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SHORT COMMUNICATION

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Investigation of antioxidant and catalytic properties of some biologically active substances by voltammetry

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Abstract Antioxidants play a major role in protecting biological systems against many incurable diseases. The biological activity of 12 plant aqueous–alcohol extracts, some standard antioxidants (vitamin C, glucose, resorcinol, and catechol), Na_2SO_3, humic acids, phthalocyanines, and chlorophyll have been investigated in this work together with evaluation of their influence on the kinetics of the oxygen electroreduction. Finally the use of these substances for prophylactic purposes has been recommended.

Keywords Antioxidant · Biologically active substances · Oxygen · Voltammetry

Introduction

Protection of humans against oxidative stress as well as development of prophylactics for cancer and other incurable diseases are important directions in medicine and biochemistry over the world. Oxidative stress arises in a biological system after increased exposure to oxidants, a decrease in the antioxidant capacity of the system, or both. It often leads to the generation of reactive oxygen species (ROS), including free radicals [1]. Antioxidants play a major role in protecting biological systems against many incurable diseases. Antioxidants have been widely used in different fields of industry and medicine as substances which interrupt radical-chain oxidation processes, improve general health, help cell rejuvenation, and prevent cancer [2]. This work offers new approach to the determination of antioxidant and biological activity of biologically active substances (BAS).

Voltammetry is a convenient methodology for study of antioxidant properties and for the determination of antioxidant activity of biological systems. Nowadays the development of bioelectroanalytical chemistry is successful and is conditioned by the nature of the matter, because electrochemical mechanisms make up the basis of the majority of biochemical processes. Moreover a mechanism of electrochemical oxygen reduction (ER O_2) at the mercury film electrode is more convenient process for modeling the biological activity of the systems, because it is similar to the oxygen reduction in tissues [3]. It proceeds at the cathode in several stages with formation of the active superoxide anion–radical of oxygen ($O_2^{\cdot-}$):

$$O_2 + e^- \overset{k}{\rightleftarrows} O_2^{\cdot-} \quad (1)$$

$$O_2^{\cdot-} + H^+ \rightleftarrows HO_2^{\cdot} \quad (2)$$

$$HO_2^{\cdot} + H^+ + e^- \rightleftarrows H_2O_2 \quad (3)$$

$$H_2O_2 + 2H^+ + 2e^- \rightleftarrows 2H_2O \quad (4)$$

The superoxide anion–radical is highly reactive and toxic and it can cause oxidation of biomacromolecules as well as initiating radical-chain oxidation in tissues. For this reason numerous mechanisms of antioxidant defense are treated as a natural means of cell defense against the consequences of oxidation stress.

Therefore substances which can react rapidly with superoxide are of major importance among antioxidants. They result in a decrease of its toxic action and stop the radical-chain processes.

Electrochemical oxygen reduction at the mercury film electrode is a quasi-reversible process. It depends on diffusion as well as on the rate of the kinetic interconversion. In the limiting case of semi-infinite diffusion where $t \to 0$ under standard boundary conditions the solution of the problem yields the following equation for the oxygen reduction current on the mercury film electrode [4]:

$$I = z\ F\ S\ k\ c\left[H^+\right], \quad (5)$$

where I is the electrochemical reduction current of oxygen, z is the number of electrons involved in the limiting stage of the process, F is the Faraday constant, S is the area of the electrode surface in cm^2, c is the oxygen concentration at the electrode in mol L^{-1}, k is the rate constant of the limiting stage (1) of the process and $[H^+]$ is the concentration of hydrogen at the electrode in mol L^{-1}. It

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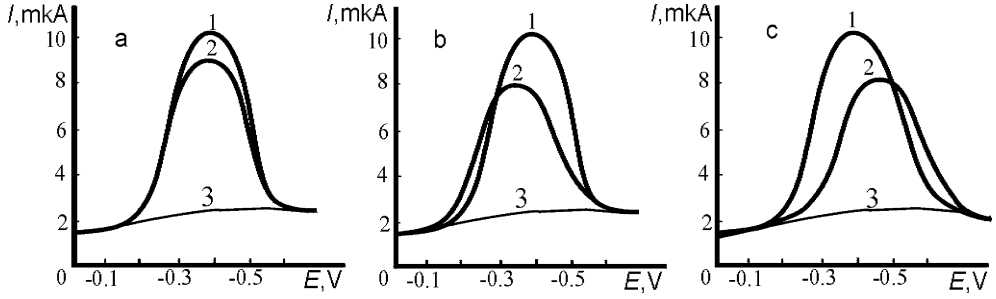

Fig. 1 Voltammograms of the ER O_2 current in the supporting electrolyte 0.1 mol L^{-1} Na_2SO_4 (*1*) without and (*2*) with (**a**) 0.002 g mL^{-1} Na_2SO_3; (**b**) 0.005 g mL^{-1} glucose; and (**c**) 0.02 g mL^{-1} extract of Siberian rose. *3* residual current without oxygen and the BAS in solution

should be noted that I does not depend on the diffusion coefficient in the above mentioned limiting case.

