ANTIRADICAL ACTIVITY OF 3-SUBSTITUTED COUMARINS AND THEIR EFFECT ON IRON-DEPENDENT CHEMILUMINESCENCE

Yu. A. Vladimirov, É A. Parfenov, O. M. Epanchintseva, and L. N. Smirnov

UDC 615.272.014.425:547.857.51].07

KEY WORDS: chemiluminescence; lipid peroxidation; antioxidants; 3-substituted coumarins; iron chelating agents.

Oxidative stress, which is based on activation of molecular oxygen by ions of the transitional metals (mainly iron) not present in a composition of biological structures, is now considered to be the cause of many pathological states (radiation damage, inflammatory states, autoimmune diseases, cancer, diseases of the cardiovascular and central nervous system, aging) or makes an important contribution to their molecular picture [6]. To prevent a pathological state, activity of the intrinsic antioxidant protective system of the body [7] is of the utmost importance, and if it is deficient, the administration of extrinsic antioxidants (AO) assumes a therapeutic role. However, it must be recalled that synthetic AO exhibit the properties of xenobiotics [8]. It is clear that the search for pharmacologically active AO must preferably be undertaken among biotics. Meanwhile, AO which are minor components of the diet, especially of vegetable origin [5], are known to have a therapeutic value. Of these natural AO, the extensive group of phenolic AO obtained from plants, and in particular, coumarins and flavonoids, have attracted considerable attention. The coumarins also have many other attractions: they are easily accessible, they have a simple structure, and they can be used as intermediates for constructing other types of AO [2].

In this investigation we studied the properties of 3-substituted coumarins.

EXPERIMENTAL METHOD

To study the ability of previously known coumarins, namely 3-aminocoumarin (I), 3-oxycoumarin (II), 3-acetylaminocoumarin (III) and 3-coumarinocarboxylic acid (IV) to inhibit lipid peroxidation (LPO), a standard chemiluminescence system (SChS) was constructed from hens' egg yolk lipoproteins [3]. A suspension of lipoproteins in phosphate buffer (40 mM KH_2PO_4, 100 mM KCl, pH 7.47) was kept at 4°C for 1 week. LPO in SChS was inhibited by the addition of Fe(II) ions to it in a final concentration of 2.5 mM. Measurements were made at a temperature of 37°C with constant mixing. The levels of chemiluminescence (ChL) in the test samples were measured by means of the apparatus described in [1], and the LPO level in parallel tests was estimated by measuring accumulation of products reacting with thiobarbituric acid (the TBA test) [9].

EXPERIMENTAL RESULTS

The classical AO ionol and many other known AO quench ChL arising during LPO. This phenomenon is used to develop methods of determination of activity of AO [1, 3]. Data on the effect of ionol on ChL Or an SChS, which we obtained, are given in Fig 1. Clearly with an increase in concentration of the antioxidant the intensity of ChL decreases simultaneously with the decrease in the content of TBA-active products formed in the system Table 1 gives data on the antioxidative activity of ionol, determined as accumulation of LPO products.

N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'nol Biologii i Meditsiny, Vol. 112, No, 10, pp. 358-360, October, 1991. Original article submitted March 13, 1991.

