Oxidation of ascorbic acid in the presence of phthalocyanine metal complexes. Chemical aspects of catalytic anticancer therapy.
1. Catalysis of oxidation by cobalt octacarboxyphthalocyanine

E. G. Girenko,[a]★ S. A. Borisenkova,[b] and O. L. Kaliya[a]

[a]Federal State Unitary Enterprise ´State Research Center "Research Institute of Organic Intermediate Products and Dyes," ´ Building 4, 1 ul. B. Sadovaya, 101999 Moscow, Russian Federation. Fax: +7 (095) 254 1200. E-mail: jerrik@cityline.ru
[b]Department of Chemistry, M. V. Lomonosov State University, Leninskie Gory, 119899 Moscow, Russian Federation. Fax: +7 (095) 932 8846. E-mail: borisenk@petrol.chem.msu.ru

The kinetics of oxidation of ascorbic acid in the presence of cobalt octa-4,5-carboxyphthalocyanine sodium salt (Teraphthal) was studied. A kinetic equation was obtained and a scheme of the process was proposed. According to the scheme, the first stage is the formation of a complex of the catalyst with dioxygen, and the limiting stage is the reaction of dioxygen with the ascorbate monoanion. The influence of the pH of the medium and the presence of a transport protein (albumin) on the state and catalytic activity of Teraphthal was studied. The involvement of hydrogen peroxide and oxygen-centered radicals in the catalytic oxidation of ascorbic acid was proved.

Key words: cobalt phthalocyanine, ascorbic acid, oxidation, catalysis, anticancer therapy.

The method of catalytic anticancer therapy using free oxygen-centered radicals as cytotoxic agents was proposed, tested, and patented in 1995.[1] The radicals are formed in the oxidation of ascorbic acid (AH_2) with dioxygen catalyzed by phthalocyanine metal complexes (PcM). The relative catalytic activity of several substituted PcM in this reaction was tentatively estimated in 1996.[2] Further development of the method required a deeper study of the kinetics and mechanism of the reaction to establish quantitative criteria for the evaluation of catalysts and recommendations on controlling the efficiency of the catalytic generation of cytotoxic agents. We have recently[3] analyzed the published data on the oxidation of AH_2 with dioxygen both for autooxidation and in the presence of different catalysts and determined the main problems to be studied.

In this work, we present the first results obtained with cobalt octa-4,5-carboxyphthalocyanine (PcCo) as the catalyst. The sodium salt of PcCo named Teraphthal (TP) is most actively being studied in various biological aspects[4] and is already undergoing the second-phase clinical tests as a component of a catalytic system for anticancer therapy. The kinetic characteristics of AH_2 oxidation in an aqueous buffer solution and in a solution of albumin, which models, to some extent, the blood plasma, were obtained. The scheme of the reaction was proposed.

Experimental

Cobalt octa-4,5-carboxyphthalocyanine sodium salt $CoPc(COONa)_8$, *viz.*, Teraphthal (TP), was synthesized at the Federal State Unitary Enterprise ´State Research Center "Research Institute of Intermediate Products and Dyes" ´ and its quality corresponds to the Pharmacopeial Enterprise Clause (PEC No. 42-0047-1680) advocated by the Pharmacopeial Committee of the Russian Federation. The electronic absorption spectrum (EAS) of TP in an aqueous 0.025 *M* phosphate buffer, pH 6.9, has maxima at λ = 674 nm (ε = $1.2 \cdot 10^5$ mol^{-1} L cm^{-1}) and 333 nm.

Ascorbic acid AH_2 (pharmaceutics grade, Russia) was used. Its EAS in an 0.025 *M* buffer solution with pH 6.9 has a maximum at λ = 267 nm, $\varepsilon = 1.4 \cdot 10^4$ mol^{-1} L cm^{-1}.

