ANTIRADICAL ACTIVITY OF COUMARIN RUDUCTONES

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Oxidative stress and, in particular, its clearest manifestation, namely free-radical lipid peroxidation (LPO), may be the cause of many diseases [7, 11]. In such situations the development of pathology becomes possible also due to a deficiency of the body's own antioxidant defensive system. In these cases medical intervention to correct this defect is indicated. One approach may be to use therapeutic agents to activate the intrinsic antioxidant protective system or to compensate its insufficiency. The most desirable substances for use in clinical practice are natural compounds, and it is therefore logical to look for pharmacologically active agents among the minor components of the diet, which possess the properties of antioxidants (AO), and such research is being successfully carried out [5, 8].

The most extensive group of natural phenolic AO is the coumarins [10]. The availability, simple structure, and ease of their chemical modification make the coumarins convenient "synthons" for conversion into other types of AO [3]. Previously [2] we examined 3-heterosubstituted coumarins, the AO properties of which are determined by their structural analogy with dehydroalanine. In the present investigation we turned to 3,4-diheterosubstituted coumarins, which structurally speaking are analogs of ascorbic acid. AO of this type have been given the group name "reductones" [12].

EXPERIMENTAL METHOD

To study the ability of 3-amino-4-oxycoumarin (I), 3,4-diaminocoumarin (II), 3-amino-4-methylaminocoumarin (III), 3-amino-4-benzylaminocoumarin (IV), 3-amino-4-di-*p*-propylaminocoumarin (V), and 3-amino-4-*s*-benzylcoumarin (VI) to inhibit LPO, a standard chemiluminescence system (CLS) was prepared from lipoproteins from hens' egg yolks [4]. A suspension of lipoproteins was prepared in phosphate buffer (40 mM KH_2PO_4, 10 mM KCl), pH 4.7 and kept at 4°C for a week. Induction of LPO in the CLS was carried out by introducing Fe(II) ions into it in a final concentration of 2.5 mM. Measurements were made at 37°C with constant mixing. Levels of chemiluminescence (ChL) were measured in samples by an apparatus described in the monograph [1], and LPO levels in parallel samples were estimated from the accumulation of products reacting with thiobarbituric acid (the TBA test) [9]. For comparison, the synthetic phenolic AO ionol and a natural reductone, namely ascorbic acid, were used.

EXPERIMENTAL RESULTS

The structural similarity between the coumarin reductones to that of ascorbic acid, which plays an important role in the natural AO protective system of man and other animals [6], suggests that the coumarin reductones may closely resemble ascorbic acid in other properties also. To study the AO action of coumarin reductones the effect of these substances was studied on the intensity of ChL of a CLS. Kinetic curves for coumarin IV are given in Fig. 1. It can be seen that with an increase in concentration of coumarin IV, as of all other coumarin reductones studied, the intensity of Chl of the CLS was observed to diminish. The comparative antioxidative activity for all preparations, determined as the concentration reducing the intensity of ChL by half at the maximum, is given in Table 1, which also gives data on antioxidative

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TABLE 1. Comparative Antioxidative Activity of Coumarin Reductones, Ionol, and Ascorbic Acid (M ± m)

Compound	Conc. of AO (in moles/liter) reducing by air		Coeff. of correlation for the dependence	
	intensity of ChL at maximum	Concentration of products reacting with TBA	$I_0-I/\sqrt{I_0 \cdot I}=f(C)$	$I/I_0=f$ [lg (1/C)]
I	$(3,4\pm0,1)\cdot10^{-6}$	$(7,0\pm0,2)\cdot10^{-6}$	0,88	0,91
II	$(4,0\pm0,3)\cdot10^{-7}$	$(3,5\pm0,2)\cdot10^{-6}$	0,35	0,83
III	$(3,8\pm0,4)\cdot10^{-7}$	$(8,0\pm0,3)\cdot10^{-7}$	0,82	0,84
IV	$(1,0\pm0,1)\cdot10^{-7}$	$(1,8\pm0,1)\cdot10^{-6}$	0,86	0,99
V	$(7,5\pm0,6)\cdot10^{-6}$	$(9,2\pm0,4)\cdot10^{-6}$	0,59	0,94
VI	$(2,2\pm0,1)\cdot10^{-5}$	$(1,5\pm0,1)\cdot10^{-4}$	0,94	0,96
Ionol	$(6,0\pm0,4)\cdot10^{-7}$	$(7,4\pm0,2)\cdot10^{-7}$	0,90	0,72
Ascorbic acid	$(1,8\pm0,1)\cdot10^{-4}$	—	0,91	0,96

