ANTIRADICAL ACTIVITY OF COPPER(II) COUMARIN CHELATES

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UDC 617.747:616.831

KEY WORDS: chemiluminescence; lipid peroxidation; antioxidants; copper(II) complexes; coumarin ligands

Metalloenzyme modeling is a trend in bioinorganic chemistry whereby, using only simple resources, it is possible not only to make a contribution to our understanding of the mechanisms of enzymic catalysis [2], but also to search for active agents. Convincing examples may include synthesis and study of the properties of carboxylate complexes of the transition metals, notably copper and manganese, possessing catalytic activity of superoxide dismutase (SOD) [6, 11]. A remarkable feature of the best studied of the salicylate complexes of copper and manganese is that not only are they not inferior in catalytic activity to their own prototype (and superoxide dismutase is one of the most active enzymes [7]), but may often exceed it [13]. SOD is a component of the intrinsic antioxidant protective system of the organism [7] and administration of exogenous SOD is of therapeutic value [9]. Investigation of salicylate complexes of copper(II) has led to the discovery of the high anti-inflammatory, antitumor, radioprotective, and anticonvulsant activity [11], which is connected in all probability with their ability to inhibit the superoxide anion-radical. In the investigation described below the antiradical activity of new carboxylate chelates of copper(II), based on coumarin ligands, was studied.

EXPERIMENTAL METHOD

The test objects were carboxylate complexes of copper(II) based on coumarin ligands: PQ 92 (I), PQ 83-3 (II), PQ-156-3 (III), PQ 101 (IV). For comparison we used salicylate complexes of copper and manganese: bis/salicylato/di/aquo/copper(II) dihydrate (V) and salicylato/tris/aquo/manganese(II) semihydrate (VI), and also the commercial antioxidant (AO) ionol, and superoxide dismutase. A standard chemiluminescent system (SCS) was prepared from lipoproteins from hens' egg yolks [4]. The lipoprotein suspension prepared in phosphate buffer (40 mM KH_2PO_4, 100 mM KCl, pH 7.47) was kept at 4°C for a week. LPO was induced in SCS by the addition of Fe^{2+} ions in a final concentration of 2.5 mM. The measurements were made at 37°C with constant mixing. Levels of chemiluminescence were measured in selected samples by the use of an apparatus described in the monograph [1], and LPO levels were estimated in parallel tests on the basis of accumulation of products reacting with TBA (the TBA test). To study the mechanism of the antioxidative action of copper-containing carboxylate complexes based on coumarin ligands, their action also was studied on chemiluminescence (Chl) of liposomes. Phospholipids for preparation of liposomes were isolated from hens' egg yolks by the method in [5]. Chl of liposomes was induced by the addition of ferrous ions in a final concentration of 100 μM. Measurements were made with constant mixing in Tris-HCl buffer at 37°C (pH 7.4).

N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 5, pp. 479-481, May, 1992. Original article submitted April 8, 1991.

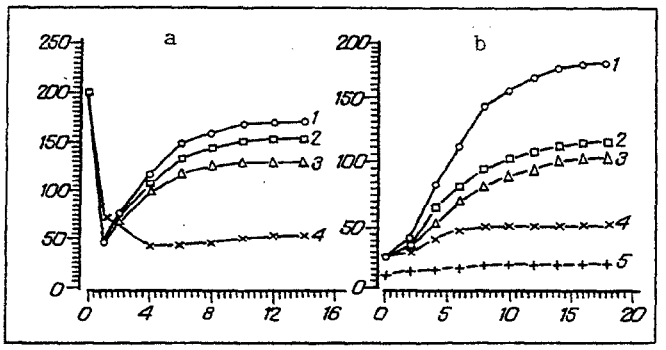

Fig. 1. Kinetic curves of iron-dependent chemiluminescence of egg yolk lipoproteins in the presence of various substrate concentrations: A) SOD: 1) 0 unit/ml, 2) 30 units/ml, 3) 70 units/ml, 4) 150 units/ml; B) preparation III: 1) 0 nM, 2) 0.71 nM, 3) 7.1 nM, 4) 7.1 nM, 5) 710 nM. Abscissa, time (n min). Ordinate, intensity of chemiluminescence (conventional units).

EXPERIMENTAL RESULTS

This investigation showed that carboxylate complexes of copper(II) based on coumarin ligands, in concentrations of 10^{-8}-10^{-3} M, exhibit the properties of inhibitors of chemiluminescence induced by Fe^{2+} ions in SCS. Under these circumstances the shape of the chemiluminescence curves remained unchanged but the amplitude of the luminescence was reduced with an increase in concentration of the preparation added. This is clearly seen in Fig. 1, in which curves showing dependence of chemiluminescence kinetics on concentration for SOD (Fig. 1a) and for a carboxylate complex of copper III (Fig. 1b) are compared. Similar curves also were obtained for the other compounds studied. These data show that copper complexes of coumarins possess antioxidative activity, whose magnitude can be expressed through the concentration of the antioxidant reducing the intensity of Chl by half. These concentrations and the quantity of TBA-active products are given in Table 1.

These concentrations, as will be seen, were about equal for ionol and compounds II, V, and VI, whereas compounds I, III, and IV undoubtedly quenched Chl more effectively than they inhibited MDA formation. The latter may be due to the fact that these copper complexes are quenchers of excited states of molecules as well as their true antioxidant action.

