Effect of Ascorbate and α-Tocopherol on Resistance of β-Carotene to Oxidation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 7, pp. 59-62, July, 2000
Original article submitted March 8, 2000

The efficiency of ascorbate and α-tocopherol as stabilizers of β-carotene, which is widely used in complex therapy and prevention of some diseases accompanied by oxidative stress, was studied. The latency of induced β-carotene oxidation linearly depends on ascorbate concentration, while steady-state rate nonlinearly depends on the concentration of α-tocopherol, which attests to involvement of antioxidants in various stages of chain oxidation of β-carotene.

Key Words: *β-carotene; α-tocopherol; ascorbate; oxidation*

Phytopigment β-carotene widely spread in nature plays an important role in biological systems mainly as vitamin A precursor. Both central and peripheral double bonds in the polyenic chain of β-carotene molecule undergo enzymatic cleavage yielding retinoid compounds [15], which participate in vision and regulation of reproduction, cell growth, and differentiation [11]. The presence of an extended system of conjugated bonds in β-carotene molecule explains its high reaction capacity towards oxygen and free radicals [5, 12], so β-carotene and other carotenoids are considered as vitals elements of antioxidant protection in cells and biological fluids. Strong evidence is now available on the positive (prophylactic and protective) effects of β-carotene in some diseases accompanied by oxidative stress (cataract, chronic infections, inflammation, cancer, and cardiovascular pathology) [4,7]. Utilization of β-carotene in nonspecific oxidation reactions with phenoxyl radicals formed from endogenous, nutrient, drug, and environmental phenols is considered as a factor promoting oxidative stress [14]. It is possible that nonspecific oxidation competes with enzymatic conversion of β-carotene, which is of particular importance during oxidative stress due to the absence of natural mechanisms of β-carotene regeneration.

Therefore, the problem of β-carotene stabilization in multivitamin preparations and food additives is actual. Our aim was to study the effect of ascorbate and α-tocopherol in a broad concentration range on oxidation of β-carotene.

MATERIALS AND METHODS

We used 4% β-carotene suspension (BASF AG), water-soluble inductor azobis(2-amidinopropane) hydrochloride (ABAP), α-tocopherol (Fluka), and sodium ascorbate.

Oxidation of β-carotene was performed in 0.1 M phosphate buffer (pH 7.5) at 37°C under constant concentrations of β-carotene (10^{-5} M) and ABAP (10^{-2} M). Oxidation of β-carotene was assessed by a decrease in optical density at 435 nm on a Beckmann DU-70 spectrometer.

RESULTS

In the examined range of α-tocopherol concentrations differing by more than one order of magnitude (0.25-8.50×10^{-5} M) the reaction of inhibited β-carotene oxidation proceeded with a constant rate until substrate exhaustion (Fig. 1, *a*). The inhibiting effect of α-to-

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0007-4888/00/0007-661$25.00

copherol was assessed by the degree of inhibition *I*, calculated by the formula $(1-V_i/V_0)\times100\%$, where V_i and V_0 are the initial rates of β-carotene oxidation in the presence and absence of the inhibitor, respectively. The dependence of I on α-tocopherol concentration was described by a saturation curve (Fig. 2). Even when α-tocopherol concentration was 0.56×10^{-5} M (*i.e.* at β-carotene:α-tocopherol 2:1 w/w ratio), *I* reached 50%, while at a concentration of 2.2×10^{-5} M (1:2 w/w ratio) α-tocopherol provided 90% protection of β-carotene from oxidation.

Another character of inhibition of β-carotene oxidation was observed in the presence of ascorbate: at all inhibitor concentrations (2×10^{-5}-40×10^{-5} M) the kinetic curves of inhibited β-carotene oxidation were characterized by an induction period, while the steady-state oxidation rate did not differ from that without inhibitors (Fig. 1, *b*, curve *6*). The duration of lag phase linearly depended on ascorbate concentration (Fig. 3).

The combined effect of α-tocopherol and ascorbate was studied for α-tocopherol concentration of 0.56×10^{-5} M (corresponding to 2:1 β-carotene:α-tocopherol ratio) and for a broad range of ascorbate concentration. An additive effect of these antioxidants was revealed: oxidation of β-carotene in the presence of α-tocopherol and ascorbate (Fig. 1, *b*, curve *7*) was characterized by an induction period corresponding to ascorbate concentration (Fig. 1, *b*, curve *6*), while after the end of the induction period the rate of steady-state reaction decreased by 50% due to the action of α-tocopherol (Fig. 1, *b*, curve *3*).

