., Liu Q.* (2017) Hydrogen peroxide sensing based on inner surfaces modification of solid-state nanopore. Nanoscale Res. Lett., **12**, 1–10. DOI: 10.1186/s11671-017-2190-x
33. *Tan S.W., Gu D.J., Liu H., Liu Q.J.* (2016) Detection of a single enzyme molecule based on a solid-state nanopore sensor. Nanotechnology, **27**(15), 155502. DOI: 10.1088/0957-4484/27/15/155502
34. Sigma-Aldrich. Certificate of Analysis. Peroxidase from horseradish. Type VI-A, essentially salt-free, lyophilized powder, ≥250 units/mg solid (using pyrogallol), 950–2000 units/mg solid (using ABTS). Product Number P6782, Batch Number SLCK8071. Retrieved March 21, 2024, from: https://www.sigmaaldrich.com/certificates/COFA/P6/P6782/P6782-BULK_____SLCK8071__.pdf
35. *Berglund G.I., Carlsson G.H., Smith A.T., Szöke H., Henriksen A., Hajdu J.* (2002) The catalytic pathway of horseradish peroxidase at high resolution. Nature, **417**(6887), 463–468. DOI: 10.1038/417463a
36. *Sanders S.A., Bray R.C., Smith A.T.* (1994) pH-Dependent properties of a mutant horseradish peroxidase isoenzyme C in which Arg38 has been replaced with lysine. Eur. J. Biochem., **224**, 1029–1037. DOI: 10.1111/j.1432-1033.1994.01029.x
37. Merck. Enzymatic Assay of Peroxidase (EC 1.11.1.7) 2,2-Azino-Bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) as a Substrate Sigma Prod. No. P-6782. Retrieved March 21, 2024, from: https://www.sigmaaldrich.com/RU/en/technical-documents/protocol/protein-biology/enzymeactivity-assays/enzymatic-assay-of-peroxidase-abts-as-substrate

Received: 25. 04. 2024.
Revised: 04. 07. 2024.
Accepted: 22. 07. 2024.

РЕГИСТРАЦИЯ АКТИВНОСТИ ЕДИНИЧНОЙ МОЛЕКУЛЫ ПЕРОКСИДАЗЫ ХРЕНА С ПОМОЩЬЮ ДЕТЕКТОРА НА БАЗЕ ТВЕРДОТЕЛЬНОЙ НАНОПОРЫ

Ю.Д. Иванов[1], А.Н. Аблеев[1], А.В. Виноградова[1], Е.Д. Неведрова[1], И.Д. Шумов[1]*, В.С. Зиборов[1], А.Ф. Козлов[1], И.А. Иванова[1], Н.В. Ваулин[2,3], Д.В. Лебедев[2,3], А.С. Букатин[2,3], И.С. Мухин[2,3], Е.А. Пономаренко[1], А.И. Арчаков[1]

[1]Научно-исследовательский институт биомедицинской химии имени В.Н. Ореховича, 119121, Москва, ул. Погодинская, 10; *эл. почта: shum230988@mail.ru
[2]Академический университет имени Ж.И. Алферова, 194021, Санкт-Петербург, ул. Хлопина, 8, корп. 3
[3]Институт аналитического приборостроения, 190103, Санкт-Петербург, ул. Ивана Черных, 31-33

В настоящей работе продемонстрировано использование твердотельного нанопорового детектора для мониторинга активности единичной молекулы модельного фермента — пероксидазы хрена (ПХ). Этот детектор включал в себя измерительную ячейку, разделенную на цис- и транс-камеры чипом из нитрида кремния (SiN структуры) с нанопорой диаметром 5 нм. Для встраивания единичной молекулы ПХ в нанопору, в цис-камеру помещали электрод и прикладывали к нему отрицательное напряжение, после чего в эту камеру добавляли раствор ПХ. После этого проводили реакцию окисления субстрата ПХ — 2,2-азино-бис(3-этилбензотиазолин-6-сульфоната (АБТС) — этой молекулой в присутствии пероксида водорода. В процессе этой реакции проводили мониторинг функционирования встроенной в нанопору единичной молекулы ПХ путём регистрации зависимости ионного тока, протекающего через нанопору, от времени. Предложенный в нашей работе подход может позволить в дальнейшем исследовать функционирование различных ферментов на уровне единичных молекул, что является важным шагом в развитии энзимологии единичных молекул.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: нанопоровый детектор; ферментативная активность; нанопора; энзимология единичных молекул

Финансирование. Работа выполнена в рамках Программы фундаментальных научных исследований в Российской Федерации на долгосрочный период (2021–2030 годы) (№ 122030100168-2).

Поступила в редакцию: 25.04.2024; после доработки: 04.07.2024; принята к печати: 22.07.2024.