0007-4888/91/0010-1398$12.50

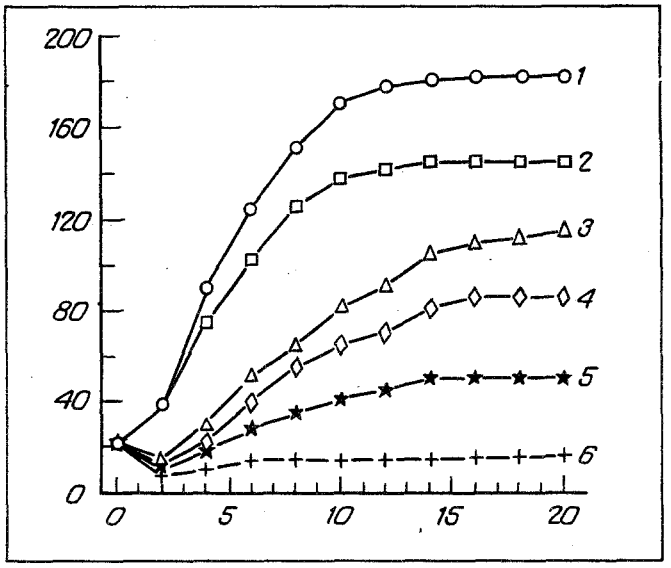

Fig. 1. Kinetic curve of ion-dependent ChL of egg yolk lipoproteins in presence of different concentrations of ionol. Ionol concentration: 1) 0 μM, 2) 0.2 μM, 3) 0.5 μM, 4) 0.7 μM, 5) 0.9 μM, 6) 1.4 μM. Abscissa, time (in min); ordinate, intensity of ChL (in conventional units).

TABLE 1. Antioxidative Properties of Coumarins I-IV, determined as Accumulation of TBA Active Products

Compound tested	C 50%, moles/liter
Ionol	$(7,4\pm0,3)\cdot10^{-7}$
Coumarin I	$(4,0\pm0,2)\cdot10^{-5}$
Coumarin II	$(5,0\pm0,4)\cdot10^{-5}$
Coumarin III	$(1,2\pm0,1)\cdot10^{-3}$
Coumarin IV	$(0,8\pm0,1)\cdot10^{-3}$

Legend. C 50%) Concentration of antioxidants (in moles/liter) depressing maximum of intensity of ChL in a standard system by half.

Clearly ionol not only quenches luminescence but also depresses MDA accumulation To study the antioxidative action of coumarins, the effect of these substances also was studied on accumulation of peroxidation products in an SChS (Table 1). It was found that in all cases all the coumarins have an equal inhibitory effect, i.e., that they are AO. The concentration of the compounds reducing the quantity of accumulated MDA by half can be used as a measure of anti-oxidative activity. These concentrations also are given in Table 1. Clearly even the most active coumarins (I and II) possess significantly weaker (by about two orders of magnitude) antioxidative activity than ionol, whereas compounds II and IV are as much as 20-25 times less active. Kinetic curves for coumarins I-IV are given in Fig. 2 and dependence of the intensity of luminescence on the concentration of the different coumarins is given in Fig. 3. In the case of coumarin IV (Figs. 2a and 3a) quenching of ChL is observed, just as in the case of ionol. However, all other coumarins gave activation of ChL in the early states of development of the process (with a maximum at about 2 min). This could be seen particularly clearly in the case of compound II (Fig. 2c), where the intensity of ChL in the course of 2 min was increased 15-fold in a concentration of 150 μM. This is clearly visible also in Fig 3c, where the intensity of ChL is shown as a function of concentration. An appreciable increase in ChL in the early stages of the process (1-8 min) also is characteristic of compound I, but at the same time the intensity is reduced in the later stages (see Fig. 2d). It was suggested previously [4] that two different ChL reactions may be responsible for the development of ChL in the presence of ferrous ions. One is connected with LPO (the

