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Redox-Reactions of Active Oxygen Species on the Surface of Model Biomembranes

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Abstarct—Reactions of active oxygen species with a series of amino acids on the surface of the hydrophilic side of biomembrane models was investigated. As these models the following polymers adsorbed on a copper cathode were used: polyacrylamide (PAA), albumin, and polyaniline (PANI). The characteristics of the polymers' adsorption were studied. It is shown that PANI and PAA, in contrast to albumin, facilitate the reduction of molecular oxygen, accelerating the formation of its reactive species. It is established that the reaction of oxygen reactive species with amino acids is catalyzed by PANI and is decelerated by PAA, whereas albumin has practically no influence on this process. Assumptions are reported on the mechanism of the polymers nature influence on the oxygen reduction and the reaction of its active species with amino acids of various structure.

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Redox reactions, transport of small and fairly large molecules and ions, in particular, of oxygen and amino acids in biosystems occur as a rule on the surface of membranes [1, 2]. Especially important are the reduction reactions of molecular oxygen, which on the one hand, are involved in energy supply and metabolism, on the other, can produce radicals that damage vital body systems [3, 4]. Besides it is on the surface of biomembranes is an interaction active forms of oxygen with antioxidants, able to slow or prevent the damaging effects of active oxygen reduction products.

The study of redox reactions at biomembranes *in vivo* is a very difficult experimental problem associated with the action of many factors that are difficult to program, and which substantially affect the outcome of the experiment. Therefore, one of the most promising approaches to such research is to model the surface of biological membranes *in vitro*, in particular the external hydrophilic parts of the macromolecules, which may be involved not only in reactions with electron transfer, but also in the transport of biologically active compounds. According to existing notions, the transport of these compounds [1], as well as the electron transport through biomembranes are underlain by the electrochemical mechanisms [5].

Thus, the value of the transmembrane potential is approximately –0.2 V, but due to the fact that the membrane thickness is about 10 nm, the intensity of the electric field in it reaches 2×10^5 V cm^{-1}, which is close to the intensity of the electric double layer (EDL) [5]. Therefore the electrochemical methods provide unique opportunities for the simulation of redox reactions on biomembranes.

It should be emphasized that when the electron transfer to the molecule of oxygen in the respiratory chain of biological systems occurs through a dozen intermediate stages due to the difference of redox potentials of donor and acceptor, with the participation of coenzymes [6], while the electrochemical reduction of oxygen is programmed through a simple control of the electrode potential. The ability to create the conditions for the transport on the oxygen both one and two electrons is especially valuable, such the possibility is provided by the method of differential pulse voltammetry on a copper cathode [7, 8].

From our point of view, the organic models of the hydrophilic side of biological membranes is advisable to create by the adsorption on the electrode surface of water-soluble polymers containing functional groups inherent to the organic compounds of biomembranes,

