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Antiradical and Antioxidant Activity of Biologically Active Carboxylic Acids

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Abstract — The antioxidant and antiradical activity of a series of biologically active carboxylic acids were studied, their effectiveness series were constructed, and optimal concentrations for inhibition of active oxygen formation were found. It is shown that the mechanism of the antioxidant action of these acids can be studied by their effect on different stages of oxygen reduction.

Growing recent attention has been focussed on free-radical processes involving oxygen, as well as the mechanisms and ways of their chemical regulation. This interest is explained by intensification in living bodies, under conditions of shaply worsened ecological situation, consequences of Chenobyl accident, and other extremal factors, of free-radical oxidation processes which may be associated with the so-called "oxygen stress" (sharply increased concentration of active oxygen reduction products in the organism) [1, 2].

Retardation of such processes is achieved by using compounds possessing antioxidant properties, such as biologically active carboxylic acids. The problems of choosing effective antioxidants and determining the antioxidant activity of chemical compounds (potential antioxidants) are rather intricate.

The mechanism of antioxidant action has long been associated with the ability of antioxidants to deactivate free radicals in the organism [3, 4]. Therefore, the relative effectiveness of antioxidants has commonly been measured by their reactivity toward diphenylpicrylhydrazyl whose alcohol solution gives a well-defined visible absorption band [5].

From our viewpoint, successful application of carboxylic acids largely depends on whether one knows the mechanism of their action under conditions of "oxygen stress", as well as the driving forces of their antioxidant and antiradical activity. Attempted separation of the latter two terms have been made in [6, 7]. However, the antioxidant activity has been determined as the ability to hinder oxidation as a whole. At the same time, it is known that antioxidants affect different stages of redox processes in the organism [8, 9].

The aim of the present work was to study the antiradical and antioxidant activity of a series of carboxylic acids, based on the information of their reaction with products of different stages of electrochemical reduction of oxygen on biometals, such as copper.

As we showed earlier [10, 11], under certain conditions of polarization of a copper electrode in a 0.1 M solution of NaCl, the oxygen reduction process occurs in three stages with the following waves:

I wave $\quad ^{\bullet}OH + \bar{e} \longrightarrow {}^{-}OH,$

II wave $\quad O_2 + 2\bar{e} + 2H^+ \longrightarrow H_2O_2,$

III wave $\quad H_2O_2 + \bar{e} \longrightarrow H_2O_2^{-} \longrightarrow {}^{-}OH + {}^{\bullet}OH$

$\qquad\qquad\qquad \longrightarrow + 2\bar{e} + 2H^+ \longrightarrow 2H_2O.$

By the intensity of the oxygen reduction waves, varied by addition of the compounds studied into the electrochemical cell, one can measure their effect on molecular oxygen and its active reduction intermediates (hydrogen peroxide and hydroxyl radicals).

We showed that all the carboxylic acids studied affect, more or less, the electrochemical reduction of oxygen, but the strength of this effect on different stages of the process varies. Thus, in the presence of citric acid, all the oxygen reduction waves get lower (Fig. 1a). Therewith, stronger suppression is characteristic of the hydroxyl and hydrogen peroxide reduction waves (I and III waves). The II wave is lowered not so strongly, but its maximum is shifted to positive potentials, implying that citric acid facilitates reduction of molecular oxygen. As known, citric acid is an effective antioxidant and radioprotector. The presented experimental evidence establishes that the antiradical properties of citric acid are underlied by its reaction

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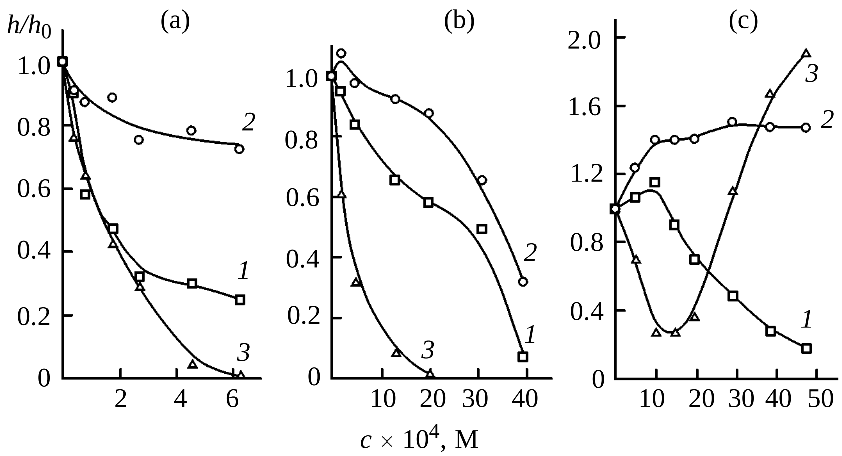

