THE INFLUENCE OF LIPID COMPOSITION AND β-CAROTENE ON LIPID PEROXIDATION IN LIPOSOMES

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ABSTRACT

Oxidative damage to membrane lipid is one of the prime events occurring in aging and other undesirable physiological processes. In this study experiments were performed on liposomes (prepared either from crude erythrocyte phospholipids or purified egg yolk phosphatidylcholine) as models of lipid bilayer portion of biomembranes. The effects of β-carotene, and phospholipid composition on peroxidation process, initiated by Fe^{2+}, were studied. It was found that β-carotene does not show any noticeable antioxidant effect on the peroxidation process initiated by Fe^{2+} in liposomes prepared from erythrocyte phosphatides, whereas it effectively suppressed the same process in egg yolk phosphatidylcholine (EYPC). It is concluded that the anti-/pro-oxidant activity of β–carotene is also dependent on the membrane lipid composition and this may provide an explanation about the conflicting reports on its role in ordinary or promoted oxidation experiments.

Keywords: β-carotene, Liposomes, Lipid peroxidation, Erythrocyte phospholipids, Iron-induced peroxidation, Egg yolk phospholipids, Antioxidant.

INTRODUCTION

Carotenoids are not just "another group of natural pigments". They are substances with very special and remarkable properties that no other groups of substances possess and these form the basis of their many varied functions in all kinds of living cells. Traditionally often thought of as plant pigments, carotenoids have a much wider distribution and occur extensively in animals and microorganisms (1-3). Without carotenoids, photosynthesis and life in an oxygen atmosphere would be impossible (1).

The natural functions and actions of carotenoids obviously are determined by the physical and chemical properties arising from their molecular structures. First of all their overall molecular geometry (size, shape, and presence of functional groups) is vital for ensuring their fitness into cellular and subcellular structures (correct location and orientation) allowing them to function efficiently. Secondly, their conjugated double bond system determines the photochemical and chemical properties underlying their physiological functions. In addition, their specific interactions with other molecules in their immediate vicinity are crucial for their appropriate functioning (1).

More than 600 naturally occurring carotenoids have been identified and beta-carotene is one of them (2, 3). Foot and colleagues first demonstrated the exceptional singlet oxygen quenching action of beta-carotene in 1968 (4, 5). Krinsky, Burton and Ingold later documented the peroxyl radical scavenging properties of β-carotene and indicated that β-carotene acts as a previously unknown type of chain-breaking antioxidant especially effective at low partial oxygen pressures (4, 6, and 7). The antioxidant action of β-carotene has also been observed in the case of lipids in solution (8-12), phospholipids in liposomes (9, 11, 12), and microsomes (15-17). Inhibition of oxidative modification of low-density lipoproteins by β-carotene has also been reported (18). Unlike vitamin E and vitamin C that reveal their antioxidant activity through hydrogen donation, β-carotene acts via addition reaction to its double bonds yielding a resonance stabilized, carbon-centered, conjugated radical (8). Dietary β-carotene has been postulated, through epidemiologic evidence and laboratory studies, as an effective agent for prevention of lung cancer and perhaps other cancers (19, 20). However, a recent randomized trial found no benefit in long-term β-carotene supplementation in normal, healthy subjects (21). Moreover, three intervention trials with heavy smokers suggested that β-carotene may actually increase the risk of cigarette smoke-induced lung cancer (22-24).

β-carotene is insoluble in water and has limited solubility in organic solvents. In addition, β-carotene is easily oxidized both by light and by some components of the physiological milieu, e.g.

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metals and reactive oxygen species. These factors plus differential solubility of β-carotene complicate the study of β-carotene metabolism, and may provide an explanation for the disparate results obtained by different laboratories (25).

In this paper, we report the results of our investigations on: a) the role of β-carotene and lipid composition of membranes on lipid peroxidation in liposomes and, b) the catalyzed reactions of Fe^{2+} in promoting the spontaneous lipid peroxidation.

MATERIALS AND METHODS

Materials

Trichloroacetic acid, 2-thiobarbituric acid, ferric sulfate II ($FeSO_4.7H_2O$), EDTA (Titriplex III), L-ascorbic acid, α-tocopherol and all organic solvents were analytical reagent grade purchased from Merck (Darmstadt, Germany). β-carotene was purchased from Fluka (Buchs, Switzerland). Human red blood cells (RBC) were provided by the Blood Transfusion Organization of Iran, Tehran.

