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An Attempt to Create Stable Biomimetic Sensors for Express Analysis of Hydrogen Peroxide in Water Solutions

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Abstract

The physico-chemical properties of a new type catalase sensor, the so-called biomimetic sensors, modulating some of the catalase biosensor functions were investigated. These sensors have technological advantages over their biological analogs because of properties usually attributed to chemical sensors. The developed electrochemical system lies between bio- and chemical sensors.

Index Entries: Hematin; biomimic; biomimetic electrode; catalase fragment; alumina; Pattex; hydrogen peroxide; electrochemical potential; autooscillation; diffusion.

Introduction

Construction of systems exhibiting enzymic properties is one of the most challenging trends in modern biotechnology. Biosensors are successfully applied for environmental control, medical diagnostics, and on-line monitoring in the commercial production of numerous products *(1)*. Enzymes are the active components of biosensors. The high sensitivity and substrate specificity of sensors based on biological systems make them very useful for practical applications. However, all biological systems are sensitive to operational conditions and are more or less readily inactivated in the course of their use. Substitution of the active part of biosensors (biopolymers) for their chemical analogs (biomimics) *(2)* solves the problem of their instability and some other problems. This involves the construction of a real model (simulator) mimicking the objects and processes of enzyme

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catalysis in terms of their fundamental characteristics, e.g., selectivity, mild conditions, mechanism of active center functioning, and so forth. Because only some enzymic properties are simulated, biomimics cannot be considered as an adequate model of the selected enzyme, although they can successfully substitute for the biocatalysts.

In recent years, progress in understanding the structure and catalytic mechanism of catalase, peroxidase, and cytochrome P-450 has been achieved. Structural simulation of these enzymes relies on the following propositions:

1. Catalytically functional acid–base and oxidation–reduction groups should exhibit favorable geometry that structurally resembles the active site of the corresponding enzyme *(3)*.
2. The structure of an activated complex should correspond to that of a biochemical analog.
3. It should have structural units protecting the catalytic groups from undesirable interaction with other species of the reactive system *(4)*.

Taylor et al. *(5,6)* describe the techniques of preparation of stable porphyrins by replacing the pyrrole hydrogen atoms with electronegative groups and the bridge atoms with phenyl radicals. This strategy allows one to synthesize highly stable iron (III) perfluorinated tetraphenylporphyrin, which catalyzes benzene hydroxylation into phenol by hydrogen peroxide *(7)*.

The stereochemistry of formation of catalase complexes was discussed in ref. *8*. A heterogeneous iron protoporphyrin biosimulator was proposed to mimic the catalase reaction *(9,10)*. Activation and stabilization of similar catalase biosimulators were studied in ref. *11* using heme-containing catalysts deposited on various inorganic acid–base supports (Al_2O_3, $Al_2O_3 \times SiO_2$, and SiO_2). The data indicate that PP $Fe^{3+}OH/Al_2O_3$ is most active *(11)*. Presumably, aluminum oxide is more suitable to perform the functions of acid–base active site.

The effect of catalyst $PPFe^{3+}OH/Al_2O_3$ modified with tyrosine on the peroxide activity is absent, whereas the catalase activity of nonmodified catalysts is about three times as high *(12)*. The described biomimetic catalysts function in liquid phase, as do the enzymes themselves. Their activity depends on the solvent's nature, pH of the reaction medium, and cage effects.

The procedure of deposition of Fe (III) perfluorotetraphenylporphyrin on activated neutral alumina has been developed to prepare an effective inorganic biomimic of cytochrome P-450 *(13)*. The synthesized catalyst was preliminarily (before monooxygenation) tested for catalase activity in the liquid phase. This approach, as our experience shows, is effective with these catalytic systems. We continued the tests in order to reveal its stability to the action of high concentrations of the oxidizer (H_2O_2). The biomimic was periodically kept in a 30% aqueous hydrogen peroxide solution for 6 mo. Throughout this period it exhibited the same constant catalase activity,

which suggests that the catalyst has a high-performance stability and unique resistance to the oxidizer action.