The kinetics of β-carotene oxidation attests to peculiar interaction between the antioxidants and β-carotene in the examined systems. The experiments were performed with β-carotene in the form of a microdispersed hydrosol, whose particles were surrounded by a protective polymer shell [10]. It is possible that the observed difference in the effects of α-tocopherol and ascorbate on the reaction of induced β-carotene oxidation is explained by different distribution of hydrophilic ascorbate and hydrophobic α-tocopherol between the phases of this system. Oxidation of β-carotene proceeds according to the free radical chain mechanism [1,8], and the inhibitor can affect various stages of the chain process. In this case, ascorbate dissolved in water phase is probably involved in the reaction even at the initiation stage, *i.e.*, peroxide radicals of the inductor formed in the aqueous phase are captured by ascorbate rather than attack β-carotene molecules embedded in a protective shell. Under these conditions the chain reaction of β-carotene oxidation should be suppressed to complete exhaustion of the inductor, the duration of lag phase being proportional to inhibitor concentration, which agrees with our data. Hence, ascorbate and products of ascorbate interaction with initiating radicals do not penetrate into β-carotene micelles, so after complete exhaustion of the inhibitor the reaction rate corresponds to the rate of uninhibited oxidation (Fig. 1, *b*, curve *6*). Similar mechanism of inhibition was previously proposed in analysis of induced oxidation of multilamellar dilinoleilphosphatidylcholine liposomes in water [9]. Oxidation of liposome bilayer was induced by lipid- and water-soluble inducers. Ascorbate inhibited oxidation induced only in the aqueous phase. The authors conclude that inhibition occurs at the stage of induction is a result of interaction of inducing radicals with ascorbate before their penetration into the liposome bilayer.

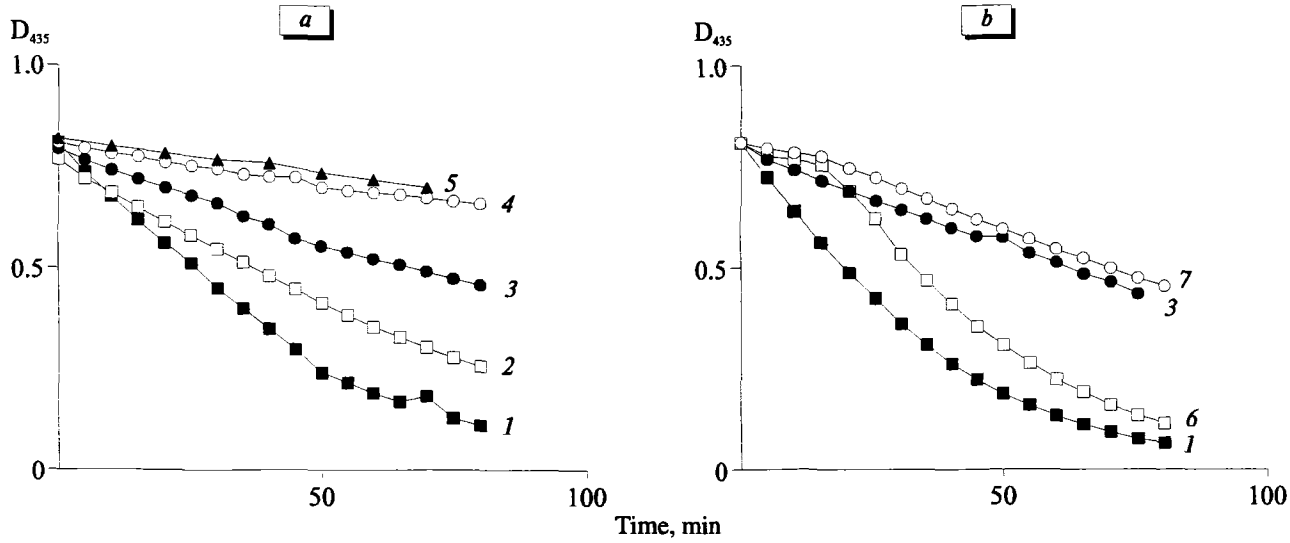

Fig. 1. Kinetic curves of β-carotene oxidation in the presence of α-tocopherol in various concentration (*a*) and under combined action of ascorbate and α-tocopherol (*b*). *1*) without inhibitors, α-tocopherol, $\times10^{-5}$ M: *2*) 0.26, *3*) 0.56, *4*) 1.4, *5*) 2.26; *6*) ascorbate 1.27×10^{-5} M, *7*) ascorbate+α-tocopherol, 2:1:2 β-carotene: α-tocopherol:ascorbate ratio.