Oxidation of ascorbic acid in the presence of TP was studied using a Specord UV VIS spectrophotometer in 1-cm quartz cells. All experiments were carried out in buffer solutions with pH 5.5—7.4. The concentration of ascorbic acid was determined from the absorption at λ = 267 nm with a correction for the absorption of TP and products of the catalyst destruction in this spectral region. The concentration of TP was calculated from the absorption in the visible region. The concentration intervals for the reactants were $3.5 \cdot 10^{-5}$—$5.0 \cdot 10^{-3}$ mol L^{-1} and $0.35 \cdot 10^{-5}$—$0.35 \cdot 10^{-4}$ mol L^{-1} for AH_2 and TP, respectively, and corresponded to those of therapeutic doses of these substances. Dioxygen—dinitrogen mixtures were prepared for studying the dependence of the reaction rate on the par-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 7, pp. 1137—1142, July, 2002.

tial dioxygen pressure. The temperature in experiments was 18—37 °C. The initial rate of ascorbic acid oxidation ($W^0_{AH_2}$) was determined from the tangents to the initial regions of the kinetic curves. The non-catalytic component of the rate of ascorbic acid oxidation is negligible in the concentration interval studied of the reactants and the catalyst, which follows, in particular, from the rather accurate extrapolation to zero of the dependence of the oxidation rate on the catalyst concentration.

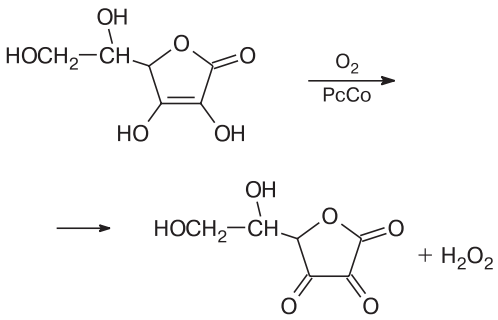
Effective activation energy was determined in an 0.025 *M* phosphate buffer, pH 6.9 (j = 0.1) in air in the 18—37 °C temperature range.

Superoxide dismutase (SOD) (Serva), catalase (Olaine Plant of Chemical Reagents, Latvia), and bovine serum albumin (BSA) (Sigma) were used as received. The specific trap of hydroxyl radicals, *viz.*, *p*-nitroso-*N*,*N*-dimethylaniline (NDMA, reagent grade), was purified before use by double crystallization from benzene.

Results and Discussion

1. Kinetics and mechanism of the reaction

The oxidation of ascorbic acid is the first-order reaction with respect to both the catalyst and ascorbic acid (Fig. 1).



The plots of $W^0_{AH_2}$ *vs.* P_{O_2} (Fig. 2, *a*) are described by the equation

$$W^0_{AH_2} = K'P_{O_2}/(1 + K''P_{O_2}) \quad (1)$$

(K' and K'' are the constants in the Michaelis—Menten equation), which is confirmed by the corresponding linear anamorphoses (Fig. 2, *b*). The data obtained allow the estimation of the rate of AH_2 oxidation in the living organism (points corresponding to the dioxygen content in the venous and arterial blood are marked in curve *2* (Fig. 2)) and prediction that it can substantially be increased by an increase in the dioxygen concentration in the blood using, *e.g.*, hyperbaric oxygenation.

The effective activation energy of ascorbic acid oxidation in the presence of Teraphthal was determined in the 18—37 °C temperature interval in air as 31±1 kJ mol^{-1}. The effective activation energy needs to be estimated because the catalytic system under study should be optimized as applied to biological conditions. It is also necessary to evaluate whether a combination of hyperthermism and catalytic anticancer therapy is reasonable or not.

