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EFFECT OF ASCORBIC ACID ON DECOMPOSITION OF ARACHIDONATE-15-HYDROPEROXIDE IN THE PRESENCE OF IRON SALTS AND COMPLEXES

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KEY WORDS: lipid peroxidation, 15-hydroperoxyarachidonate, iron salts and complexes

A central role in the initiation and development of free-radical lipid peroxidation (LPO) reactions in vivo is played by ions of metals of variable valency and their complexes with intracellular ligands [8, 12]. It has been suggested that metals of variable valency participate in Fenton's reaction (initiation of LPO) and also in reactions of decomposition of hydroperoxides of polyenic fatty acids (chains lengthening reactions) [3]. In both types of reactions, the reduced states of metals with variable valency are active, and electron donors are needed to maintain them [4]. Components of electron-transport chains (enzyme systems of LPO induction), and also low-molecular-weight reducing agents (nonenzymic LPO) may act in the latter role in the cell. One such universal reducing agent, responsible for induction of LPO in vivo and also widely used in model system in vitro, is ascorbate [4]. However, high ascorbate concentrations have an inhibitory action on LPO, and the mechanisms of this effect have not been adequately studied. It is considered that ascorbate, in high concentrations, behaves as a trap for radicals, leading the oxidation chain [11]. However, the character of dependence of LPO inhibition by ascorbate on the Fe^{2+} concentration cannot be explained on the basis of this mechanism [2, 5, 14]. Other hypotheses have therefore been put forward recently to explain the mechanism of inhibition of LPO by high ascorbate concentrations [1].

The aim of the present investigation was to study the effect of ascorbic acid on decomposition of hydroperoxides of polyenic fatty acids (of 15-hydroperoxyarachidonate, 15-HPA) in the presence of iron and some of its complexes.

EXPERIMENTAL METHOD

15-HPA was synthesized by incubating arachidonic acid with soy lipoxygenase in 10 mM phosphate buffer, containing 0.2% deoxycholate (pH 7.4 at 25°C). The 15-HPA was purified from unreacted arachidonic acid and by-products of the reaction by extraction with chloroform followed by distributive extraction in a two-phase system containing a mixture of methanol: petroleum benzin:water in the ratio of 3:1:1. The formation and decomposition of 15-HPA were recorded spectrophotometrically on the basis of the characteristic absorption maximum of the conjugated dienes (monohydroperoxides) in the UV-spectrum at 234 nm [1] on a "Perkin-Elmer 552" spectrophotometer. In the experiments with ascorbic acid, 10% less ascorbic acid (50% less in the presence of EDTA) was added to the comparison cuvette than into the measuring cuvette. The ascorbate concentration at the end of the reaction was determined from the optical density at 264 nm [10] in the comparison cuvette.

The following reagents were used: ascorbate (from "Reanal," Hungary), soy lipoxygenase, deoxycholic acid, and arachidonic acid (from "Sigma," USA), and Na_2HPO_4, NaOH, EDTA, and

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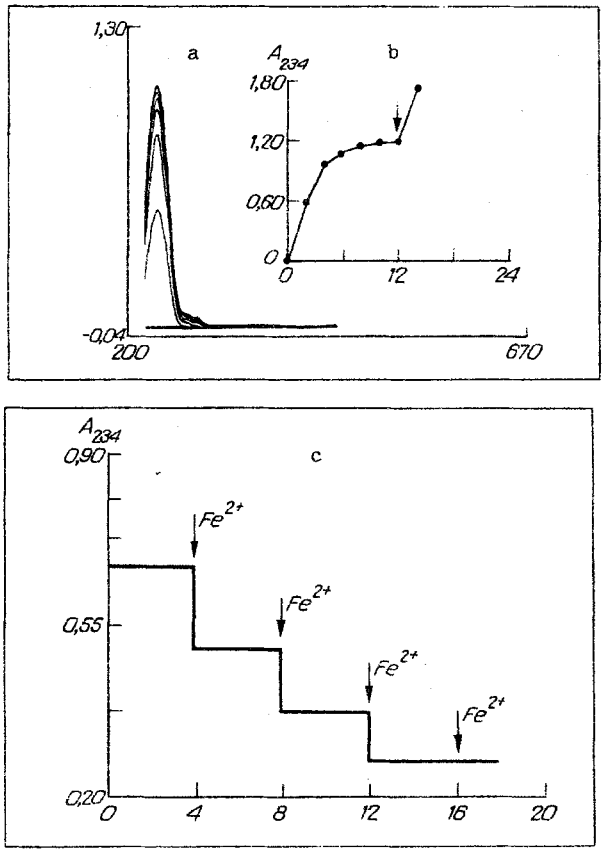


Fig. 1. Oxidation of arachidonic acid: a) UV absorption spectra of arachidonic acid during its oxidation, catalyzed by soy lipoxygenase. Spectra recorded at intervals of 2 min. During the reaction optical density rises to a maximum at 234 nm. Incubation medium: K,Na-phosphate buffer (10 mM, pH 7.4, at 25°C), sodium deoxycholate (0.2%), arachidonic acid (50 mM), and lipoxygenase (175 EU/ml). Abscissa, wavelength (in nm); ordinate, optical density. b) Changes in optical density at 234 nm during oxidation of arachidonic acid in the presence of soy lipoxygenase. The same conditions of incubation. Arrow indicates repeated addition of lipoxygenase. Abscissa, incubation time (in min). c) Changes in UV-absortion of 15-HPA at 234 nm on addition of Fe^{2+} (30 mM) to the reaction medium. Abscissa, incubation time (in min).