Antioxidants reacting with the superoxide decrease its concentration at the electrode under otherwise equal conditions. The electrochemical reduction current of oxygen decreases and therefore it can be used as a comparative value of the antioxidant activity in the solution being analyzed.

Investigations on the influence of various biologically active substances (BAS) on the process of ER O_2 and its kinetics could be treated as the modeling of biological antioxidant or catalytic activity of the substances in vitro.

It is known that the majority of BAS as well as plant extracts have good antioxidant properties. However they act via different mechanisms in tissues. BAS react with reactive oxygen species (ROS) and either decrease (antioxidant capacity) or increase (catalytic capacity) the electrochemical reduction current of oxygen (first wave at E=–0.3 V). The dependence of degree of these changes on BAS concentration in the solution has been suggested to be a coefficient of the biological activity of the systems (K):

$$K = \frac{\Delta j}{(j_{or} - j_{res})\Delta c}, \qquad (6)$$

where Δc is the change of BAS concentration in g mL^{-1}, Δj is change of the oxygen reduction current density after addition of BAS, j_{or} is the limiting current of the oxygen reduction without BAS in the solution, and j_{res} is the residual current without oxygen in the solution.

The voltammetric methodology allows evaluation of the antioxidant and catalytic activity of BAS and, moreover, consideration of their influence on the kinetics of the ER O_2 and the suggestion of mechanisms of interaction of BAS with O_2 and its species in the model systems.

In addition, K characterizes reduction ability of substances. The greater the value of K the easier reduction ability of the substance. It can be used in electroanalytical chemistry to study the electrochemical behavior of the substances and the development of methodology for analysis of the substances [5].

Experimental

The technique of this method is simple. It involves the recording of voltammograms of the cathodic reduction of oxygen by means of any voltammetric analyzer using differential voltammetry under the following conditions: potential rate scan is 50 μV s^{-1}, potential range is E=0 to 0.6 V.

The electrochemical cell (V=10 mL) was connected to the analyzer and consisted of a working mercury film electrode (MFE), a silver–silver chloride reference electrode with KCl saturated ($Ag|AgCl|KCl_{sat}$) and a nitrogen (or inert gas) supply tube. An open type cell can be used in this investigation. The reference electrode and the working electrode were held in the electrochemical cell. The working electrode potential was initially set at 0 V for about 30 s. During this step the solution was stirred by a magnetic stirrer. After stirring the potential was scanned negatively, causing oxygen reduction, which gives a current first wave at E=–0.1 to –0.3 V. Its value is proportional to the amount of oxygen in the solution bulk. Oxygen concentration in the solution bulk was monitored by potentiometric oxygen analyzer (P5972.MARA-ALVRO, Poland).

As supporting electrolytes, 0.1 mol L^{-1} Na_2SO_4 in aquatic and 0.1 mol L^{-1} $NaClO_4$ in non-aquatic solutions were chosen. The solutions of ascorbic acid and other standard BAS were prepared from the chemically pure corresponding substances by dissolving in twice-distilled water. Twelve plant extracts were prepared by removing solvent from 70% water–alcohol extracts and drying under the standard conditions. Humic acids were removed as water extracts from peat.

Results

Biological activity of 12 plant aquatic-alcohol extracts, some standard antioxidants (vitamin C, glucose, resorcinol, and catechol), Na_2SO_3, humic acids, phthalocyanines, and chlorophyll have been investigated in this work together with evaluation of their influence on the kinetics of the ER O_2.

In order to investigate the antioxidant or catalytic activity of the BAS, voltammograms of the oxygen reduction current in supporting electrolyte containing the BAS at the MFE were recorded (Figs. 1 and 2). Curves of the limiting current of the oxygen reduction against the BAS

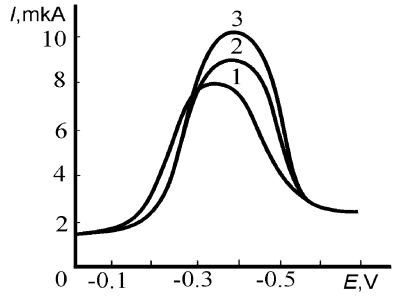

Fig. 2 Voltammograms of the ER O_2 current in the supporting electrolyte 0.1 mol L^{-1} $NaClO_4$ without (*1*) and with 0.002 g mL^{-1} (*2*) or 0.005 g mL^{-1} (*3*) chlorophyll in solution

Table 1 Antioxidant activity coefficients of the plant extracts being investigated and some standard antioxidants

N	The BAS name	K (mL g^{-1})	Sr	N	The BAS name	K (mL g^{-1})	Sr
1	Vitamin C	137.15	0.04	10	Arrow-wood (*Viburnum*)	281.5	0.08
2	Glucose	48.50	0.03	11	Milfoil (*Achillea millefolium*)	456.0	0.07
3	Resorcinol	24.15	0.03	12	Oat grass (*Avena fatua*)	68.8	0.04
4	Catechol	42.94	0.08	13	Clover (*Trifolium* gen.)	578.3	0.05
5	Na_2SO3	349.40	0.08	14	Lingonberry (*Vaccinium vitisidaea*)	297.6	0.04
6	Ashberry (*Sorbus aucuparia*)	80.4	0.05	15	Pine needle (*Pinus*)	129.7	0.03
7	Seeds of an alder (*Alnus*)	53.9	0.04	16	Labrador tea (*Ledum groenlandicum*)	358.4	0.07
8	Four-rowed barley (*Hordeum vulgare*)	101.1	0.05	17	Thistle (*Carduus* gen.)	121.8	0.02
9	Agrimony (*Agrimonia*)	93.5	0.06				

Sr is experimental error

concentration in the supporting electrolyte were plotted in this work.