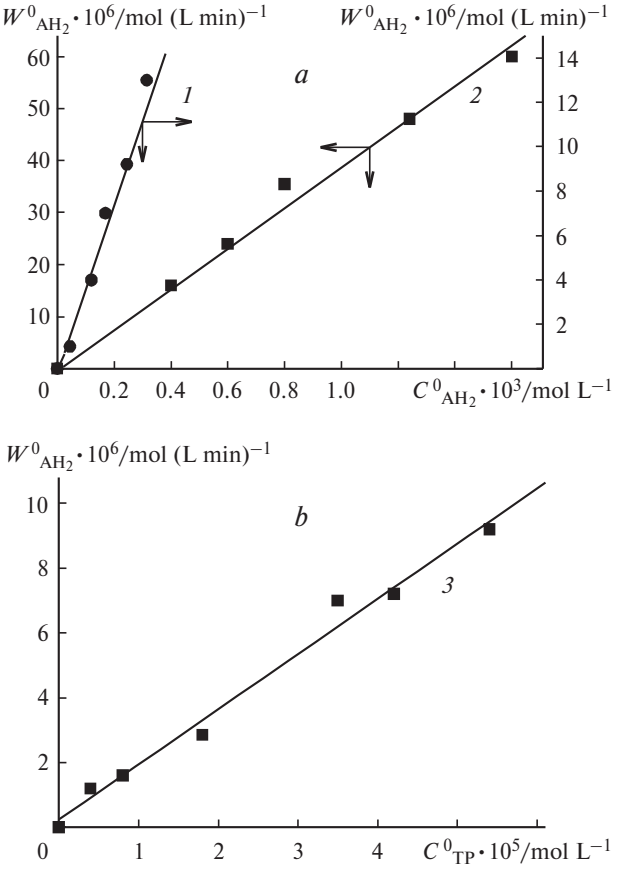


Fig. 1. Initial rate of ascorbic acid oxidation ($W^0_{AH_2}$) as a function of the initial concentrations of the substrate (*a*) and catalyst (*b*); $C_{TP} = 0.35\cdot10^{-4}$ (*1*), $0.8\cdot10^{-5}$ (*2*), and $C_{AH_2} = 7\cdot10^{-5}$ mol L^{-1} (*3*), 18 °C, pH 6.86.

The overall experimental kinetic equation takes the form (2), and the corresponding simplest scheme of the reaction is presented by reactions (3a) and (3b) if we take into account that the total concentration of Teraphthal is analytical.

$$W^0_{AH_2} = K_\Sigma[AH_2][Ct]P_{O_2}/(1 + K''P_{O_2}), \quad (2)$$

$$Ct + O_2 \underset{}{\overset{K''}{\rightleftharpoons}} CtO_2, \quad (3a)$$

$$CtO_2 + AH_2 \xrightarrow{k} \text{(intermediate stages)} \longrightarrow \longrightarrow Ct + \text{products}. \quad (3b)$$

According to this scheme, a complex of dioxygen with the catalyst (CtO_2) is formed in the first stage as it has been assumed in Ref. 5 for non-substituted PcCo. The reaction of this complex with AH_2, more precisely, with the AH^- ascorbate anion (because under our conditions (pH 6.1—7.4) ascorbic acid (pK_{A_1} 4.25) almost entirely exists as the monoanion), limits the process.

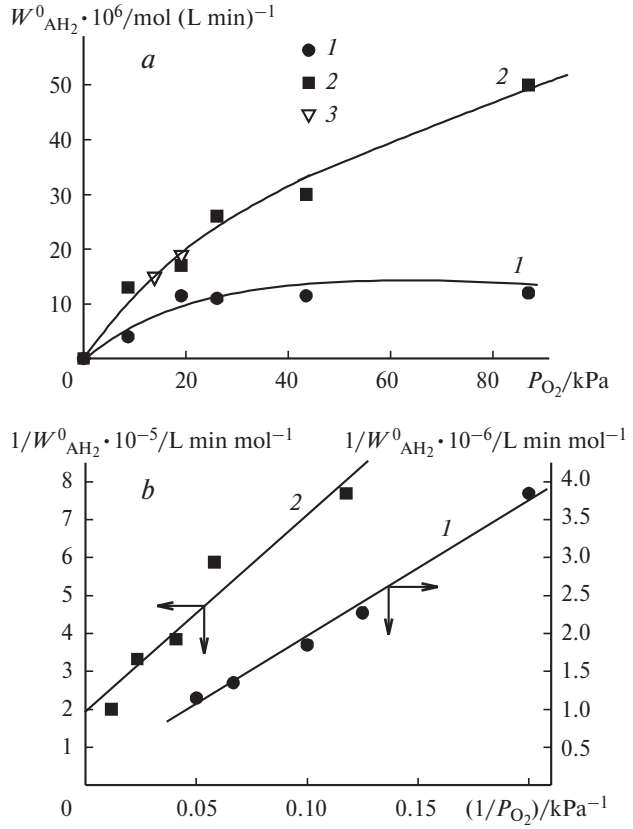


Fig. 2. Initial rate of AH_2 oxidation as a function of the dioxygen concentration (*a*) and linear anamorphoses of the curves (*b*); $C^0_{(TP)} = 0.35 \cdot 10^{-5}$, $C^0_{(AH_2)} = 7.00 \cdot 10^{-5}$ mol L^{-1}. Temperature: 18 °C (*1*) and 31 °C (*2*), *3*, content of dioxygen in the venous and arterial blood.