$FeSO_4 \cdot 7H_2O$ (from "Serva," West Germany).

EXPERIMENTAL RESULTS

Organic hydroperoxides can oxidize Fe^{2+}, with conversion into alkoxy-radicals, which take part in subsequent reactions with the formation of a variety of molecular products. The formation of hydroperoxides of polyenic fatty acids is accompanied by migration of methylene-interrupted double bonds and the appearance of diene conjugation with characteristic absorption maxima in the UV-spectrum [1, 13].

It will be clear from the data in Fig. 1a that during lipoxygenase-catalyzed oxidation of arachidonic acid, mono- and dihydroperoxides accumulate, with absorption maxima at 234 and 260-275 nm, respectively. After 15-20 min of incubation the reaction proceeds much more slowly, probably because of inhibition of the enzyme (since the addition of a new portion of enzyme reactivates the reaction of oxidation of arachidonate, Fig. 1b); decomposition of hydroperoxides in the presence of Fe^{2+} leads to a decrease in absorption at 234 nm (Fig. 1c, Fig. 2). By ordinary spectrophotometric recording, the kinetics of decomposition of 15-HPA cannot be recorded, because of the high reaction velocity. The quantity of hydroperoxide decomposed is determined by the concentration of added Fe^{2+} (Fig. 1c). Within the concentration range of added Fe^{2+} from 10^{-5} to $0.6 \cdot 10^{-4}$ M, the optical density of the solution recorded at 234 nm decreased as a quasilinear function of the increase in Fe^{2+} concentration (the first two additions of Fe^{2+}). Accordingly, in subsequent kinetic measurements conditions were chosen under which the changes in optical density did not exceed 50% of the original value. The presence of EDTA did not affect Fe^{2+}-catalyzed decomposition of 15-HPA (Fig. 2), recorded spectrophotometrically. Incidentally, identical results were obtained both when a mixture of arachidonate oxidation products was used, when Fe^{2+} was added directly after the end of the lipoxygenase reaction, and with preparations of 15-HPA, previously purified by selective extraction, and with lipoxygenase.

The reaction of decomposition of organic hydroperoxides in the presence of Fe^{2+} to peroxy-radicals [7, 13], known from the literature, under the experimental conditions used proceeds very slowly and does not lead to changes in optical density in the UV-spectrum in the course of 10-15 min (Fig. 2). The same is valid also for complexes of Fe^{3+} (for example, with rutin or EDTA) (Fig. 2).

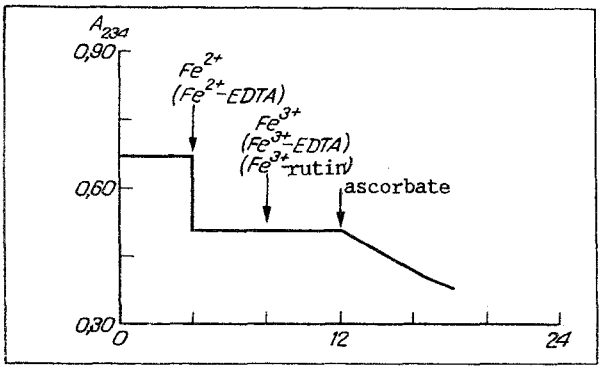
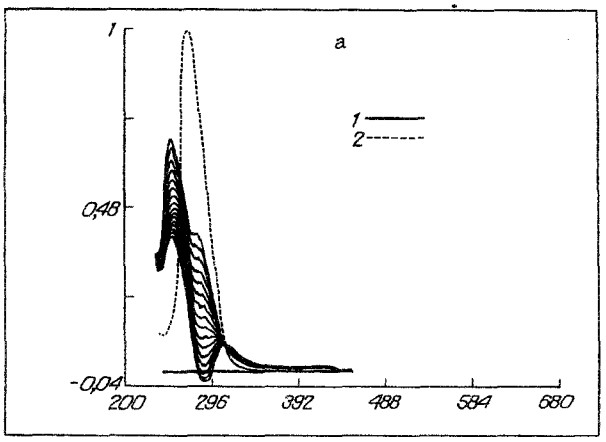


Fig. 2. Effect of Fe^{2+}, Fe^{3+}, and their complexes on UV-absorption of 15-HPA at 234 nm. Conditions of incubation (Fig. 1) Fe^{2+} 30 mM, EDTA 100 mM, ascorbate 100 mM, rutin 60 mM. Abscissa, incubation time (in min).