Fig. 1. Relative heights (h/h_0) of the oxygen reduction waves on a copper electrode in the presence of (a) citric, (b) nicotinic, and (c) ascorbic acids vs. acid concentrations. (*1*) Reduction wave of hydroxyl radicals (I wave), (*2*) reduction wave of molecular oxygen (II wave), and (*3*) reduction wave of hydrogen peroxide (III wave).

with hydroxyl radicals, while the antioxidant activity of the acid, with its reactions both with molecular oxygen and with the hydrogen peroxide formed.

Salicylic and acetylsalicylic acids similarly affect the oxygen reduction waves but at higher concentrations. Thus, the concentration producing a 10% lowering of the I and III waves for salicylic acid (1.13×10^{-4} and 2.60×10^{-4} M, respectively) is lower than for acetylsalicylic acid (2.85×10^{-4} M for the I wave and 4.80×10^{-4} M for the III wave), which is higher by an order of magnitude than for citric acid (0.22×10^{-4} M for the I wave and 0.14×10^{-4} M for the III wave). Therewith, salicylic and acetylsalicylic acid, too, facilitate reduction of molecular oxygen (the II reduction wave is shifted by ~60 mV to positive potentials).

Nicotinic acid most effectively suppressed the III wave (hydrogen peroxide) (Fig. 1b) which almost vanished at the acid concentration $\sim 2 \times 10^{-3}$ M. The I wave (reduction of the hydroxyl radicals formed by reduction of hydrogen peroxide), too, was suppressed considerably. These data reveal a high antioxidant activity of nicotinic acid, associated with its reaction with hydrogen peroxide, which is fully consistent with the pharmacological activity of this acid and its derivatives. The clinical activity of such compounds is explained by their prevention of peroxide oxidation of lipids in biological membranes [9].

We arranged the carboxylic acids in series in terms of their relative effectiveness measured by the concentration producing a 10% lowering of the reduction waves of hydroxyl radicals (I wave) and hydrogen peroxide (III wave): by the I wave: citric > salicylic > acetylsalicylic > nicotinic; and by the III wave: citric > nicotinic > salicylic > acetylsalicylic.

Of particular interest was to compare the resulting data with the effect of ascorbic acid on the electrochemical reduction of oxygen, since this acid plays an important role in redox processes in the organism. Addition into the solution studied of small amounts of ascorbic acid changed the patterns of the voltammetric curves (Fig. 1c). Thus, in the presence of less than 9×10^{-4} M of ascorbic acid, the height of the reduction wave of hydroxyl radicals increased only slightly, while the III wave (hydrogen peroxide) was suppressed considerably. At concentrations higher than 10^{-3} M, the I wave sharply decreased and the III wave increased, implying a certain shift of the system state to decreased amounts of radical reduction products and increased amounts of peroxide reduction products.

It is known that ascorbic acid plays a protective role as a biological radical trap [8], but under certain conditions it can exhibit prooxidant properties. These data agree with our results. According to the proposed interpretation of the resulting data as applied to biosystems, one can suppose that ascorbic acid primarily acts as a factor of protection from radical oxygen reduction products. Probably, the mechanism of its action is associated with the fact that, containing a dienol moiety, ascorbic acid exhibits strong reductive properties, and, as a strong electron donor, favors conversion of hydroxyl radicals to hydroxide ions, thus acting as an antioxidant and reducing the level of radical compounds in the system.

The resulting data suggest that the mechanism of action of ascorbic acid in biosystems largely depends on its concentration. Thus, at concentrations lower than 9×10^{-4} M, it can exhibit prooxidant properties.