The stages following the limiting stage were not earlier considered in the works devoted to catalysis of AH_2 oxidation in the presence of PcM. We believe that in the catalytic system under study the same processes as in the non-catalytic variant mainly occur in these stages.[6]

The limiting stage affords primary radicals, namely, superoxide radical anion $O_2^{\bullet -}$ and ascorbate radical $A^{\bullet -}$ (4.1). The transformations of these radicals in the non-catalytic variant (4.2)—(4.6) were described and characterized by the corresponding rate constants.[7—13] All of them form hydrogen peroxide except for the recombination of the ascorbate radicals ($A^{\bullet -}$) and their reaction with dioxygen. Subsequent reactions are variants of transformations of H_2O_2 (4.7)—(4.10) and chain termination stages (4.12)—(4.15). Thus, the scheme of ascorbic acid oxidation in the presence of Teraphthal (PcCo)

$$AH^- + O_2(TP) \xrightarrow[+H^+]{} A + H_2O_2 + TP, \qquad (4)$$

A is dehydroascorbic acid, can be presented by the following series of stages beginning from the limiting one:

I. Formation of primary radicals

$$AH^- + O_2(TP) \rightarrow H^+ + A^{\bullet -} + O_2^{\bullet -}\,(TP). \qquad (4.1)$$

II. Reactions of primary radicals

$$O_2^{\bullet -} + AH^- + H^+ \rightarrow A^{\bullet -} + H_2O_2, \qquad (4.2)^7$$

$$O_2^{\bullet -} + A^{\bullet -} + H_2O \rightarrow A + HO_2^- + OH^-, \qquad (4.3)^7$$

$$O_2 + A^{\bullet -} \rightarrow A + O_2^{\bullet -}, \qquad (4.4)^8$$

$$O_2^{\bullet -} + O_2^{\bullet -} + 2\,H^+ \rightarrow H_2O_2 + O_2, \qquad (4.5)^9$$

$$A^{\bullet -} + A^{\bullet -} + H^+ \rightarrow A + HA^-. \qquad (4.6)^{10}$$

III. Subsequent reactions

1. *Decomposition of hydrogen peroxide*

$$H_2O_2 + PcCo^{II} \rightarrow PcCo^{III}\,(HO^-) + {}^{\bullet}OH, \qquad (4.7)$$

$$H_2O_2 + O_2^{\bullet -} + H^+ \rightarrow O_2 + H_2O + {}^{\bullet}OH, \qquad (4.8)^9$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow HO_2^{\bullet} + H_2O, \qquad (4.9)^{11}$$

$$PcCo^{III}(OH) + H_2O_2 \rightarrow \text{destruction of PcCo}. \qquad (4.10)$$

2. *Reactions of OH$^{\bullet}$ radicals*

$$^{\bullet}OH + PcCo^{II} \rightarrow PcCo^{III}(OH), \qquad (4.11)$$

$$^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2, \qquad (4.12)^{9,12}$$

$$^{\bullet}OH + O_2^{\bullet -} \rightarrow OH^- + O_2, \qquad (4.13)^9$$

$$^{\bullet}OH + AH^- \rightarrow A^{\bullet -} + H_2O. \qquad (4.14)^{13}$$

Reaction	k/mol L^{-1} s^{-1}	Reaction	k/mol L^{-1} s^{-1}
4.2	$5.75 \cdot 10^4$	4.8	6.4
4.3	$2.6 \cdot 10^8$	4.9	$3.7 \cdot 10^7$
4.4	$5 \cdot 10^2$	4.12	$5 \cdot 10^9$
4.5	<100	4.13	$1 \cdot 10^{10}$
4.6	$1 \cdot 10^6$	4.14	$1 \cdot 10^{10}$

When reactions occur in biological objects, one has to consider three additional termination stages (4.15), which, actually, form the basis of the therapeutic effect of the TP—AH_2—O_2 catalytic system. These stages are considered as termination stages because radicals which can be formed are more stable than the initial radicals and cannot propagate the kinetic chain.

$$\left.\begin{matrix} {}^{\bullet}OH \\ O_2^{\bullet -} \\ A^{\bullet -} \end{matrix}\right| + \text{cell organelles} \rightarrow \text{cell damage} \qquad (4.15)$$

Stages (4.7) and (4.10) reflect the participation of TP in hydrogen peroxide decomposition. The catalytic ac-