Instruments

These include: UV-vis spectrophotometer, Shimadzu model 160A (Tokyo, Japan), vortex mixer (Heidolph, Germany), bench top pH meter (Wilhelm, Germany), probe type sonicator (model MK2-3.75, MSF, France), rotovap (model NAJ, Eyela, Japan), and refrigerated ultracentrifuge (model 6-5, Imaco, Iran).

Methods

Preparation of human RBC

Packed cells of male human with A^+ blood group were used. Red blood cells (RBC) were washed three times with normal saline (9 g/l NaCl) and separated from the buffy coat. During the last wash, the cells were centrifuged at 1000 RPM for 10 min.

Extraction of lipids

Erythrocyte phosphatides were extracted by a simple and low toxicity method (26). Purified egg yolk phosphatidylcholine (EYPC) was obtained by column chromatography on alumina following the method by Singleton (27) and purity assayed by TLC in comparison with commercial standard samples. Purity of phospholipids thus obtained was much higher than those of the commercial standard samples.

Vesicle preparation

Liposomes were prepared by the method described by Janson (28). β-carotene was dissolved in chloroform and added to phospholipid solutions and the solvent removed by evaporation under nitrogen in a rotary vacuum evaporator. Aliquots of 7.8 mM PBS (Phosphate Buffered Saline), pH 7.4, were added to the dried lipid to give a phospholipid concentration 7 mM and an antioxidant concentration of 0.2-2 mol % with respect to phospholipids. The suspensions were sonicated via the probe sonicator for 5 min at 4^oC under a nitrogen atmosphere to yield unilamellar liposomes (29). The vesicle dispersions were then centrifuged for 15 min at 3500 RPM to remove any undesired particle (lipid aggregates, metal debris, etc.).

Peroxidation of lipid vesicles

Vesicles obtained from freshly prepared lipids were subjected to stimulated peroxidation by iron salt (Fe^{2+}) at a concentration of 150μM. Lipid peroxidation was monitored through the formation of thiobarbituric acid (TBA) reactive material as malondialdehyde (MDA) at 535 nm (30). Susceptibility of different lipid samples to peroxidation was determined by monitoring the appearance of the conjugated dienes by UV differential spectroscopy (at 234 nm). In this set up the formation of conjugated dienes in the sample phospholipids was measured against the pristine phospholipids as the reference (31).

β-Carotene bleaching

The change in β-carotene concentration in liposomes was monitored by following the decrease in the absorbance at 466 nm and 497 nm (λ_{max} of β -carotene) by UV-vis spectrophotometry (32).

RESULTS

The ability of beta-carotene to protect phospholipids against iron induced lipid peroxidation in liposomal solutions is revealed in Figs. 1-3 where the time course of lipid peroxidation in liposomes prepared from erythrocyte phospholipids, carrying various levels of β-carotene, in solutions containing either Fe^{2+}, Fe^{2+} + EDTA or Fe^{2+} + ascorbic acid has been studied. It is seen that lipid peroxidation is initiated after about 200 minutes and beta-carotene has no profound effect on the extent and the time course of the process (30).

Fig. 4 shows the enhancing effect of Fe^{2+} on the lipid peroxidation in liposomes prepared from egg yolk phosphatidylcholine containing different amounts of beta-carotene. It is clearly seen that lipid peroxidation is initiated right from the start and beta-carotene has a pronounced effect on inhibiting the process (30).

Fig. 5 shows the appearance of conjugated dienes (absorbance at 234 nm) as a function of time in liposomes prepared from erythrocyte

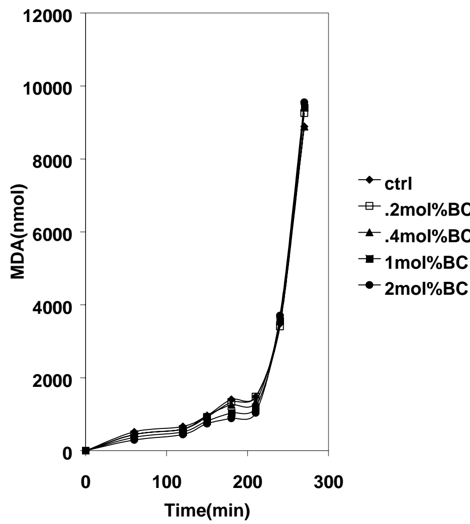


Figure 1. Peroxidation of liposomes made of erythrocyte phospholipids (containing different levels of beta-carotene) induced by 150 μM $FeSO_4$ in 7.8 mM PBS buffer.