Admixture ions (Cr and Mg) oppositely affect the catalase activity of aluminosilicate-based biomimetic catalysts with respect to that of $PPFe^{3+}OH/Al_2O_3$. It was shown that $PPFe^{3+}OH$/aluminochromosilicate is most active and gives 700 turnovers/h. These results lead us to conclude that ions (activators) substantially influence the catalytic activity of supported metalloporphyrins changing the acid–base characteristics of aluminosilicate carriers *(14)*. It cannot, however, be ruled out that the ions added can themselves participate in H_2O_2 decomposition.

We have lately considered the biomimetic sensors that can be used as the work elements of biomimetic electrodes of the catalase sensors. Now we shall consider the work elements directly in catalase biomimetic sensors.

Among known catalase biosensors, the microbiosensor (MBS) for express assay of hydrogen peroxide is of great interest *(15)*. This biosensor includes Clark's electrode with the polyacryl amide gel–immobilized catalase. The restricted intervals of pH and temperature, low sensitivity, and expensive cost are the drawbacks of this microbiosensor. High concentrations of hydrogen peroxide destroy MBS causing enzyme dissociation.

The amperometric enzyme sensor for glucose definition was the most studied. It is constructed on the basis of Clark's electrode or on the basis of definition of H_2O_2 concentration *(16)*. The amperometric enzyme electrode for detection of H_2O_2 is more sensitive and sharply decreases the sensitivity of the electrode for the changes of oxygen concentration in analyzed solution.

The Pt-electrode is traditionally used as indicator for amperometric H_2O_2-dependent enzyme electrodes.

Utilization of carbon materials for indicator electrode and immobilized enzyme support is a new method for creating glucose biosensors *(17)*. The possibility of covalent coupling directly with the surface of the electrode is the benefit of this method.

The penetration of biosensors and their mimetic analogs into analytical market is determined by their price and simplicity of utilization.

For comparison of biosensors with existing methods of analysis, the price of one biomimetic sensor for numerous utilizations is less than $2 (US), but for devices of single utilization it is less than 50 cents (US) (18).

The goal of this study was the development of catalase mimicking sensors based on two approaches. The first one used just the active center of the enzyme, Fe (III) protoporphyrin, and the second operated with the tryptic digest of catalase.

Materials and Methods

The electrochemical model of catalase biomimetic sensor consisting of the electrode of comparison ($Ag/AgCl/Cl^-$) and biomimetic electrode was suggested.

Two types biomimetic electrodes were made. Hematin-containing biomimic was synthesized by hemin adsorption from water–alcohol solution *(19)*. Activated neutral alumina was used as a support.

Aluminum wire and aluminum foil were used as electrodes.

To obtain appropriate catalase fragments, the enzyme immobilization was performed using different organic and inorganic supports, such as, alumina, Diasorb DEAE, agarose, followed by trypsin treatment. Pattex glue and 7.5% polyacrylamide gel were used as coupling agents between electrode and the biomimic.

Inorganic mimic was prepared according to the methods in refs. *20* and *21*. The catalase purchased from Sigma (St. Louis, MO), partially purified catalase from human blood erythrocytes, and trypsin were used in this work.

To create the biomimetic sensor, the aluminum wire (diameter 2 mm) and the aluminum foil (size 20 × 10 × 1 mm) were used as the electrode on which the work element was fixed by two methods:

1. Thin film of glue and biomimic were put on the aluminum foil or wire one after another (as a "sandwich").
2. Some glue and biomimetic were mixed and spread on the surface of aluminum foil or wire.

Changes of electrode potential owing to the catalase reaction were measured with B722A and F4843 voltmeters.

Simultaneous definitions of pH and E changes of hydrogen peroxide in water solution were carried out for possible correlation between pH-metrical and potentiometrical measures of the enzymes activities of catalase biomimetic sensors. The electrochemical installation for investigation was supplied with a magnetic mixer. The results of work are expressed on the graphs.

Results and Discussion

To determine low concentrations of hydrogen peroxide on the base of immobilized biosimulators of catalase, we have created and elaborated the cheapest potentiometrical biomimetic sensors with good hydrodynamic properties and speed of response. These biomimetic sensors are very simple to use.

Experimental data of the catalase activity of hematin-containing biomimetic electrode in 0.03% hydrogen peroxide in water solution are shown (Fig. 1). A comparison of the catalase activities of aluminum electrode and aluminum electrode with glue are also shown on this figure.