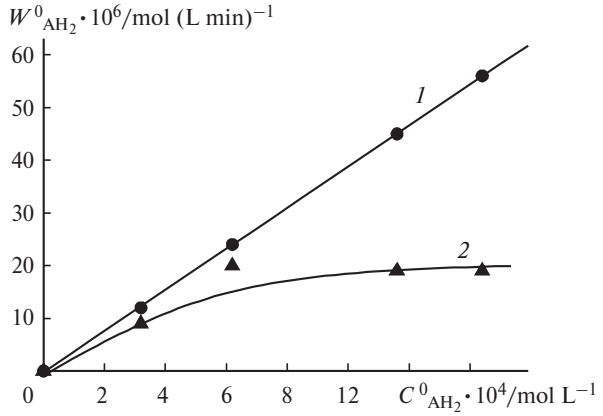


Fig. 3. Influence of superoxide dismutase (SOD) on the rate of AH_2 oxidation in the presence of Teraphthal: in the absence (*1*) and presence of SOD (*2*), C_{SOD} = 0.05 g L^{-1}, C_{TP} = $0.35 \cdot 10^{-4}$ mol L^{-1}.

tivity of cobalt phthalocyanine in this reaction is well known.[14—16]

We confirmed that the $O_2^{\bullet -}$ radical anion is involved in the oxidation of AH^- by a series of experiments in which superoxide dismutase (SOD) catalyzing $O_2^{\bullet -}$ disproportionation was introduced into the reaction mixture. The reaction rate decreases in the presence of SOD, and the magnitude of the effect depends on the ratio of the concentrations of ascorbic acid to SOD (Fig. 3).

Comparison of the rate constants for different stages suggests that the stage of the reaction of the monoanion with the $^{\bullet}OH$ radical can substantially contribute to the rate of ascorbic acid oxidation. According to the scheme proposed, the $^{\bullet}OH$ radicals necessary for the reaction are generated upon the decomposition of hydrogen peroxide, *viz.*, intermediate reaction product. If this assumption is valid, a decrease in the steady-state concentration of H_2O_2 should decrease the rate of ascorbic acid oxidation. We found that the introduction of catalase (Cat) decreases the reaction rate (Fig. 4). The limiting magnitude of the effect indicates that more than 80% ascorbic acid are consumed in the route of its reaction with hydrogen peroxide or its decomposition products, *i.e.*, $^{\bullet}OH$ radicals. However, it cannot be excluded that the effect of catalase is associated with the protein, which is the catalase apoenzyme, rather than with the main function of catalase. The catalase apoenzyme, like albumin (see below), can sharply decrease the activity of Teraphthal in catalysis of ascorbic acid oxidation.

The substantial role of the $^{\bullet}OH$ radicals in the process under study was confirmed by the reactions in the presence of *p*-nitroso-*N*,*N*-dimethylaniline (NDMA), which is the known trap for these radicals.[17,18] The introduction of NDMA in the equimolar (with respect to the catalyst) concentration ($3.5 \cdot 10^{-5}$ mol L^{-1}) results in both a decrease in the rate of catalytic oxidation of ascorbic acid (Fig. 5) and consumption of NDMA. Further increase in the NDMA concentration produces more complicated effects due to the interaction of TP with the trap.

The participation of the highly reactive $^{\bullet}OH$ radicals in the process is additionally argued by the fact that, according to the EAS, Teraphthal is destroyed during the oxidation of AH_2.

2. pH-Dependence of the rate of oxidation of ascorbic acid

The TP+AH_2 system in aqueous solutions is characterized by phase stability only at pH ≥ 6.4. At pH < 6.0, TP is transformed into the corresponding acid insoluble in water. At pH 6.0—6.3, TP in a solution is strongly aggregated and precipitates when AH_2 is introduced (pH of the solution remains unchanged). The maximum rate of the catalytic reaction is observed at pH ~6.5 (Fig. 6).

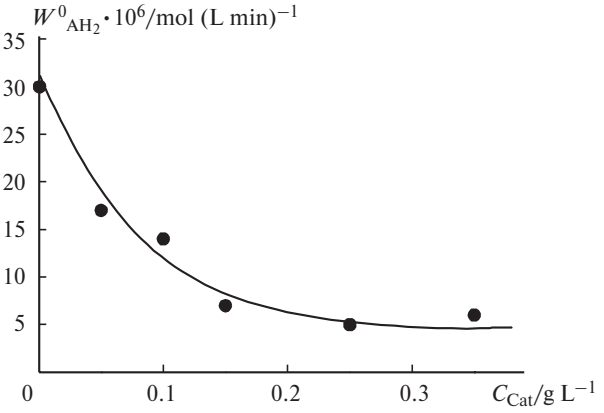


Fig. 4. Initial rate of ascorbic acid oxidation ($W^0_{AH_2}$) as a function of the catalase concentration; C_{TP} = $0.35 \cdot 10^{-4}$ mol L^{-1}, $C_{AH_2} = 8 \cdot 10^{-4}$ mol L^{-1}.