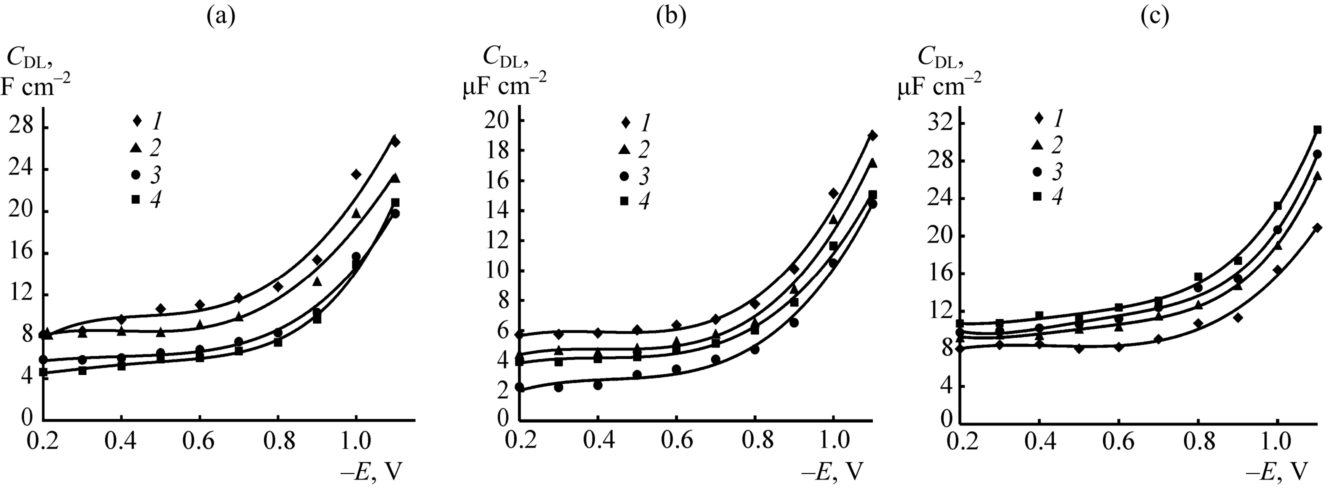

Fig. 1. Differential capacitance of copper cathode on the background of 0.1 M NaCl solution in water (*1*), in the presence of polymers: (a) PANI: (*2*) 0.5, (*3*) 1.5, (*4*) 2.5×10^{-3}%; (b) PAA: (*2*) 0.098, (*3*) 0.37, (*4*) 0.45×10^{-2}%, and (c) albumin (*2*) 0.0125, (*3*) 0.047, (*4*) 0.0876%.

as well as by the adsorption of polymer possessing the electronic conductivity.

Given the above, the electrochemical reduction of oxygen on a copper cathode was investigated in the presence of polyacrylamide (PAA), which contains amide groups capable of adsorption on a negatively charged surface and able to influence the charge of a protein; of albumin, which serves as a carrier of molecules of many poorly soluble in water transported by the blood endogenous compounds as well as exogenous drugs like penicillin, sulfanilamide, and others; of the water-soluble polyaniline (PANI), which possesses an electronic conductivity, so important for modeling electron transport that in particular occurs in the transmission of nerve impulses. Besides, the influence of the above-mentioned polymers was studied on the interaction of molecular oxygen and the active products of its reduction with endogenous antioxidants like cysteine, acetylcysteine, methionine, histidine, and carnosine.

Our data obtained by the impedance spectroscopy enable to evaluate the adsorption of the polymers on a copper cathode. According to Fig. 1 where plots are presented of the dependence of the electric double layer capacitance (*C*) on the potential (*E*) of the copper electrode in 0.1 M solution of NaCl are presented of each of the polymers characteristic of the adsorption change of the capacitance of the electric double layer is observed characteristic of the adsorption compared to the capacitance of the background over the entire range of investigated potentials. In the presence of PANI a reduction of the capacitance of the electric double layer (Fig. 1a) is observed, depending on the concentration of the polymer. The addition of PAA to the background solution also results in a significant reduction of the capacitance of the electric double layer of the copper electrode (Fig. 1b).

As follows from the analysis of adsorption isotherms (Fig. 2a), at the reduction potentials of molecular oxygen and its active forms the degree of surface coverage of the copper cathode with PANI depends on its concentration attains ~0.9 with the saturation at 1.5×10^{-3}%.

The the degree of surface coverage of the copper cathode with PAA as show its adsorption isotherms (Fig. 2b) does not exceed 0.8.

In the presence of albumin the *C*–*E* curves increase with its growing concentration in the same range of potentials (Fig. 1c). These data suggest that the adsorption of albumin is accompanied by a specific interaction of its functional groups with the surface of the copper cathode. And responsible for this interaction are clearly sulfhydryl groups, the so-called anchor groups from such fragment of albumin as cysteine, whose adsorption is also accompanied by the increased capacitance of the electric double layer of the copper electrode (Fig. 3a) in the negative range of potentials.

This explanation is confirmed by a special experiment. The curves of the *C*–*E* background were taken (Fig. 3b), then of the background, containing 0.57×10^{-3} M of cysteine, after which the electrode was

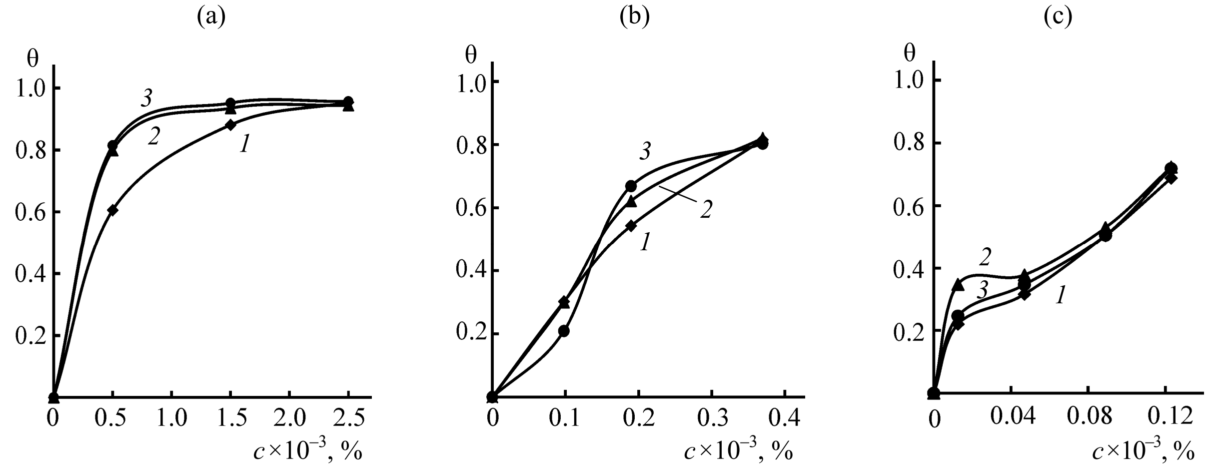

Fig. 2. Adsorption isotherms for copper cathode: (a) PANI, (b) PAA, and (c) at the potentials: (*1*) 0.2, (*2*) –0.6, and (*3*) –1.1 V.