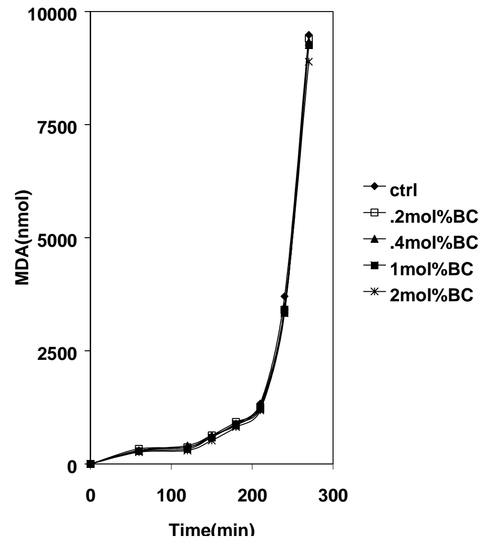


Figure 2. Peroxidation of liposomes made of erythrocyte phospholipids (containing different levels of beta-carotene) induced by 150 μM $FeSO_4$ and EDTA in 7.8 mM PBS buffer.

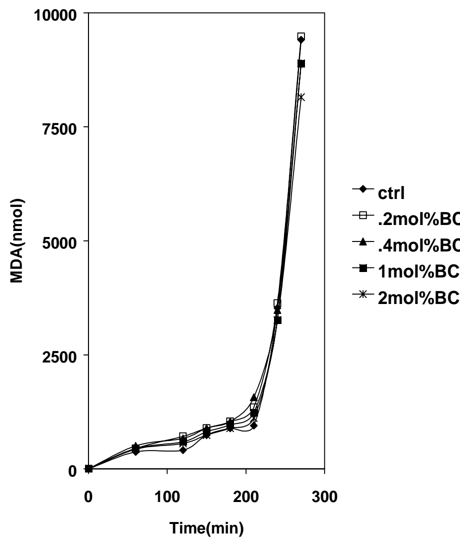


Figure 3. Peroxidation of liposomes made of erythrocyte phospholipids (containing different levels of beta-carotene) induced by 150 μM $FeSO_4$ and 600 μM Ascorbic acid in 7.8 mM PBS buffer .

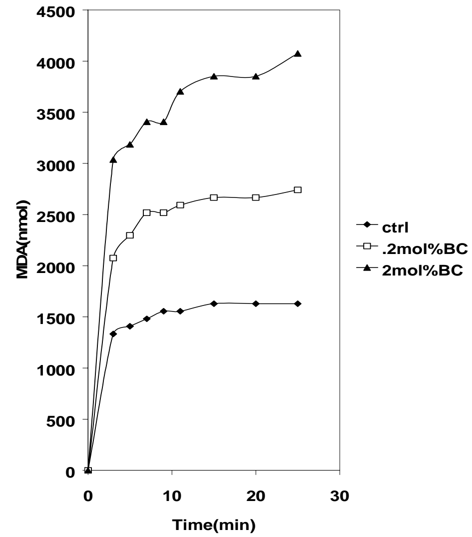


Figure 4. Peroxidation of liposomes made of egg yolk phosphatidylcholine (containing different levels of beta-carotene) bathed in 7.8 mM PBS buffer containing 150 μM $FeSO_4$.