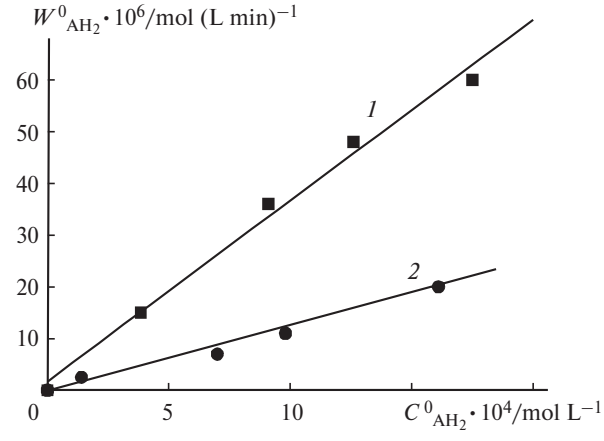


Fig. 5. Initial rate of AH_2 oxidation ($W^0_{AH_2}$) as a function of $C^0_{(AH_2)}$ in the presence of TP (*1*) and TP+NDMA (*2*); C_{TP} = $3.5 \cdot 10^{-5}$ mol L^{-1}, $C_{NDMA} = 3.5 \cdot 10^{-5}$ mol L^{-1}.

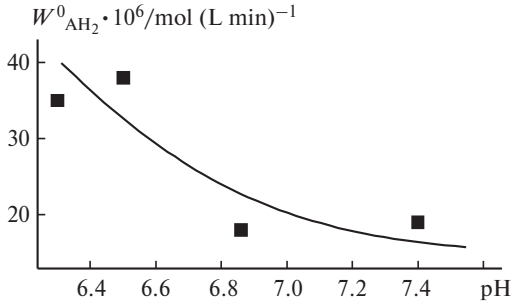


Fig. 6. Initial rate of ascorbic acid oxidation ($W^0_{AH_2}$) as a function of the pH: $j = 0.1$; $C_{TP} = 0.35 \cdot 10^{-4}$ mol L^{-1}, $C_{AH_2} = 7.0 \cdot 10^{-4}$ mol L^{-1}.

Table 1. Rate of AH_2 oxidation in the presence of TP at different ionic strengths of the solution

$C^0_{AH_2} \cdot 10^4$ /mol L^{-1}	$W^0_{AH_2} \cdot 10^6$ /mol (L min)$^{-1}$		
	I	II	III
3.5	8	20	18
7.0	18	33	30
10.5	25	40	38

Note. Conditions: pH 6.9, $C_{TP} = 0.35 \cdot 10^{-4}$ mol L^{-1}; I — $j = 0.1$ (phosphate buffer), II — $j = 0.2$ (NaCl), III — $j = 0.2$ ($NaClO_4$).

The dependence of the reaction rate on the ionic strength of the solution (Table 1) is in complete accordance with the assumption about the interaction of the similarly charged ions (monoanion AH^- and polyanion, which is a complex of TP with dioxygen) in the limiting stage. The effect of the ionic strength is independent of the nature of the salt, which creates this ionic strength. This fact rules out alternative explanations, such as an axial coordination of the anion of the salt.

3. Influence of albumin on the rate of oxidation of ascorbic acid in the presence of Teraphthal

The challenges of using TP in therapy of oncological diseases caused urgency of the study of its catalytic properties under conditions simulating biological media. This concerns, first, the problem of the influence of albumin, which is the main transport protein, whose concentration in the blood is 35—50 g L^{-1} or $(5.2—7.5) \cdot 10^{-4}$ mol L^{-1}.

3.1. *Interaction of Teraphthal, albumin, and ascorbic acid*

Some information on the interactions of the components of the catalytic system with albumin was obtained by analysis of the electronic absorption spectra of the individual components, pairs, and the whole system.