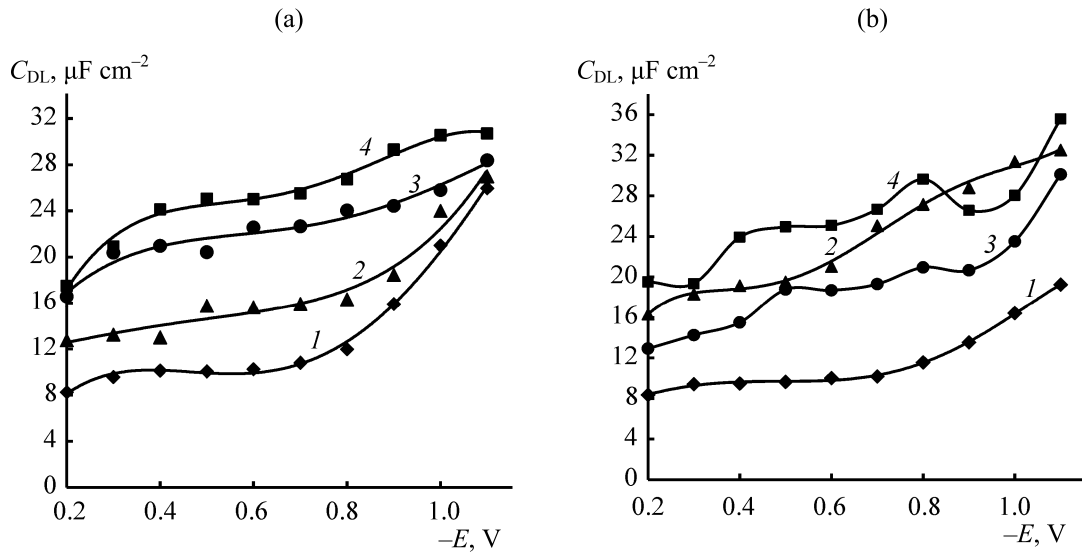

Fig. 3. Differential capacitance of copper cathode on the background of 0.1 M NaCl solution in water (*1*): (a) at different concentrations of cysteine: (*2*) 0.19, (*3*) 0.39, (*4*) 0.91×10^{-3} M, (b) (*2*) in the presence of cysteine concentrations of 0.57×10^{-3} M; (*3*) the new background solution, after washing the electrode, (*4*) after a 3 min exposure of the electrode in a solution of cysteine of concentration 0.57×10^{-3} M.

removed, rinsed with background solution, and *C*–*E* curve was measured in the new background solution. As seen in Fig. 3b, the capacitance in this case is higher than that of the background over the entire potential range, indicating that the specific adsorption of cysteine (Fig. 3b, curve *3*) occurs. Additional exposure of the electrode to a 0.1 M solution of NaCl, containing 0.57×10^{-3} M cysteine for 3 min leads, after a thorough washing with a background solution, to a much greater increase in the capacitance curve compared to the original one (Fig. 3b, curve *4*). This effect is most likely due to the increased charge density of the electric double layer [10], which is obviously due to the loss of a proton of the sulfhydryl group and the formation of the anion (R–SH → RS^-) during the adsorption of cysteine and albumin on the copper electrode, as it occurs during the formation of self-assembled structures by thiol compounds [11–13]. A similar phenomenon is observed in the adsorption of acetylcysteine.

Adsorption isotherms of albumin (Fig. 2c) are complex, probably due to the change in the conformation of macromolecules, to the increase of their concentration on the electrode surface and the change in the orientation of functional groups with respect to the electrode.