It is clear (Fig. 1) that the presence of hydrogen peroxide in the system leads to a sharp increase of potential in all cases (Al, Al with glue, and biomimetic electrode). First of all, it is bound up with formation of new surface film on the boundary between the electrode and solution. After a while, the equilibrium surface film is fixed and potential on the boundary between aluminum and solution and aluminum–glue–solution does not change.

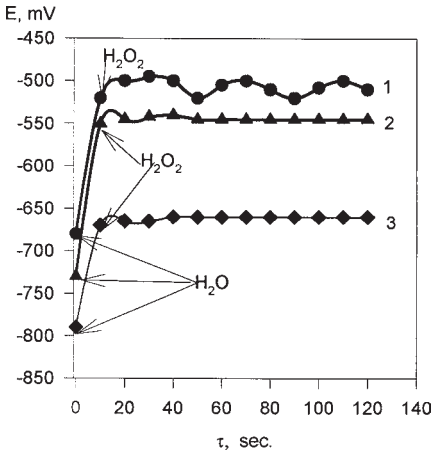

Fig. 1. The change of the electrochemical potential of system in dependent on the time: 1, hematin-containing biomimetic electrode; 2, aluminum electrode; 3, aluminum electrode with glue. t = 20°C, concentration of H_2O_2, 0.03%.

Refractometrical measurements have shown that aluminum did not decompose low concentrations of hydrogen peroxide. At the same time the electrochemical potential in the system of biomimetic electrode/H_2O_2/Cl^-/AgCl/Ag continued to sinusoidally change to complete decomposition of hydrogen peroxide (Fig. 1). (Decomposition of hydrogen peroxide was tested by titration with potassium permanganate.)

We consider the following reactions to take place in electrochemical system:

catalase reaction

$$2\ H_2O_2 \rightarrow H_2O + O_2 \qquad (1)$$

electrochemical reaction

$$O_2 + 4e^- + 2H^+ \rightarrow 2\ OH^- \qquad (2)$$

Hydrogen peroxide is known to be a weak two-base acid. Therefore, as a result of catalase activity of biomimetic electrodes, the pH of hydrogen peroxide is changed in both reactions. If the above-mentioned reactions take place on the biomimetic electrode, the solution at the end of reaction must have a pH equal to bidistilled water.

To establish the mechanism of H_2O_2 decomposition, we have studied the dynamics of the changes of pH until the complete decomposition of H_2O_2. Direction of curve has shown (Fig. 2) that after a while the pH of the solution exceeded the pH of bidistilled water and reached pH = 7.0.

The fact that pH of reaction exceeds pH of bidistilled water undoubtedly points to Eqs. 1 and 2 proceeding in the system. When there is an absence of the electrochemical reaction (Eq. 2) and complete decomposition of H_2O_2, pH of solution must correspond to pH of bidistilled water (pH = 6.2).

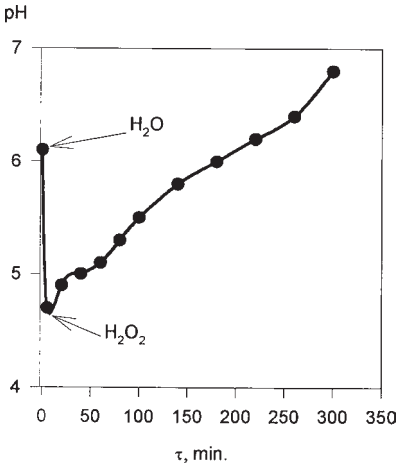

Fig. 2. The change of pH dependency on time. T = 20°C; concentration of H_2O_2, 0.01%.

The existence of two processes on the biomimetic electrode is an important result, and it follows that:

1. Some oxygen molecules accumulated on the surface of biomimetic electrode (catalase reaction) must pass through the film of glue to the surface of the electrode. Hence, special demands for glue material are the following:
   a. It must give enough stability to the connection of mimic with electrode.
   b. It must have low adsorption to oxygen molecules.
2. The glue material must be inert.
3. OH^- groups generated as a result of electrode reaction (Eq. 2) must have high adsorption to the glue.
4. There is superficial oxygen in the electrochemical reaction (Eq. 2).