What is the mechanism of the antioxidant action of copper chelates with coumarin? The most natural answer is to suggest that it is similar to the mechanism of action of the copper-containing antioxidant enzyme SOD. To confirm the correctness of this hypothesis to some degree, we studied the action of SOD and of compound III on the kinetics of chemiluminescence of liposomes. The results are given in Figs. 2a and 3a for SOD and Figs. 2b and 3b for compound III. Comparison of these curves shows that the action of the two substances is similar, namely: 1) the amplitude of the slow flash of chemiluminescence is reduced (Fig. 2a, b); 2) the amplitude of the steady-state emission is reduced; 3) on the addition of these compounds to the stage of steady-state emission, a flash of chemiluminescence arises, probably due to hydrogen peroxide formation from superoxide radicals (Fig. 3a, b). In this respect SOD and compound III differ from the effects of other coumarin antioxidants, which, when added a second time, do not give flashes of Chl. Moreover, it has to be pointed out that on the addition of compound III in a concentration of $3 \cdot 10^{-6}$ M the latent period was lengthened, whereas SOD shortened it (data not given). This suggests that the antioxidant effect of compound III also is partly due to the coumarin part of the complex. The results indicate that the determining mechanism of the antioxidative action of copper-containing chelates with coumarins is similar to the action of Cu–Mn–SOD with a certain contribution of the coumarin effect as a free radicals trap. Table 1 shows that the substances obtained differ in their antioxidative activity; activity of substances II and III was higher than that of an antioxidant so well known as ionol. In view of their ability to exchange with bio-

TABLE 1. Comparative Antioxidative Activity of Carboxylate Complexes of Copper Compounds (I-IV), and Salicylate Complexes of Copper, Manganese, and Ionol

Compound	Concentration of antioxidants (M), reducing by half:	
	intensity of Chl at maximum	quantity of TBA-active products
I	$(1,5\pm0,1)\cdot10^{-7}$	$(9,0\pm0,5)\cdot10^{-7}$
II	$(7,0\pm0,3)\cdot10^{-8}$	$(7,0\pm0,4)\cdot10^{-8}$
III	$(3,5\pm0,2)\cdot10^{-8}$	$(9,0\pm0,5)\cdot10^{-8}$
IV	$(3,5\pm0,2)\cdot10^{-6}$	$(8,0\pm0,4)\cdot10^{-6}$
V	$(8,0\pm0,4)\cdot10^{-7}$	$(6,0\pm0,4)\cdot10^{-7}$
VI	$(1,6\pm0,2)\cdot10^{-3}$	$(2,2\pm0,3)\cdot10^{-3}$
Ionol	$(6,0\pm0,4)\cdot10^{-7}$	$(7,4\pm0,2)\cdot10^{-7}$
SOD	(125 ± 20) Units/ml	(130 ± 15) Units/ml

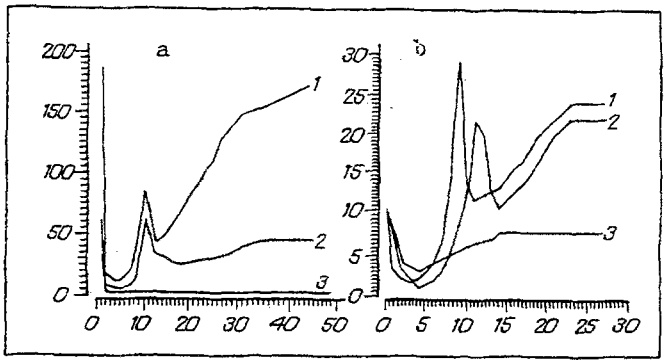

Fig. 2. Kinetic curves of iron-dependent chemiluminescence of liposomes in the presence of different substrate concentrations: a) SOD: 1) 0 unit/ml, 2) 10 units/ml, 3) 300 units/ml; b) compound III: 1) 0 μM, 2) 3 μM, 3) 10 μM.

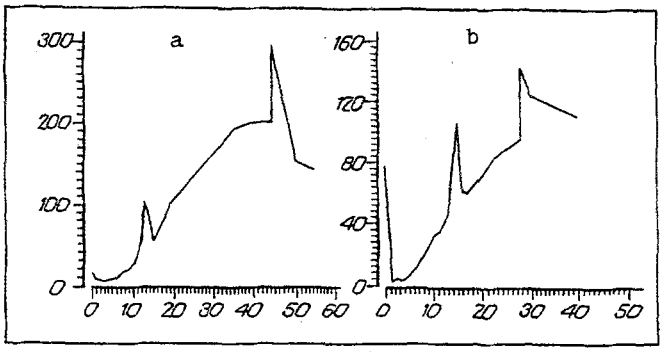

Fig. 3. Flash of chemiluminescence on addition of substrate to suspension of liposomes at stage of steady-state emission. a) SOD (300 units/ml), b) compound III (3 μM).

ligands, the carboxylate complexes of transition metals are insufficiently stable for long existence in biological systems, whereas salicylate complexes of copper(II) have been shown to have high bioaccessibility [11]. On this basis, complexes of this kind can also be regarded as transport substances for supplying trace elements to biological targets, or possessing intrinsic activity of ligands present in their composition, and this additionally justifies the search for pharmacologically active agents among complex compounds of transition metals based on coumarin and additional ligands.

All the substances studied thus possess antioxidative properties. The mechanism of the antioxidant action of copper-containing coumarins closely resembles the action of Cu-Mn-SOD.

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