Comparison of the EAS of solutions of TP and a TP—BSA mixture shows that the spectra are identical within the experimental error. This implies that either no interactions of TP with the protein occur or such interactions are not manifested in the EAS. In our opinion, the latter is more probable because albumin is known to interact with a broad array of compounds, including those containing charged fragments (anions, cations)[19] and developed aromatic structures, for example, anthraquinone.[20]

When solutions of BSA and AH_2 are mixed, the intensity of the band with $\lambda = 279$ nm increases sharply, which, according to published data,[21,22] indicates a change in the conformation of the protein molecule. In our case, this is a consequence of the interaction of ascorbic acid with albumin. According to the EAS data, this interaction is disturbed when Teraphthal is introduced in concentrations above some value minimum for this ratio of the acid to protein. In other words, TP prevents the formation of adducts of BSA with AH_2 because it likely binds to the protein globule.

3.2. *Catalytic activity of TP in the oxidation of ascorbic acid in the presence of albumin*

We carried out three series of experiments in which the TP concentration differed by an order of magnitude ($0.35 \cdot 10^{-5}$ and $0.35 \cdot 10^{-4}$ mol L^{-1}) and the C_{TP}/C_{BSA} ratio and the interval of C_{AH_2} change corresponded to the region in which, according to the above data, no changes in the albumin conformation occur.

The experiments showed (Fig. 7) that for $C_{BSA}/C_{TP} \geq 1$ the rate of AH_2 oxidation decreases sharply. The influence of albumin on the reaction rate is much less pronounced in the systems where C_{TP} substantially exceeds C_{BSA}.

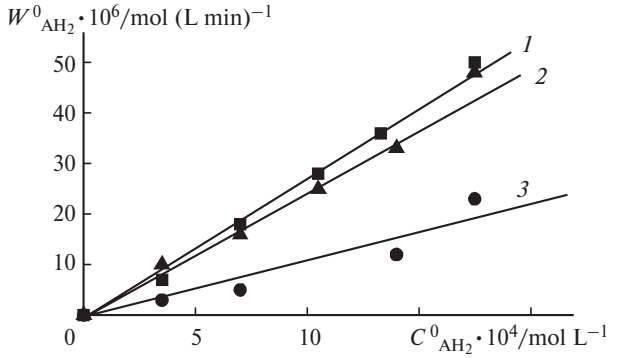


Fig. 7. Initial rate of AH_2 oxidation ($W^0_{AH_2}$) in the presence of TP ($C^0_{TP} = 0.35 \cdot 10^{-4}$ mol L^{-1}) as a function of the initial concentration of AH_2 in the absence (*1*) and presence of BSA (*2*, *3*): $2.3 \cdot 10^{-6}$ (*2*) and $1.08 \cdot 10^{-5}$ mol L^{-1} (*3*).

Thus, the results of catalytic studies evidence the interaction of TP and albumin in solutions and also show that TP in a complex with the protein looses its catalytic activity. These results are of great significance for anticancer therapy: when TP is injected into the blood, it does not catalyze the oxidation of AH_2, *i.e.*, the reaction does not occur in the blood and can begin only after TP was transported to the cell and its complex with albumin dissociated.

These results along with the corresponding published data[23—26] suggest the most probable types of effects of intermediates of the catalytic system on the cell. These effects are responsible for its high cytotoxicity with respect to malignant cells.

The data of our studies leave virtually no doubts that the ascorbate radical, superoxide radical anion, hydrogen peroxide, and hydroxyl radicals are formed in the system. According to the published data,[23,24] the former is cytotoxic and can play an independent role. Three others form a successive chain of intermediates, which in a normal cell could be destroyed or rendered harmless by special protective systems designed for oxidative stress control. However, the content of superoxide dismutase, catalase, vitamin A, and other antioxidants in tumor cells is lower than in normal cells.[24—26] Therefore, under conditions of fast local ejection of strong cytotoxic agents, this apparatus unlikely perform its functions to an entire extent, due to which the cytotoxic properties of the discussed catalytic system can appear. In addition, the oxidation of ascorbic acid in a tumor decreases the oxygen level in the cell, serving as yet another factor for the decay of cancerous cells.[24]