The data shown (Fig. 3) are additional experimental proof of item 4. The changes of pH and potential in the system of hematin-containing mimetic electrode/H_2O_2/Cl^-/AgCl/Ag are shown in (a). The experiment when the mimic was immersed in solution without fixing with electrode surface is shown in (b).

The curves (b) show a sharp decrease of potential in the system. At the same time according to the data, the pH of the system increased.

The experiment without the mixing of solution is of interest (the mixing of solution with a magnetic mixer was used in all experiments). The fact is that part of the molecular oxygen produced by catalase reaction (Eq. 1) leaves the solid phase and produces the gaseous "cover" around mimetic electrode on the boundary between the solid phase and the volume of reaction medium. This circumstance will hamper H_2O_2 diffusion on biomimetic electrode and decrease speed of catalase reaction (Eq. 1) pro-

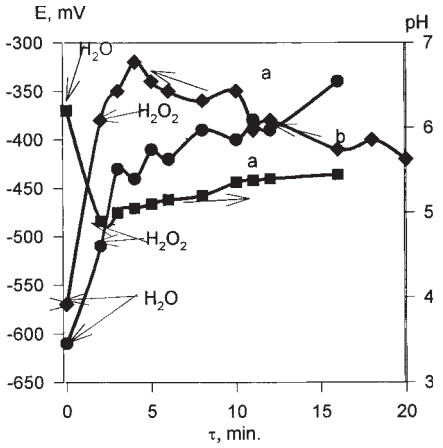

Fig. 3. The change of the electrochemical potential of system and pH of hydrogen peroxide in water solution dependent on the time: a, hematin-containing biomimic glued together with aluminum foil; b, the biomimic was in the volume. T = 20°C; concentration of H_2O_2, 0.01%.

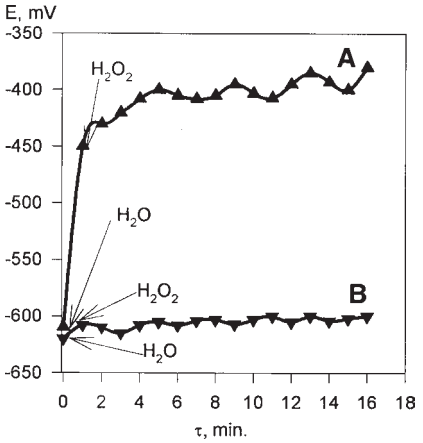

Fig. 4. The mixing influence on the change of the electrochemical potential of system: **(A)** with mixing; **(B)** without mixing. T = 20°C; concentration of H_2O_2, 0.01%.

moting the decrease of electric potential. The mixing of solution can help to remove these hindrances. Comparing the data (Fig. 4) we can see the influence of mixing on the process.

Though the mixing of the solution promotes the decrease of diffusion influence on the catalase reaction, it was necessary to carry out the experiment to determine the dominant role of diffusion or kinetic factor.

We have studied the temperature influence on the changes of pH and electrode potential. The results of experiments are shown in Fig. 5. As is seen, the pH of the reaction was constant though the temperature of reaction increased two times. That is typical for reactions proceeding in condi-

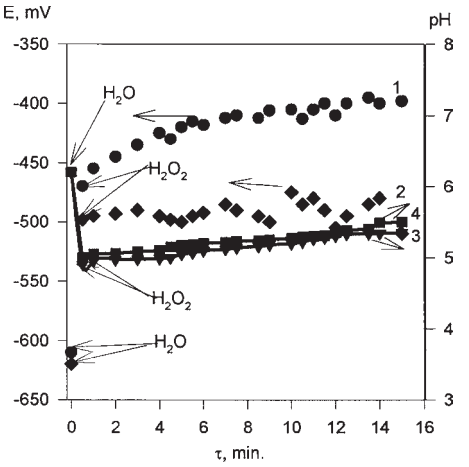

Fig. 5. The temperature influence on the change of electrochemical potential and pH of system: 1 and 2, electrochemical potential (at 20 and 40°C, respectively), 3 and 4, pH (at 20 and 40°C, respectively). Concentration of H_2O_2, 0.01%.