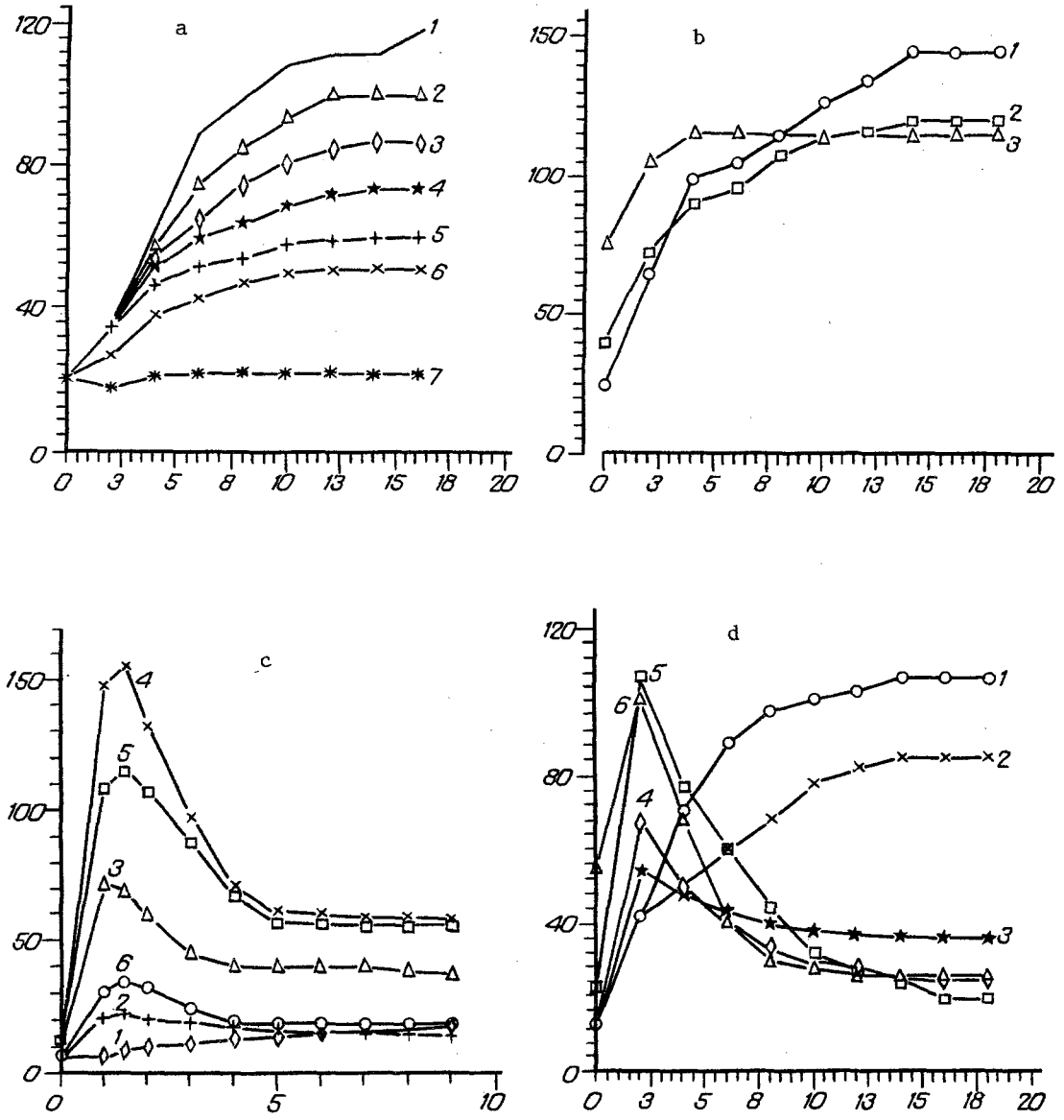

Fig. 2. Kinetic curves of iron-dependent ChL of egg yolk lipoproteins in the presence of different concentrations of substrate (in mM) a) Coumarin IV: 1) 0, 2) 0.26, 3) 0.52, 4) 1.57, 5) 2.6, 6) 3.6, 7) 4.7; b) coumarin III: 1) 0, 2) 0.24, 3) 1.2; c) coumarin II: 1) 0, 2) 0.003, 3) 0.03, 4) 0.15, 5) 0.3, 6) 1.5; d) coumarin I: 1) 0, 2) 0.03, 3) 0.09, 4) 0.155, 5) 0.3, 6) 1.55. Abscissa, time (in min): ordinate. intensity of ChL (in conventional units).

stages of fast and slow flashes of ChL), whereas the other is due to reactions of trivalent iron (the stage of stationary luminescence). It will be evident that coumarins I and II, which are AO (see Fig. 2 and Table 1), nevertheless activate ChL in the stage of the slow flash of luminescence. It can be tentatively suggested that they are activators of ChL, brought about by chain oxidation reactions of lipids. Compound III also possesses weak ability to activate luminescence of ChL at the slow flash stage and to inhibit LPO at the stationary luminescence stage. The structural formula of coumarins I-III contains an olefine fragment with electron-donor and electron-acceptor substituent located at the same center (C_3), as well as olefines possessing a so-called captodative effect [11], i.e., they stabilize the center of localization of the unpaired electron during their capture of the radical particle, i.e., they exhibit the properties of typical antioxidants.