As a result all investigated BAS were selected on four groups, which had different effect on the oxygen current and potential of the oxygen reduction (Fig. 1a, b, c), as a consequence of different mechanisms of the interaction between the BAS and oxygen species (ROS). One of them (Na_2SO_3) decreased the oxygen current under the constant potential and potentiometric oxygen analyzer showed simultaneously decrease of the oxygen concentration in the solution (Fig. 1a). Obviously It is conditioned by O_2 interaction with the BAS in the solution:

$$2Na_2SO_3 + O_2 \rightarrow 2Na_2SO_4 \quad (7)$$

The second and third groups of the BAS (all plant extracts, vitamin C, glucose, resorcinol, and catechol) decreased oxygen current with shift of the ER O_2 potential to the positive (Fig. 1b) or negative (Fig. 1c) ranges. This could reflect surface reactions between the BAS and oxygen species (ROS) at the MFE surface via different mechanisms. In accordance with these results and Ref. [4] we could suppose following mechanisms of the interaction for the second group of BAS:

$$O_2 + e^- \overset{k}{\rightleftarrows} O_2^{\cdot -} + R - OH \rightleftarrows HO_2^{\cdot} + R = O \quad (8)$$

$$HO_2^{\cdot} + R - OH \rightleftarrows H_2O_2 + R = O \quad (9)$$

and for third group of BAS:

$$O_2 + 2R - OH \rightleftarrows H_2O_2 + 2R = O \quad (10)$$

where R-OH is the reduced form of the antioxidants and R=O the oxidized form of the antioxidants. The measurements of the oxygen analyzer did not show changes of the oxygen concentration in the solution.

The fourth group of BAS (humic acid, phthalocyanines, and chlorophyll) increased the oxygen current (Fig. 2) resulting in a catalytic effect on the kinetics of the ER O_2. In this case the BAS as oxidants reacted with oxygen species (ROS) and increased the reversibility of the ER O_2 process. Consequently oxygen concentration at the surface of the MFE increased:

$$O_2 + e^- \overset{k}{\rightleftarrows} O_2^{\cdot -} + R \rightleftarrows O_2 + R^{\cdot -} \quad (11)$$

Table 2 Catalytic activity coefficients of the BAS being investigated

N	The BAS name	K (mL g^{-1})	(P.C.$\times 10^{17}$) spin g^{-1}	Sr
1	Humic acid	1805.24	2.8	0.06
2	Chlorophyll	1490.14	1.6	0.08
3	Phthalocyanine Co	3566.12	–	0.05
4	Phthalocyanine Ni	2023.22	–	0.06
5	Phthalocyanine Cr	4365.23	–	0.05

Sr is experimental error, P.C. is the quantity of paramagnetic centers (P.C.) in the samples

Curves of the relative change of the oxygen current against the BAS concentration in the supporting electrolyte were plotted in this work. The linear part of these curves in a small range of concentrations was suggested for evaluation of the biological activity of the BAS according to Eq. (5). As a result the coefficients of the antioxidant and catalytic activity of the BAS have been determined in this work. All plant extracts, as is to be expected, showed excellent antioxidant properties in comparison with the standard antioxidants (Table 1). A correlation was found between quantity of common flavonoids and the coefficients of the antioxidant activity of the plant extracts.

Humic acid, phthalocyanines and chlorophyll had catalytic effect on the ER O_2 process. Coefficients of the catalytic activity of the BAS were evaluated in this work (Table 2). Analysis by the method of paramagnetic resonance (PMR) has shown that greater quantity of paramagnetic centers (P.C.) in the samples gives better values of the coefficients of their catalytic activity in humic acid and chlorophyll.

It should be noted that the BAS being studied are not adsorbed on the surface of the working mercury film electrode in the range of the oxygen reduction, E=–0.3 to –1.0 V. The corresponding investigations were carried out by scanning voltammograms of capacity current in a wide potential range: E=0 to –2.0 V, using alternating voltage voltammetry.

Conclusion

Investigations of the influence if BAS on the kinetics of the ER O_2 have enabled to draw conclusions about mechanisms of their interaction with oxygen at the electrode. It could be a key to comprehension of the mechanisms of antioxidant protection of organism. The use for medical and prophylactic purposes of the BAS investigated could be recommended. The second and third groups of the BAS could be used not only as antioxidants and also anti-inflammatory medicine. Humic acid, phthalocyanines and chlorophyll could be used as a substances for improving plant growth.

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