References

1. Patent RF No. 2106146, 1997, US Pat. 6,004,953, Dec. 21, 1999.
2. M. E. Vol´pin, N. Yu. Krainova, I. V. Moskaleva, G. N. Novodarova, G. N. Vorozhtsov, M. G. Gal´pern, O. L. Kaliya, E. A Luk´yanets, and S. A. Mikhalenko, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 2105 [*Russ. Chem. Bull.*, 1996, **45**, 2000 (Engl. Transl.)].
3. S. A. Borisenkova, E. G. Girenko, and O. L. Kaliya, *Ros. Khim. Zh.*, 1998, 111 [*Mendeleev Chem. J.*, 1998 (Engl. Transl.)].
4. V. S. Akatov, Yu. V. Evtodienko, and A. I. Medvedev, *Dokl. Akad. Nauk*, 2000, **373** [*Dokl.*, 2000 (Engl. Transl.)].
5. Y. Ogata, K. Marumo, and Takao Kwan, *Chem. Pharm. Bull.*, 1969, **17**, 1194.
6. N. Miyaka, M. Kim, and T. Kurata, *Biosci., Biotech., Biochem.*, 1997, **61**, 1693.
7. (a) A. D. Nadezhhdin and H. B. Dunford, *Can. J. Chem.*, 1979, **57**, 3017; (b) D. E. Cabelli and B. H. J. Bielskii, *J. Phys. Chem.*, 1983, **87**, 1809.
8. O. S. Fedorova, Ph. D. (Chem.) Thesis, Novosibirsk, 1979, 20 pp. (in Russian).
9. A. Ya. Sychev and V. G. Isak, *Gomogennyi kataliz soedineniyami zheleza* [*Homogeneous Catalysis by Iron Compounds*], Shtiintsa, Kishinev, 1988, 216 pp. (in Russian).
10. S. Lagercrantz, *Acta Chem. Scand.*, 1964, **18**, 562.
11. A. Ya. Sychev, S. O. Travin, G. G. Duka, and Yu. I. Skurlatov, *Kataliticheskie reaktsii i okhrana okruzhayushchei sredy* [*Catalytic Reactions and Environmental Protection*], Shtiintsa, Kishinev, 1983, 272 pp. (in Russian).
12. T. Shiga, *J. Phys. Chem.*, 1965, **69**, 3805.
13. D. I. Metelitsa, *Modelirovanie okislitel´no-vosstanovitel´nykh fermentov* [*Modeling of Redox Enzymes*], Nauka i Tekhnika, Minsk, 1984, 293 pp. (in Russian).
14. M. R. Tarasevich and K. A. Radyushkina, *Kataliz i elektrokataliz metalloporfirinami* [*Catalysis and Electrocatalysis by Metal Porphyrins*], Nauka, Moscow, 1982 (in Russian).
15. H. Shirai, S. Higaki, and K. Hanabusa, *J. of Polymer Sci.: Polym. Chem. Ed.*, 1984, **22**, 1309.
16. H. Shirai, A. Maruyama, and J. Takano, *Macromol. Chem.*, 1980, **181**, 565.
17. G. P. Pirumyan, A. N. Martiryan, and Yu. I. Skurlatov, *Armyanskii Khim. Zh.* [*Armenian Chemical J.*], 1989, **42**, 71 (in Russian).
18. C. N. Trumbore and J. Kraljic, *J. Amer. Chem. Soc.*, 1965, **87**, 2547.
19. Yu. B. Filippovich, *Osnovy biokhimii* [*Fundamentals of Biochemistry*], Vysshaya Shkola, Moscow, 1993, 87 (in Russian).
20. V. M. Stepanov, *Molekulyarnaya biologiya. Struktura i funktsii belkov* [*Molecular Biology. Structure and Functions of Proteins*], Vysshaya Shkola, Moscow, 1996, 335 pp. (in Russian).
21. Theodore Peters J., Jr., *All about Albumin* (*Biochemistry, Genetics and Medical Applications)*, Acad. Press, Inc., San Diego, CA, 1997, p. 442.
22. *Biopolymers*, Ed. Y. Imanishi, Ser. *Kobunshi Jikkegaki Koza* (in Japan).
23. H. Sakagami, K. Satoh, and H. Ohata, *Anticancer Research*, 1996, **16**, 2635.
24. K. Asano, K. Satoh, and M. Hosaka, *Anticancer Research*, 1999, **19**, 229.
25. T. K. Basu, D. Donaldson, and M. Jenner, *Br. J. Cancer*, 1976, **33**, 119.
26. A. Bozzi, I. Mavelli, and B. Moondovi, *Cancer Biochem. Biophys.*, 1979, **3**, 135.

Received October 17, 2001;
in revised form February 13, 2002