INVESTIGATION OF THE MECHANISM OF ANTIOXIDANT ACTIVITY OF LIPIDS

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The antioxidant activity (AOA) of lipids is a universal property of all cells [3]. The presence of antioxidants in lipids of biomembranes is regarded as an essential condition for the stability of the given structure [2]. Despite wide interest in the mechanism of AOA of lipids, the contribution of individual components of the antioxidant system has not been adequately studied, and views on the effectiveness of bioantioxidants are contradictory. High constants of antiradical activity of tocopherol [1] and its low AOA in lipid substrates [7], and the inhibitory and pro-oxidant effect of phospholipids [8] have been described.

The aim of this investigation was to determine AOA of α-tocopherol, phospholipids, and mixtures of bioantioxidants by a kinetic method, using fish lipids.

EXPERIMENTAL METHOD

AOA of the lipids was determined from the period of induction of initiated oxidation at 60 ± 0.2°C. The contribution of α-tocopherol, phospholipids, and mixtures of bioantioxidants was determined by measuring the period of induction of oxidation of methyl esters of fatty acid components with the addition of $1.4 \cdot 10^{-4}$-$6.96 \cdot 10^{-3}$ M α-tocopherol or total phospholipids, mixtures of α-tocopherol and phospholipids, and total unsaponifiable components of lipids. The mechanism of action of the bioantioxidants was determined from the character of dependence of the induction period on their concentration.

Lipids from pale and dark muscles of *Coregonus peled* (Gmelin) were used. Oxidation was studied by a manometric method in a solution of chlorobenzene in the presence of $6 \cdot 10^{-3}$ M dinitrylazo-bis-isobutyric acid as an initiator of radical processes. The methods of oxidation, manometric measurements, calculation of induction periods, isolation of lipids, determination of total unsaponifiable components, and preparation and purification of methyl esters of the fatty acid components of the lipids were described previously [5, 6]; the phospholipids were isolated by precipitation in cold acetone [4]. The α-tocopherol (from "Lithe") was purified by thin-layer chromatography in a system of diethyl ether—petroleum benzene—acetic acid (10:89:1).

EXPERIMENTAL METHODS

Comparison of the rate of oxygen absorption by lipids of plane and dark muscles and by their fatty-acid components (Fig. 1) showed that lipids possess high AOA, on account of which the induction period was increased by 25 times. The shape of the kinetic oxidation curves shows that slow development of autoacceleration and high concentrations of oxygen (60-80 mm^3) at which the induction period ends are more characteristic of lipids than of their fatty acid components.

To elucidate the nature of the AOA of lipids, oxidation of methyl esters of the fatty acid components of the lipids after the addition of α-tocopherol (from $1.4 \cdot 10^{-4}$ to $5.3 \cdot 10^{-3}$ M), phospholipids (from 25 to 800 mg %), and their mixtures, and of total unsaponifiable components of the lipids. The results showed that addition of 2.5, 25, 75, 225, 375, 400, and 800 mg % of phospholipids exhibited only weak AOA (Fig. 1). AOA of α-tocopherol depends on its concentration. The character of this relationship with respect to oxidation of methyl esters of fatty acid components of lipids from pale muscles is shown in Fig. 2. These data show that addition of α-tocopherol in concentrations up to $40 \cdot 10^{-3}$ M effectively inhibits oxidation, but a further increase in the concentration of α-tocopherol is ineffective, and

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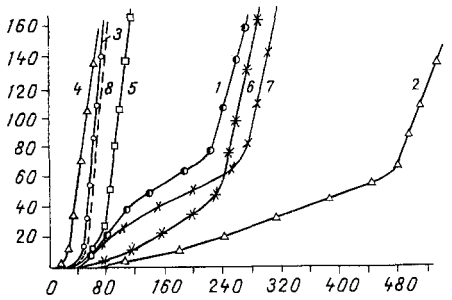

Fig. 1

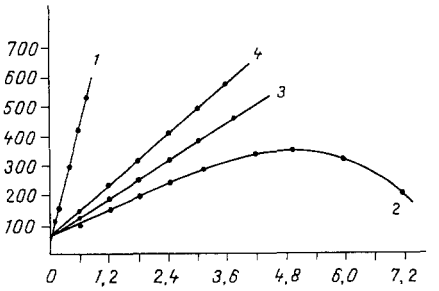

Fig. 2

Fig. 1. Kinetic curves of oxygen uptake by lipids. Abscissa, time (in min): ordinate, rate of oxygen uptake (in mm^3). 1, 2) Lipids from pale and dark muscles respectively; 3, 4) fatty acid components of lipids from pale and dark muscles respectively; 5, 6) in presence of added α-tocopherol, $0.8 \cdot 10^{-3}$ and $2.6 \cdot 10^{-3}$ M respectively; 7) in presence of mixture of $0.8 \cdot 10^{-3}$ M α-tocopherol and 800 mg % phospholipids; 8) 400 mg % phospholipids.