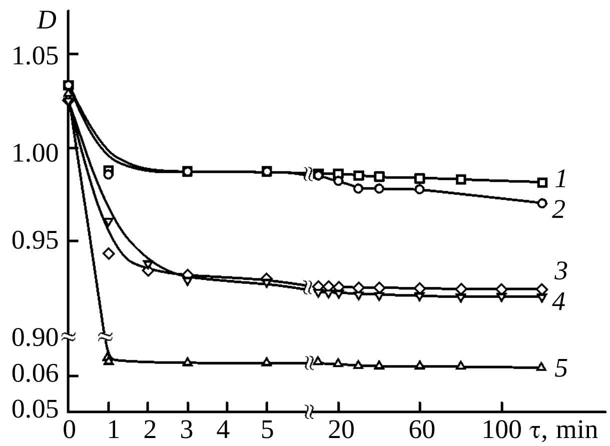

Fig. 2. Optical density of a solution of diphenylpicrylhydrazyl vs. time in the presence of (*1*) acetylsalicylic, (*2*) salicylic, (*3*) nicotinic, (*4*) citric, and (*5*) ascorbic acids.

At all the concentrations of ascorbic acid studied, the reduction wave of molecular oxygen (II wave) increased, and its maximum shifted to positive potentials (~100 mV), implying facilitation of reduction of molecular oxygen in the presence of this acid. The fact that ascorbic acid has higher effective concentrations compared with the other acids appears to be explained by a combined effect *in vivo* of introduced ascorbic acid and endogenous which is present in sufficient quantities in all organs and tissues of the organism and forming a part of its intrinsic antioxidant protective system [8, 12].

To compare the activities of the carboxylic acids studied and to gain information on their activity with respect to larger radicals (which are formed by free-radical processes in the organism), we used a traditional spectrophotometric procedure involving measurement of the rate of reaction with a stable radical, diphenylpicrylhydrazyl.

It was found that the carboxylic acids all react with diphenylpicrylhydrazyl but with different rates (Fig. 2). By the decreasing reaction rate, the antiradical activity of the carboxylic acids varies in the following series: citric > nicotinic > salicylic > acetylsalicylic.

Therewith, the effects of ascorbic acids in electrochemical studies and under conditions of its reaction with diphenylpicrylhydrazyl should be considered separately from the effects of the other acids. Ascorbic acid decolorizes a solution of diphenylpicrylhydrazyl immediately, exhibiting the highest antiradical activity among all the acids studied. The high rate of its reaction with diphenylpicrylhydrazyl may be associated with the ability to electron transfer, as well as with the fact that ascorbic acid and its dehydro derivative form a redox system readily accepting and donating hydrogen atoms [8]. However, the activity determined by this procedure may need some corrections, since the ability to decolorise a solution of diphenylpicrylhydrazyl is characteristic not only of antioxidants, but also of any good reducing agents. This circumstance much complicated interpretation of experimental results and may result in some mistakes. This is the possible reason why many authors overestimate the antioxidant activity of ascorbic acid.

Thus, our results give a deeper insight into the mechanism of antioxidant action. The employed pulse voltammetry technique provides more information, than the known diphenylpicrylhydrazyl procedure, about the antiradical and antioxidant activity of biologically active organic compounds, by following their reactions with molecular oxygen and its active reduction intermediates (hydroxyl radical and hydrogen peroxide). From the practical viewpoint, this may facilitate the choice of compounds, potential oxidants useful under "oxygen stress" conditions.

EXPERIMENTAL

The objects for study were salicylic, acetylsalicylic, ascorbic, nicotinic, and citric acids; they all (except for citric) were used as pharmacological substances. Chemical grade citric acid was twice recrystallized from twice distilled water. Solutions of acids were prepared immediately before use. The background electrolyte was a 0.1 M solution of sodium chloride, prepared from twice recrystallized NaCl and twice distilled water. The oxygen content of the solution corresponded to the equilibrium concentration of oxygen at atmospheric pressure at 20°C.

The effect of carboxylic acids at separate stages of reduction of molecular oxygen was studied using a PU-1 universal polarograph operated in the oscillopolarography mode by a three-electrode scheme; the experimental procedure was described in [10, 11].

The antiradical activity of carboxylic acids was studied by spectrophotometry by the reaction with diphenylpicrylhydrazyl whose alcohol solution gives a visible absorption maximum at 520 nm. Alcohol solutions of acid and diphenylpicrylhydrazyl were mixed in equimolar concentrations (10^{-4} M), and the reaction kinetics were followed by measuring the optical density of the resulting solution at 25°C. The absorption spectra were obtained on a Specord M-40 spectrophotometer.

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