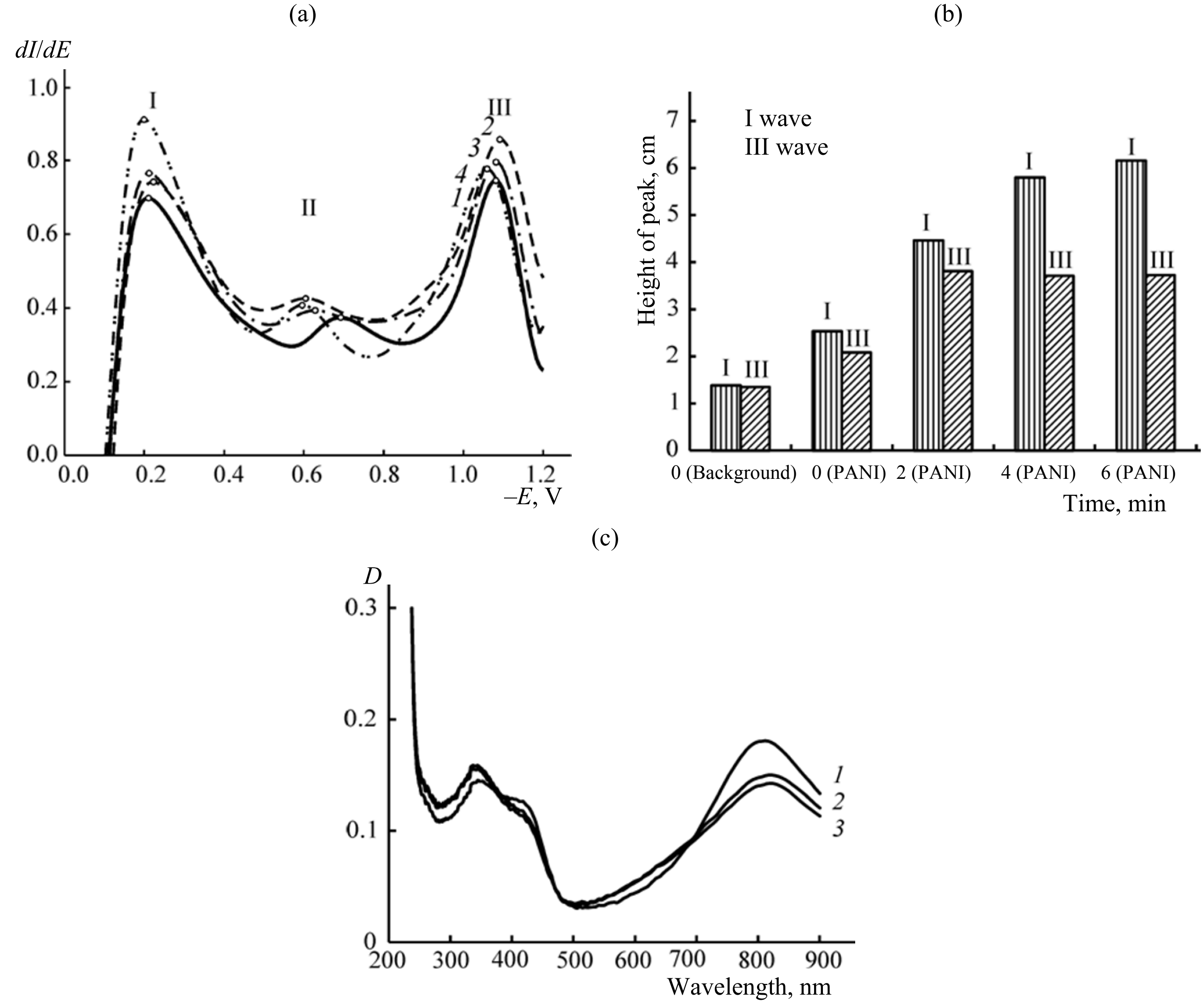

Fig. 4. (a) Differential voltammograms of oxygen reduction on copper cathode on the background of 0.1 M NaCl solution in water (*1*), in the presence of PANI of the following concentrations: (*2*) 10.0, (*3*) 20.0, (*4*) 30.0×10^{-3}%, and (b) the dependence of height of reduction wave of oxygen on the copper cathode on the time of its exposure in a cell at a concentration of PANI 20.0×10^{-3}% and (c) electronic spectra of 0.5 10^{-3}% solution of PANI: (*1*) in the presence of oxygen, and (*2*) after purging with argon for 15 min, and (*3*) After purging with argon for 30 min.

It should be noted that specially performed study showed the addition of albumin to the background solution in the absence of oxygen to result in a significantly larger rise of the capacitance curves than in the presence of oxygen. This fact and also the decrease in the capacitance of the electric double layer under the influence of proper oxygen dissolved in the 0.1 M NaCl solution indicates its adsorption on the surface of the copper cathode materially independent of the adsorption of albumin.

The effect of adsorption of the investigated polymers on the reduction of molecular oxygen on the copper cathode was judged by the changes of differential current-voltage curves in the presence of these polymers.