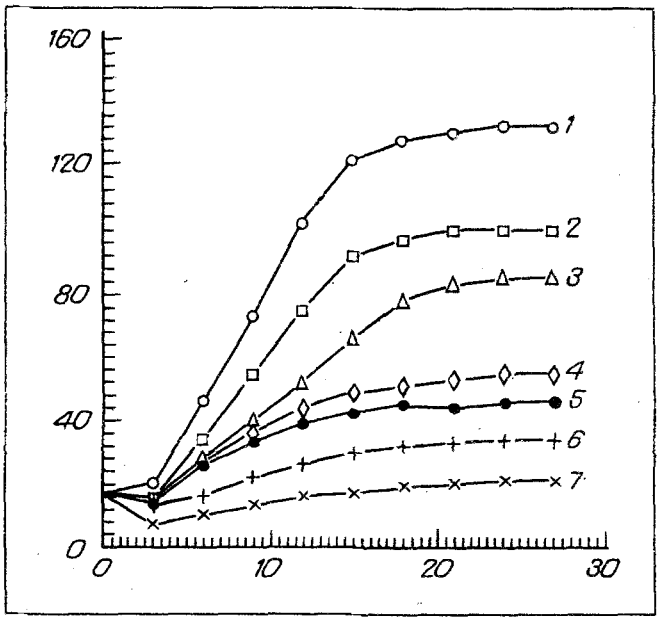

Fig. 1. Kinetic curve of iron-dependent ChL of yolk lipoproteins in presence of coumarin IV in different concentrations. Abscissa, time (in min); ordinate, intensity of ChL (in conventional units). Concentration of coumarin: 1) 0 nM; 2) 0.5 nM; 3) 1.05 nM; 4) 2.66 nM; 5) 5.53 nM; 6) 10.7 nM; 7) 21.4 nM.

activity, determined from accumulation of LPO products in the CLS. The measure of antioxidative activity in this case also was the concentration of compounds reducing the malonic dialdehyde (MDA) concentration by half. Dependence of the intensity of ChL and the content of products reacting with TBA on the concentration of added ionol (Fig. 2a) and coumarin IV (Fig. 2b), and also dependence of the intensity of ChL on concentration for coumarin IV (Fig. 2c), are shown in Fig. 2. It will be clear from Fig. 2 that concentrations reducing MDA and ChL by half differ. All curves for MDA run at a higher level, so that 50% inhibition concentrations, determined as accumulation of LPO products (TBA test) are higher than those determined from ChL (Table 1). It can be tentatively suggested that coumarin reductones, like ionol, possess both antioxidative activity and ability to reduce the quantum yield of ChL.

The compounds studied were found to be highly active AO. As Table 1 shows, compounds I-VI are much superior to ascorbic acid in their AO activity, and are on the same level as ionol. The antiradical activity of the coumarin reductones has been studied previously in a biochemical test system by the method of quenching of ChL arising in a system consisting of horseradish peroxidase – 3-oxypyridine – H_2O_2 [14]. It was found that in this system, which consists mainly of radicals of water-soluble compounds, the antiradical activity of the coumarin reductones was very high. The question arises: what is the mechanism of the AO activity of the coumarin reductones which we studied. By their mechanism of action, inhibitors of radicals can be divided into two types: inhibitors of lipid radicals (ionol) and substances acting on the state of Fe(II) and, possibly, radicals in the aqueous phase. Ascorbic acid belongs to the second type of AO. The present investigation was not

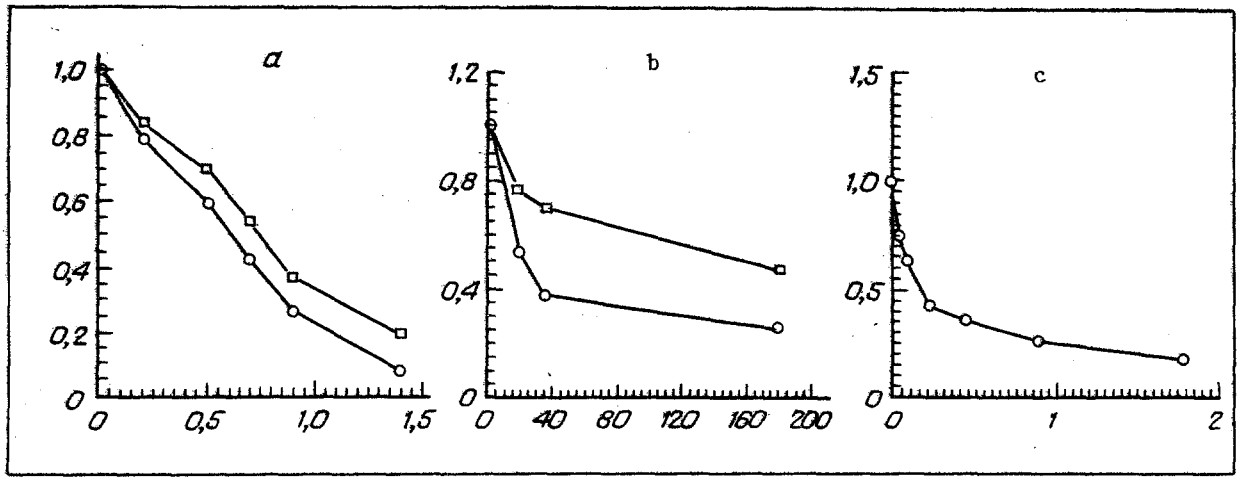

Fig. 2. Dependence of intensity of ChL (II) and accumulation of TBA-reacting products (I) on concentration of: ionol (a), coumarin VI (b), and coumarin IV (c). Abscissa, concentration of substrates: a) ionol (in μM), b) coumarin VI ($\cdot 10^{-4}$ M), c) of coumarin (in nM); ordinate, intensity (in relative units). MDA concentration (in relative units) on right.