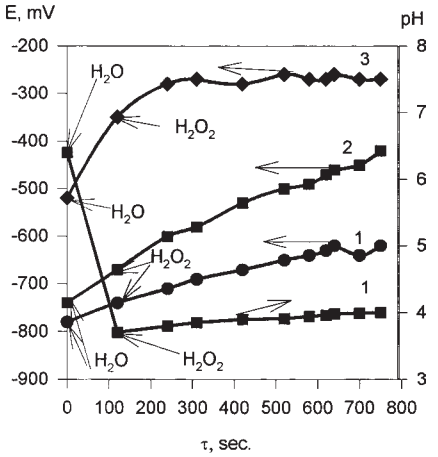

Fig. 6. The change of the electrochemical potential of system and pH of the water solution of hydrogen peroxide dependent on the time: 1, the catalase was adsorbed onto Diasorb, treated by trypsin, and glued to the surface of the aluminum foil by 7.5% polyacrylamide gel; 2, the catalase was adsorbed onto Al_2O_3, treated by trypsin, and glued together with the surface of the aluminum foil by 7.5% polyacrylamide gel; 3, the catalase was adsorbed onto Al_2O_3, treated by trypsin, and glued together with the surface of the aluminum foil by Pattex glue. T = 20°C; concentration of H_2O_2, 0.06%.

tions of diffusional compilations. Speed of catalase reaction is limited by diffusion, but not the kinetics of catalase reaction. Electrochemical indices became worse probably because of the acceleration of the process of molecular oxygen desorption into the solution, which decreased the speed of the electrochemical reaction (Eq. 2). Probably, the temperature influences the physical state of the electrode.

Electrochemical potentials have well-expressed maxima and minima (Figs. 1–6). Some courses of curves shown on the figures correspond to the diffusional area of catalase and electrochemical reactions.

During the catalase reaction (Eq. 1) the oxygen accumulating on the surface of the mimetic electrode passes through the film of glue and mimic to the electrode without leaving the surface and interacts with H^+. OH^- groups generated only by diffusional process can prepare the surface of electrode for the next portion of molecular oxygen. Thus, the speed of the electrochemical reaction (Eq. 2) is determined by correlation between diffusional speed of molecular oxygen and reverse diffusion of OH^- groups, which are the products of this reaction.

The maxima of E-curves correspond to the high speed of electrochemical reaction (Eq. 2). During the electrochemical reaction (Eq. 2), oxygen concentration decreased and at the same time the speed of electrochemical reaction (Eq. 2) also decreased the minima. When the molecular oxygen took the place of OH^- groups leaving the surface of the electrode because of diffusion, the speed of the electrochemical reaction (Eq. 2) began to increase.

So, the catalytic process together with diffusion phenomena had a sinusoidal character until complete decomposition of hydrogen peroxide.

Summing up the experimental regularities of the electrochemical process, we have concluded that the process at the electrode had an auto-oscillatory mechanism.

Undoubtedly, the auto-oscillations are the result of internal diffusion of ingredients of mimetic electrode, and the mixing of the solution cannot influence them.

The mixing of solution has a strong influence on external diffusion together with the transport of hydrogen peroxide to the mimetic electrode and removes OH^- groups from the boundary layer.

A sharp decrease of electrode potential was observed for biomimetic sensors made of the catalase adsorbed on Diasorb and agarose (with treatment by trypsin) and glued together to the surface of the aluminum electrode by Pattex glue.

Sharp oscillation of electrode potential was observed for biomimetic sensors made of catalase absorbed on Al_2O_3 (without treatment by trypsin) and glued together to the surface of the aluminum electrode by Pattex glue.

The biomimetic sensors showed better results when enzyme was absorbed onto an aluminum oxide support and treated by trypsin (Fig. 6).

Stability, long-lasting usefulness, good hydrodynamic properties, and cheap cost are the benefits of these biosensors.

In conclusion, there are catalase and electrochemical reactions in electrochemical system of biomimetic electrode/H_2O_2/Cl^-/AgCl/Ag. When the inorganic support was used, the internal and external diffusional factors are dominant. The diffusional factor influences the indices and, owing to it, the electrochemical reaction has an auto-oscillatory character.

Thus, the physicochemical regularities observed in the system of biomimetic electrode testify to the interconnection and interdependency of chemical and physical phenomena.

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