Thus at the reduction of oxygen on the copper cathode in 0.1 M NaCl at a certain interval between pulses [7] on the differential voltammograms three peaks can be observed (Fig. 4a) that characterize the reduction of the oxygen molecule (at *E* –0.6 V, II wave), the reduction of hydrogen peroxide (at *E* –1.1 V, III wave), and of hydroxyl radicals (at *E* –0.2 V, I wave) formed by one-electron reduction of hydrogen peroxide.

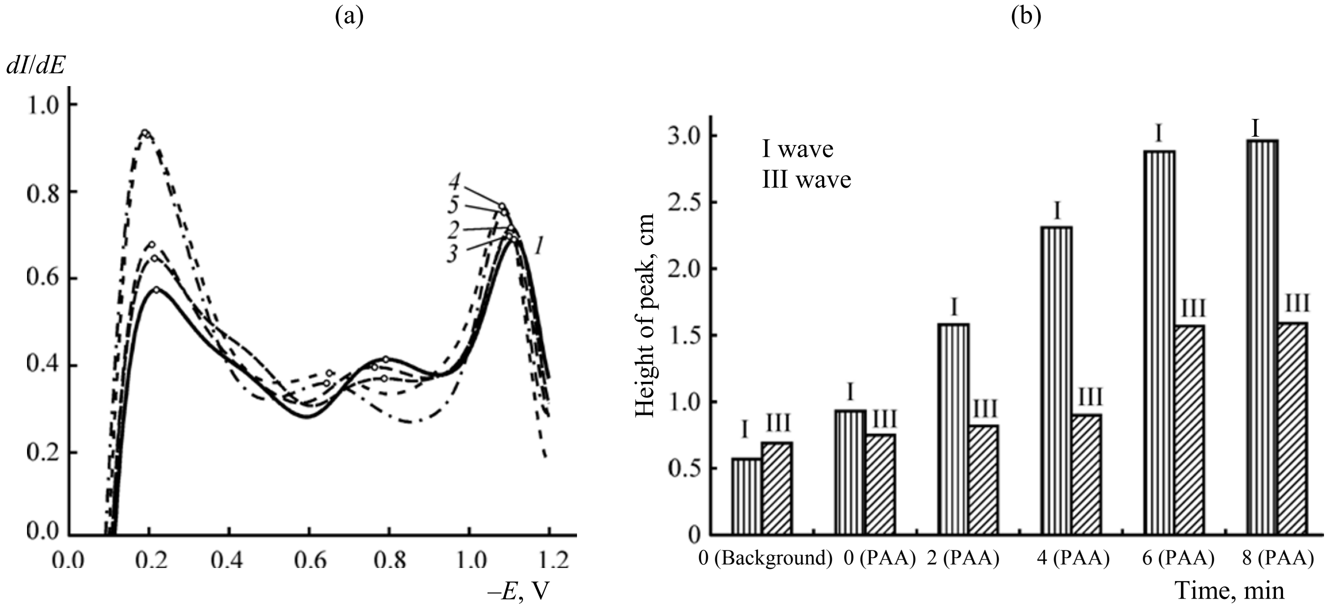

Fig. 5. (a) Differential voltammograms of oxygen reduction on copper cathode on the background of 0.1 M NaCl solution in water (*1*), in the presence of PAA of the following concentrations: (*2*) 0.09, (*3*) 0.19, (*4*) 0.37, (*5*) 0.69×10^{-2}%. (b) the dependence of height of reduction wave of oxygen on the copper cathode from the time of its exposure in a cell at a concentration of PAA 0.69×10^{-2}%.

In the presence of PANI (Fig. 4a) occurs the systematic proportional to the concentration of PANI increase and then the decrease in height of the peak of reduction of hydrogen peroxide, the growth of the height of the peak of the reduction of hydroxyl radicals, a slight growth and a significant (by 0.1 V) anodic shift of the potential of the molecular oxygen reduction peak. Moreover, the dependence of the heights (Fig. 4b) of these peaks of reduction current of hydroxyl radicals and hydrogen peroxide on the PANI residence time in the cell indicates the interaction of the latter with oxygen in the bulk of the solution followed by adsorption of the product of the interaction on the electrode. This is also evident from the electronic spectra of PANI, taken in the background solution in the presence and absence of oxygen (Fig. 4c).

The effect of PANI on the process of electrochemical reduction of oxygen originates from the features of its electronic structure owing to which [15] the molecular oxygen can be reversibly chemisorbed on the surface of PANI deposited on the electrode. Therewith the bond order of oxygen molecule chemisorbed on PANI is reduced by about 30%, and the bond length increases by 24% [15]. Due to this the activation energy of the electrochemical reduction of oxygen molecules decreases and the possibility arises of the shift of the process toward the generation of hydroxyl radicals. This is obviously a reason of the initial increase in the height of the peak of hydrogen peroxide and its decrease with further increase in the concentration of PANI, while the peak of its one-electron reduction product, hydroxyl radicals, increases.