undertaken to make a detailed study of the mechanism of action of coumarin reductones. Nevertheless, we attempted to answer the question: to what type of antiradical compounds do the substances we have studied belong. To answer this question we compared the dependence of the intensity of ChL on concentration of AO. We found that this dependence I—f(C) differs significant for ionol and ascorbic acid. In the case of ionol it was shown previously [4] that the dependence $I_0 - I/(I_0/I) = f(C)$, where I_0 stands for the intensity of the slow flash without addition of AO; I the intensity of the slow flash with AO; C the concentration of added AO, is linear, so that it is possible to estimate the antioxidative activity of drugs with the same mechanism of action. We studied the analogous relationship for coumarin reductones, but in every case it proved to be far from linear. This, in particular, can be seen from Table 1. Coefficients of correlation of the parameter $I_0I/(I_0/I)^{1/2}$ and the C concentration vary from 0.35 to 0.94 for different coumarin reductones, evidence that the mechanism of action of reductones differs from the mechanism of action of ionol. Next we attempted to choose several empirical equations which would give a near-linear dependence of the function of intensity on concentration. Of the relationships I = f(1/C), 1/I = f(C), 1/I = f(1/C), the dependence I = f[Zg(1/C)] was found to be most suitable. Table 1 gives coefficients of linear correlation between the parameters $I_0 - I/(I_0/I)^{1/2}$ and C and between I/I_0 and f[Zg(1/C)]. It is very important that a linear dependence with a high coefficient of correlation for the function $I/I_0 = f[Zg(1/C)]$ was given by ascorbic acid, whereas ionol gave a distinctly nonlinear dependence. According to this feature, the test compounds were found to be closer to ascorbic acid than to ionol, for in the last coordinates they showed a clearly linear relationship (high coefficients of correlation in Table 1).

Coumarin reductones have only recently attracted attention as physiologically active agents. Trials of 3,4-dihydroxy-substituted coumarins as antiatherosclerotic agents [13] have revealed their high antiaggregative activity, which the authors cited attribute to their oxidation—reduction properties (at pH 7.4 their redox potentials are similar in value to the redox potential of ascorbic acid) [13].

The results of this investigation indicate high antioxidative activity of the coumarin reductones, so that their antioxidant properties can be chosen as the basis for the search for pharmacologically active compounds in this series. With respect to the mechanism of their antioxidative activity, the coumarin reductones resemble their structural analog — ascorbic acid, and they differ appreciably from the phenolic AO — ionol (butylated hydroxytoluene).

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SUBSTRATE SUPPLY FOR ENERGY HOMEOSTASIS DURING STARVATION

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KEY WORDS: lipid and carbohydrate substrates; starvation; energy metabolism.

Starvation has been used in clinical practice for a long time as a method of treatment of obesity and various diseases connected with disturbances of lipid metabolism. Nevertheless, the physiological mechanisms of its therapeutic action contain many unsolved problems. Two effects of starvation are not in dispute: increased utilization of endogenous fat for the energetic needs of the tissues and stimulation of glycolysis for the same purpose. However, the use of fatty acids (FA), the basic component of fat, for carbohydrate biosynthesis is denied for mammals and man in most publications.

Sporadic biochemical and ultrastructural data have recently been published on the role of FA as substrates for glucose and glycogen formation [4, 5, 12, 13].

This paper describes a combined ultrastructural and biochemical investigation of the state of the principal lipid and carbohydrate substrates of mammals involved in energy metabolism during starvation.

EXPERIMENTAL METHOD

Experiments were carried out on 235 noninbred male rats weighing initially 300-350 g. Thirty of these rats served as the control, and remained intact, whereas the rest were subjected to total alimentary starvation, with no restriction on drinking. A daily study of fine structural changes in the liver cells was carried out by means of the IEM-7A electron microscope from the beginning of starvation until death of the animals, which usually occurred between the 7th and 14th days. In parallel experiments, uptake of lipids (staining with Sudan) and glycogen (PAS reaction) incorporation in hepatocytes and leukocytes was determined in semithin sections through the liver and in blood films, by cytochemical methods. Each day of starvation data were obtained from five animals. Biochemical investigation of the blood was carried out daily

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