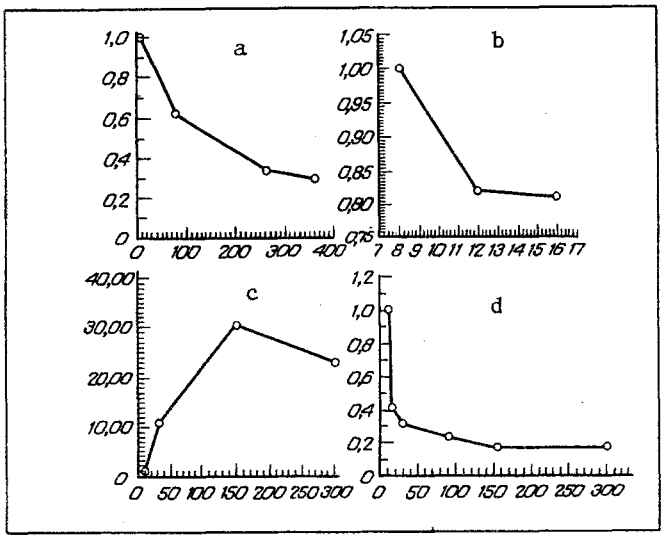

Fig. 3. Dependence of intensity of ChL on substrate concentration: a) coumarin IV; b) coumarin III; c) coumarin II; d) coumarin I Abscissa, concentration of coumarins (in μM); ordinate, intensity of ChL (in relative units).

The search for pharmacologically active compounds based on captodative olefines is a new trend in pharmacology [10]; a typical representative of these olefines is the substance AD-5,N-(*p*-methoxyphenylacetyl)-dehydroalanine, which exhibits the properties of a trap of oxygen-containing free radicals, and which inhibits LPO in rat liver microsomes Another possible mechanism of the AO action of coumarins I-III is their interaction with the LPO inducer, namely ferrous ions. To test this hypothesis we studied the effect of an olefine which has no captodative effect, but which, like coumarins I-III, can weakly bind iron ions, on ChL of coumarinocarboxylic acid (IV). It was found that compound IV quenches ChL by a method dependent on concentration (Figs. 2a and 3a), and is similar in its AO activity to coumarin III (Table 1).

LITERATURE CITED

1. T. B. Atanaev, M. P. Shesternev, and Yu. A. Vladimirov, Biofizika, **35**, No. 4, 610 (1990).
2. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
3. E. A. Parfenov and L. D. Smirnov, Khim.-Farm. Zh., **22**, No. 12, 1438 (1988).
4. Yu. O. Teselkin, I. V. Babenkova, O. S. Komarov, et al., Antioxidant Systems in Experimental and Clinical Pathology [in Russian], Sverdlovsk (1987), pp. 9-27.
5. B. N. Ames, Science, **221**, 1256 (1983).
6. B. Halliwell and G. Y. C. Gutteridge, Arch. Biochem., **246**, 501 (1986).
7. B. Halliwell, FASEB J., **1**, 3S8 (1987).
8. R. Rahl, Toxicology, **33**, 185 (1984).
9. R. C. McKnight and F. E. Hunter, J. Biol. Chem., **241**, 2757 (1966).
10. M. Robertiroid, G. Viehe, and G. Remackle, Adv. Drug Res., **16**, 1 (1987).
11. H. G. Viehe, Z. Ganousek, R. Merenyl, et al., Accounts Chem. Res., **18**, 148 (1985).