At the adsorption of PAA on the copper cathode a proportional to its concentration increase is also observed in the peaks of the reduction of hydroxyl radicals as well as of hydrogen peroxide (Fig. 5a), and also the anodic shift of the peak potential of the molecular oxygen reduction. The process develops in time, similarly to that observed with PANI, the current reaches a limiting value after 6 minutes of exposure (Fig. 5b). Thus, the adsorption of PAA on the cathode also leads to the facilitation of reduction of oxygen and its active forms. This likely is due to the possibility of the formation of hydrogen bond [16] of oxygen with an amino group of PAA that can also change the order of the bond in a molecule of oxygen.

The adsorption of albumin on copper cathode, unlike that of the previous two polymers, regardless of time of exposure has little effect on the morphology of the current-voltage curves and peak heights of oxygen reduction (Fig. 6); the latter as shown before in this case is also adsorbed on the cathode. It can be assumed that the albumin on the surface of the cathode behaves like it occurs on the surface of the outer mitochondrial membrane which is permeable to small molecules, due to pore-forming proteins, which include albumin [17].

It was established as a result that all three polymers adsorbed on the surface of a copper cathode, have some influence on the electrochemical reduction of oxygen under these conditions. The differences in the impact are associated both with the chemical structure of macromolecules, the specific interaction of functional groups with the electrode, and with the ability to change the orientation of the polymer depending on the value of the electrode surface coverage with the macromolecules.

In order to estimate the effect of the used models of biomembranes on the process of the interaction of oxygen and its active forms with amino acids let us consider the changes in the voltammograms of oxygen reduction obtained in the absence and the presence on the electrode surface of each among the adsorbed polymers by an example of the above mentioned endogenous antioxidants.

As seen in Fig. 7a, a much more dramatic decrease in the relative reduction peak heights of both hydroxyl radicals and hydrogen peroxide under the influence of cysteine occurs in the presence of PANI. A similar effect of this polymer is observed with respect to acetylcysteine, methionine, and carnosine.

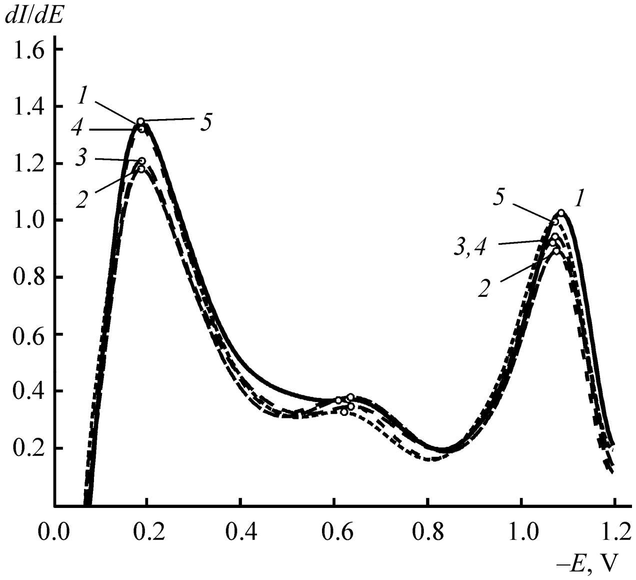

Fig. 6. Differential voltammograms of oxygen reduction on copper cathode on the background of 0.1 M. NaCl solution in water (*1*) in the presence of various concentrations of albumin: (*2*) 0.025, (*3*) 0.047, (*4*) 0.068, (*5*) 0.123%.

At the same time the presence of PAA either does not visibly affect the curves of the relative decrease in the reduction peak height of hydroxyl radicals or decelerates the decrease in the height of the reduction peak of hydrogen peroxide under the influence of cysteine (Fig. 7b), and also of acetylcysteine, methionine, histidine, and carnosine.

The effect of albumine on the reduction peaks of hydroxyl radicals and hydrogen peroxide under the influence of cysteine (Fig. 7c), and also of acetyl-cysteine, methionine, histidine, and carnosine is insignificant and ambiguous.