Addition of a reducing agent (ascorbate) to incubation medium containing 15-HPA and Fe^{3+} induced decomposition of the hydroperoxide. Under these conditions the kinetics of decomposition of 15-HPA could be recorded (Figs. 2 and 3). In the presence of ascorbate, 15-HPA decomposition also was induced by complexes of Fe^{3+} (Fe^{3+}-rutin, Fe^{3+}-EDTA; see Fig. 2). Under the experimental conditions used, 15-HPA decomposition ended after 15-20 min, long before all the ascorbate was used up (the ascorbate concentration at this time was about 50% of the initial value; Fig. 3a). It can be tentatively suggested that the observed decomposition kinetics of 15-HPA reflects a cyclic oxidation process: reduction of iron in the course of the reaction. What is responsible in this case for stopping the reaction of 15-HPA decomposition, despite the presence of the both iron and ascorbate? One of the most natural explanations is masking of the iron necessary for the redox cycle due to its complex formation with a certain reaction product. Since decomposition of 15-HPA in the absence of ascorbate is determined entirely by the concentration of added Fe^{2+}, it can be postulated that chelation of the iron takes place with one of the oxidation products of ascorbate. In fact, if previously oxidized ascorbate (in the absence of 15-HPA) is added to the incubation medium, in a standard system (Fe^{2+} + unoxidized ascorbate + 15-HPA) much less 15-HPA is decomposed.

Evidence in support of this hypothesis is given data shown in Fig. 3b. In the presence of Fe^{2+} and EDTA, the velocity of oxidation of ascorbate is higher than in the absence



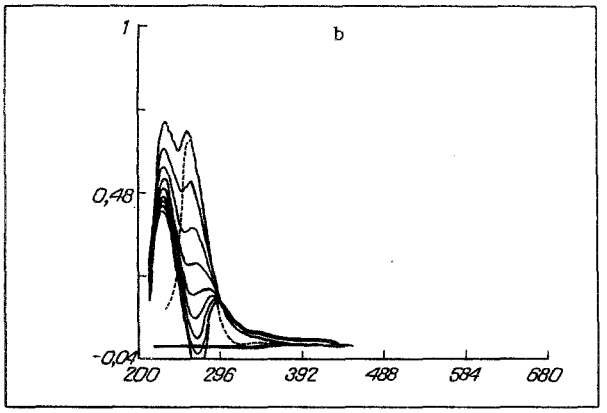


Fig. 3. UV absorption spectra of 15 HPA (1) and UV-spectrum of ascorbate (2) (at end of reaction) in presence of Fe^{2+} (a) and (b) optical density at 234 and 264 nm decreases in the course of the reaction. Conditions of incubation as in Fig. 1. Ascorbate 100 mM, Fe^{2+} 10 mM. Recordings at intervals of 2 min, EDTA 100 mM.

of EDTA (in the presence only of Fe^{3+}), and correspondingly, decomposition of 15-HPA also ceases much sooner. This probably can also be explained by the chelating action of oxidation products of ascorbate formed rapidly.

If it is accepted that Fe^{2+}-induced decomposition of hydroperoxides is an essential stage in the development of the LPO process [9, 13], the results demonstrate that one cause of inhibition of LPO by high concentrations of ascorbate is the chelation of the iron by oxidation products of ascorbate. Incidentally, this effect of oxidation products of ascorbate can inhibit Fenton's reaction.

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EFFECT OF MELATONIN AND PINEALECTOMY ON THE STATE OF THE RAT LIVER MONO-OXYGENASE SYSTEM

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KEY WORDS: pinealectomy; melatonin; mono-oxygenase system

Melatonin, the principal pineal hormone, possesses varied pharmacologic properties, including psychotropic activity [1, 13]. Meanwhile, when melatonin is combined with the use of certain neurotropic substances with a central action (apomorphine, antidepressants), it modifies their effects [2, 3]. This latter feature of melatonin may be pharmacokinetic in nature and, in particular, it may be determined by a change in the biotransformation of the drugs. It was accordingly decided to assess the state of microsomal oxidation in the rat liver during administration of melatonin and also after pinealectomy.

EXPERIMENTAL METHOD

Experiments were carried out on 44 noninbred male albino rats weighing 140-200 g.

Activity of enzymes of the mono-oxygenase system was evaluated in liver microsomes, isolated by differential centrifugation [6]. The concentrations of cytochromes P-450 and b_5 in the microsomal suspension were measured by means of the dual-beam SF-18 spectrophotometer by the method in [11], taking the value of ε to be 91,000 and 165,000 $M^{-1} \cdot cm^{-1}$, respectively. Activity of NADPH-cytochrome c-reductase was determined at 30°C on an SF-26 spectrophotometer at 550 nm. The incubation mixture, in a volume of 3 ml, contained 330 μM NaCN, 50 μM

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