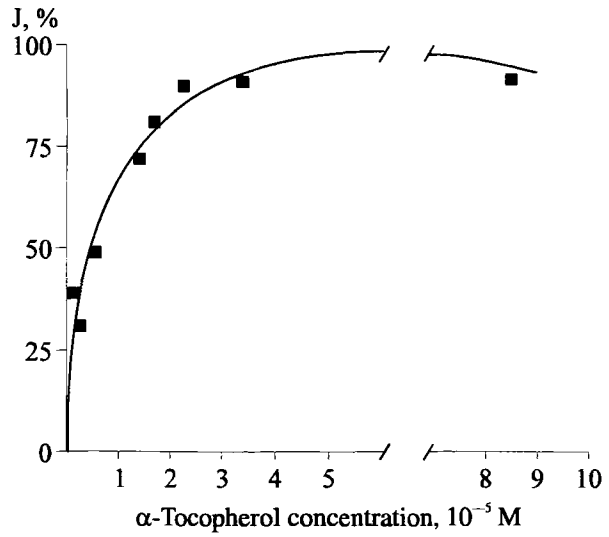

Fig. 2. Inhibition (I) of β-carotene oxidation (1.04×10^{-5} M) as a function of α-tocopher[illegible]centration.

In contrast to ascorbate, α-tocopherol penetrates into organic phase [9], which in our system was presented by β-carotene micelles. The kinetics of β-carotene stabilization by free radical inhibitors in organic media were studied previously [2]. It was shown that introduction of an inhibitor results not only in the appearance of the induction period (which may be a complex function of inhibitor concentration), but also affects the oxidation rate [2]. The authors hypothesize on a possible role of various modifications of the inhibitor during the chain process. If the inhibitor radical (for example, α-tocopheroxyl radical) can continue the chain, β-carotene oxidation will not be completely suppressed even at high inhibitor concentrations used in this work, but will proceed with significantly slower rate corresponding to a decreased reaction capacity of α-tocopheroxyl radical [6]. This approach explains not only decreased β-carotene oxidation rate in the presence of α-tocopherol, but also saturation of inhibition at high α-tocopherol concentrations. The capacity of α-tocopherol to stabilize β-carotene agrees with antioxidant hierarchical sequence for some carotenoids and tocopherol homologues (β-carotene and α-tocopherol included) revealed in the studies of interaction between a carotenoid cation radical with tocopheroxyl radical by laser flash-photolysis method [13].

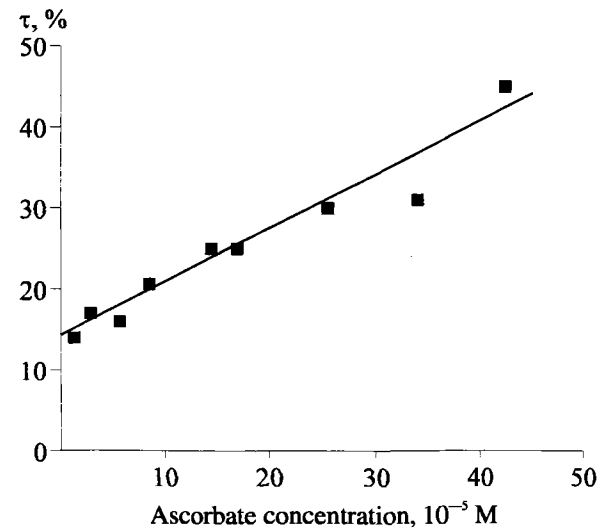

Fig. 3. Induction period (τ) of β-carotene oxidation as a function of ascorbate concentration.

Additive effects of ascorbate and α-tocopherol on β-carotene oxidation attest to the absence of direct interaction between ascorbate and α-tocopherol in the studied system, which agrees with previous data [9].

Protection of β-carotene from oxidation by α-tocopherol and ascorbate was demonstrated for vitamin preparation Vetoron containing β-carotene and α-tocopherol solubilized in clusters of surface-active substance [5]. It was also shown that α-tocopherol and ascorbate are efficient protectors of enzymatic conversion of β-carotene into retinal under the action of β-carotene-15,15'-dioxygenase [3].

The commercial preparation of encapsulated β-carotene used in the present work is a suitable model for comparative analysis of various antioxidants. Since no synergy between α-tocopherol and ascorbate was observed in the studied system, there is little point in the use of high concentrations of ascorbate for stabilization of this system. At the same time, α-tocopherol content corresponding to α-tocopherol:β-carotene 2:1 ratio effectively inhibits β-carotene oxidation.

The study was supported by the Russian Foundation for Basic Research (grant No. 99-04-48637).

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