Fig. 2. Oxidation induction period as a function of α-tocopherol concentration. Abscissa, α-tocopherol concentration (in $M \cdot 10^{-3}$) ordinate, induction period (in min). 1) Fish lipids; 2) fatty acid components of lipids; 3, 4) the same in the presence of 100 and 400 mg 2 of phospholipids respectively.

in concentrations of over $6 \cdot 10^{-3}$ M the induction period was reduced. The antioxidant effect of α-tocopherol in the region of optimal concentrations was compared with AOA of lipids. It was found that AOA of α-tocopherol does not exceed 10% of AOA of lipids (Fig. 1).

Investigations of the effectiveness of mixtures of optimal concentration of α-tocopherol and phospholipids between 25 and 800 mg % showed that synergism was exhibited by the mixtures, and that the magnitude of this synergism was critical in character.

Dependence of the induction period on phospholipid concentration was studied at optimal α-tocopherol concentrations ($0.6 \cdot 10^{-3}$, $1.2 \cdot 10^{-3}$, and $1.8 \cdot 10^{-3}$ M) (Fig. 3). The results showed that the synergic effect of phospholipids is a linear function of concentration between 10 and 50 mg %, after which the curve flattens out on a plateau at concentrations of over 200 mg %. Optimal concentrations of α-tocopherol exist for maximal synergic effects of the mixtures, for example, $1.8 \cdot 10^{-3}$ M.

Optimal mixtures of α-tocopherol and phospholipids were shown to yield only one-third of the AOA of lipids (Fig. 2).

To discover the causes of the higher AOA of lipids than of a mixture of α-tocopherol and phospholipids, the effect of a mixture of total unsaponifiable components of lipids with phospholipids was studied during oxidation of the fatty acid components of the lipids. The results showed that the effect of this mixture is greater than the effect of a mixture of α-tocopherol and phospholipids, but amounts to 60% of the AOA of lipids (Fig. 1). AOA of the total unsaponifiable components was 15% of AOA of the lipids. Total AOA of the lipids was probably due to a combination of structure and composition of the fatty acid components of the phospholipids and bioantioxidants. During saponification, re-esterification, and extraction changes occur in the structure of the fatty acid components and phospholipids, and un-

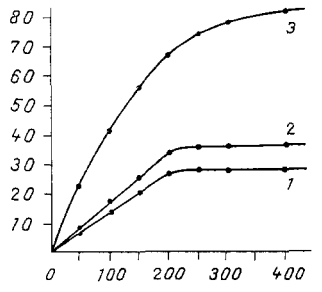

Fig. 3. Induction period as a function of phospholipid concentration with different concentrations of α-tocopherol. Abscissa, concentration of phospholipids (in mg %): ordinate, induction period (in min). 1, 2, 3) With $0.4 \cdot 10^{-3}$, $0.8 \cdot 10^{-3}$, and $1.2 \cdot 10^{-3}$ M α-tocopherol respectively.

stable bioantioxidants lose their activity. It was shown (Fig. 1) that the character of autoacceleration and the level of oxidation at which escape from the induction period takes place may be determined both by α-tocopherol in concentrations of over $2.0 \cdot 10^{-3}$ M and by mixtures of α-tocopherol with phospholipids (400-800 mg %) in concentrations characteristic of native lipids ($0.8 \cdot 10^{-3}$-$1.2 \cdot 10^{-3}$ M).

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EFFECT OF ADENOSINE, AMP, AND PAPAVERINE ON THE cAMP CONTENT IN [^{14}C]ADENINE PRELABELED THYMOCYTES

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Adenosine has a toxic action on lymphocytes [2, 12], but the mechanism of this action has not been explained. According to one hypothesis, this nucleoside affects lymphocyte function through the adenylate cyclase system, by increasing the intracellular cAMP concentration [4, 8, 14]. AMP can influence the cAMP level in lymphocytes *in vivo* more effectively still because of its higher concentration in the blood plasma than that of adenosine, and coupling of the action of 5'-nucleotidase and adenylate cyclase in the plasma membrane [6]. Papaverine also raises the cAMP concentration in cells [13] and inhibits blast transformation of thymus lymphocytes induced by concanavalin A [2]. The effectiveness and direction of action of cAMP on immune function are not always the same [3], possibly depending on the existence of special cAMP compartments, under the control of different effectors, in lymphocytes [9].

In the investigation described below the effect of various factors (adenosine, AMP, and papaverine) on the cAMP concentration was studied in thymocytes prelabeled with [^{14}C]adenine.

EXPERIMENTAL METHOD

Wistar rats aged 1.5-2 months were used. Thymocytes were isolated and then incubated in Hanks' medium, buffered with 20 mM HEPES and $NaHCO_3$ to pH 7.4. Thymocytes were labeled with [^{14}C]adenine (specific radioactivity 20 mCi/mmole) in a plastic vessel. The incubation (1 h at 37°C) the cells were washed twice and resuspended in cold isolation medium at 4°C. The incubation medium contained $3 \cdot 10^7$ cells in 1 ml and 25 μM of labeled adenine. After incubation (1 h at 37°C) the cells were washed twice and resuspended in cold isolation medium at 4°C. The resuspension was poured into test tubes in volumes of 15 ml, and after preincubation for 10 min at 37°C, Hanks' solution (control) and the test substances were added to them so that the final concentration of the latter was 100 μM and of cells $2 \cdot 10^7$/ml. After incu-

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