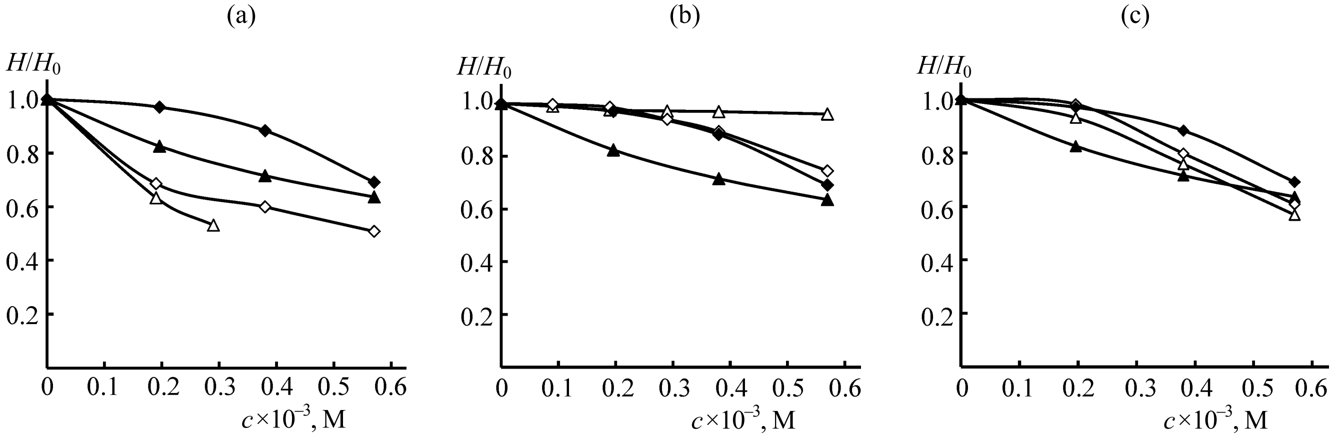

Fig. 7. The dependence of the relative height of the waves of oxygen reduction on copper cathode on the background of 0.1 M. NaCl in water on the concentration of cysteine: (♦) I wave, (▲) III wave, and the same in the presence of: (a) PANI concentration of 30×10^{-3}%. (b) the concentration of PAA 0.54×10^{-2}%, and (c) albumin concentration of 0.047%: (◊) I wave, (Δ) III wave.

The effect of polymers adsorbed on electrode on the changes in the heights of reduction peaks of hydroxyl radicals and hydrogen peroxide under the action of amino acids

Polymer	$\Delta(H/H_0) = (H/H_0) - (H/H_0)_{\text{polymer}}$									
	cysteine		acetylcysteine		methionine		histidine		carnosine	
	I	III	I	III	I	III	I	III	I	III
Polyacrylamide	–0.06	–0.32	0.01	–0.03	–0.02	–0.01	–0.22	–0.04	–0.21	–0.02
Albumin	0.09	0.04	0.07	0.02	0.05	0.07	0.06	–0.05	–0.04	0.02
Polyaniline	0.18	0.24	0.19	0.16	0.14	0.16	0.01	0.06	0.12	0.13

In order to estimate the special features and some rules of the influence produced by the studied polymers on the process of the reaction of active forms of oxygen with amino acids of different structure we compared the values of the difference between the heights of the peaks of the hydroxyl radicals and the hydrogen peroxide measured in the absence and the presence of the polymers adsorbed on the electrode. The data obtained are compiled in the table.

As seen from the table, the PANI absorbed on the electrode to greater or smaller extent decreases the effect of the shortening of the reduction peak both of the hydroxyl radical and the hydrogen peroxide under the action of each among the studied amino acids. The found effect is interesting not only from the scientific viewpoint, but can also have a practical importance for it may be used for increasing the sensitivity of the method we have developed [18] for evaluating the efficiency of antioxidants.

As to the mechanism of this influence, it is presumable that the acceleration of the reduction of the active forms of oxygen suggests the involvement of PANI in the transfer of electrons from the electrode to the active forms of oxygen.

The data in the table showing the negative effect of PAA adsorption on the decrease of the reduction peaks of the active oxygen forms from our point of view, most likely are due to the peculiarities of the mechanism of interaction of PAA with oxygen. Although on the one hand its affinity to electron grows, on the other hand the ability of the reduction products of further transformations remains unchanged, but the electrode surface can be partially blocked by the PAA macromolecules. Analogous more weak blocking of the cathode surface cannot be excluded in the case of albumin.

Thus, the used model of the hydrophilic part of biomembranes made it possible, on the one hand, to isolate the impact of polymers of different nature on the process of reduction of oxygen and the formation of its active forms, on the other, to establish the influence of the functional groups of macromolecules, which are fixed to a certain extent by the adsorption on the electrode surface, on the reaction of active oxygen forms with aminoacids– endogenous antioxidants.

EXPERIMENTAL

For the research we used an aqueous solution of polyacrylamide AK 631 A grade 1510 (PAA), purified prior to the experiment by dialysis of its aqueous solution.

Polianaline doped with polistyrenesulfonic acid (PANI-PSS) was synthesized by polymerization of aniline at 10°C in the presence of polistyrenesulfonic acid under the action of ammonium persulfate in the following ratio: aniline: PSS elementary unit = 1:1.5, aniline: ammonium persulfate = 1:1.25. The resulting aqueous solution of PANI-PSS (PANI) was purified from by-products by dialysis. The polistyrenesulfonic acid for the synthesis was isolated from its sodium salt (Aldrich).

Albumin solution from “Sigma” (Germany) in 0.1 M aqueous NaCl, due to the nature of its dissolution was prepared for a day before the experiment.

The amino acids cysteine and acetylcysteine from “Merck” (Germany). were used without further purification. Solutions of amino acids in aqueous 0.1 M NaCl was prepared just before measurements.

Background electrolyte was 0.1 M NaCl prepared from twice recrystallized sodium chloride of chemically pure grade in double distilled water. The oxygen concentration in the test solution corresponds to an equilibrium at atmospheric pressure and temperature of 20°C.

The adsorption of the compounds under investigation on the copper cathode was studied by electrochemical impedance spectroscopy [9] using a universal system of ACM Instruments Auto for a three-electrode scheme. The working electrode used was with copper face (*d* = 1.0 mm), the auxiliary electrode was Pt plate (25.0 mm×15.0 mm), the potentials were measured against the saturated silver chloride reference electrode.

Voltammetric studies were performed using the coupled with PC polarograph PU-1 in a 3-electrode cell [7, 8]. In a special pulsed mode at a copper cathode the differential current-voltage curves of oxygen reduction on the background of 0.1 M NaCl in water were measured. The potential of working electrode was set with respect to the silver chloride reference electrode. Auxiliary electrode was a platinum spiral.

REFERENCES

1. Gennis, R., *Biomembrany: Molekulyarnaya struktura i funktsii* (Biomembranes. Molecular Structure and Functions), Moscow: Mir, 1997.
2. Rubin, A.B., *Biofizika* (Biophysics), Moscow: Mosk. Gos. Univ., 2004. vol. 2.
3. Zenkov, N.K., Men'shikova, E.B., and Tkachev, V.O., *Kislorod i Antioksidanty*., 2009, no. 1, p. 3.
4. Lushhak, V.I., *Biokhim*., 2007, vol. 72, no. 8, p. 995.
5. Damaskin, B.B., Petrii, O.A., and Tsirlina, G.A., *Elektrokkhimiya* (Electrochemistry), Moscow: Khimiya, 2006.
6. Al'berts B., Brei, V., L'yuis, D., Reff, M., Roberts, K., and Watson, D., *Molekulyarnaya biologiya kletki* (Molecular Cell Biology), Moscow:: Mir, 1994, vol. 1.
7. Gromovaya, V.F., Shapoval, G.S., and Mironyuk, I.E., *Zh. Obshch. Khim*., 2002, vol. 72, no. 5, p. 828.
8. Shapoval, G.S., Gromovaya, V.F., Mironyuk, I.E., and Kruglyak, O.S., *Zh. Obshch. Khim*., 2008, vol. 78, no. 12, p. 2040.
9. Mironyuk, I.E., Shapoval, G.S., Gromovaya, V.F., and Kuhar', V.P., *Teor. Eksp. Khim*., 2001, vol. 37, no. 2, p. 105.
10. Damaskin, B.B., Petrik, O.A., and Batrakov, V.V., *Adsorbtsiya organicheskikh soedinenii na elektrodakh* (Adsorption of Organic Compounds based on Electrodes), Moscow: Nauka, 1968.
11. Bukhtiarov, A.V., Mikheev, V.V., Lebedev, A.V., Tomilov, A.P., and Kuz'min, O.V., *Zh. Obshch. Khim*., 1988, vol. 58, no. 3, p. 684.
12. Shengfu, Wang and Dan, Du, *Sensors*., 2002, no. 2, p. 41.
13. Ulman, A., *Chem. Rev*., 1996, vol. 96, no. 4, p. 1533.
14. Khlestkin, V.K., *Supramolekulyarnaya khimiya* (Supramolecular Chemistry), Novosibirsk: Novosibirsk. Gos. Univ., 2007.
15. Barsukov, V.Z., Khomenko, V.G., Katashinskii, A.S., and Motronyuk, T.I., *Elektrokhim*., 2004, vol. 40, no. 11, p. 1364.
16. Moskva, V.V., *Soros. Obrazovat. Zh.,* 1999, no. 2, p. 58.
17. Kol'man, Ya., Rem, K.G., and Virt, Yu., *Naglyadnaya biokhimiya* (Visual Biochemistry), Moscow: Mir, 2000.
18. Shapoval, G.S. and Kruglyak, O.S., *Zh. Obshch. Khim*., 2011, vol. 81, no. 7, p. 1092.