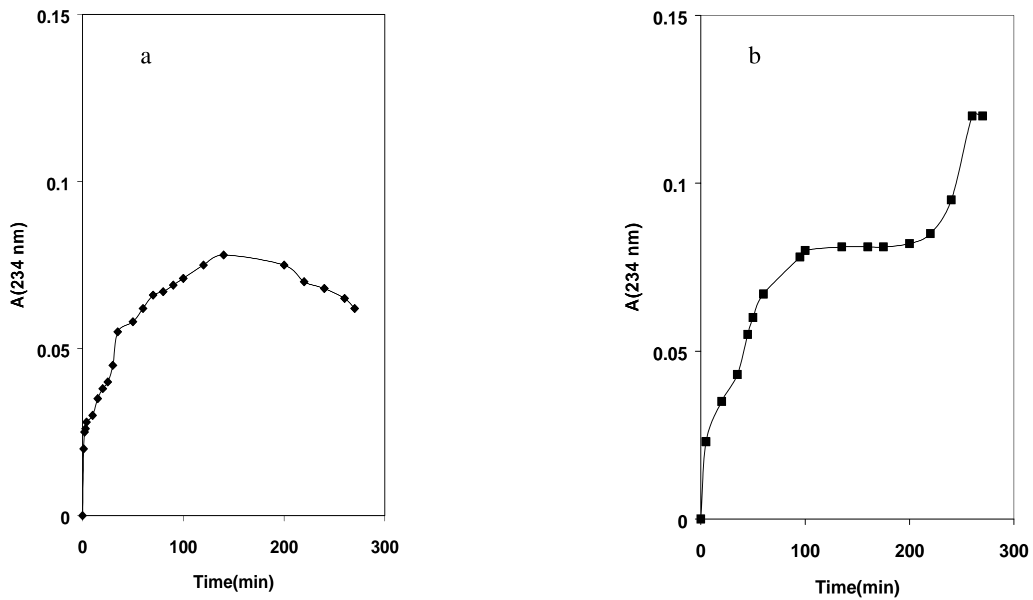


Figure 5. Formation of conjugated dienes in liposomes made from erythrocyte phospholipids bathed in PBS containing 150 μM $FeSO_4$ a) lacking beta-carotene, and b) containing 0.4 mol % β-carotene

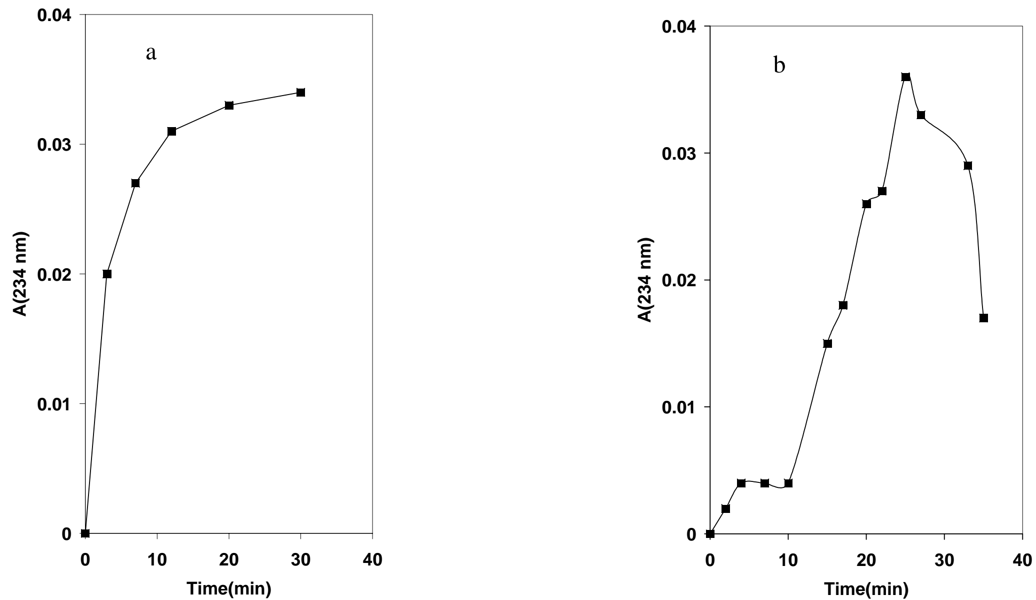


Figure 6. Formation of conjugated dienes in liposomes made from egg yolk phosphatidylcholine bathed in 7.8 mM PBS containing 150 μM $FeSO_4$ a) without β-carotene b) with 0.4 mol % β-carotene.

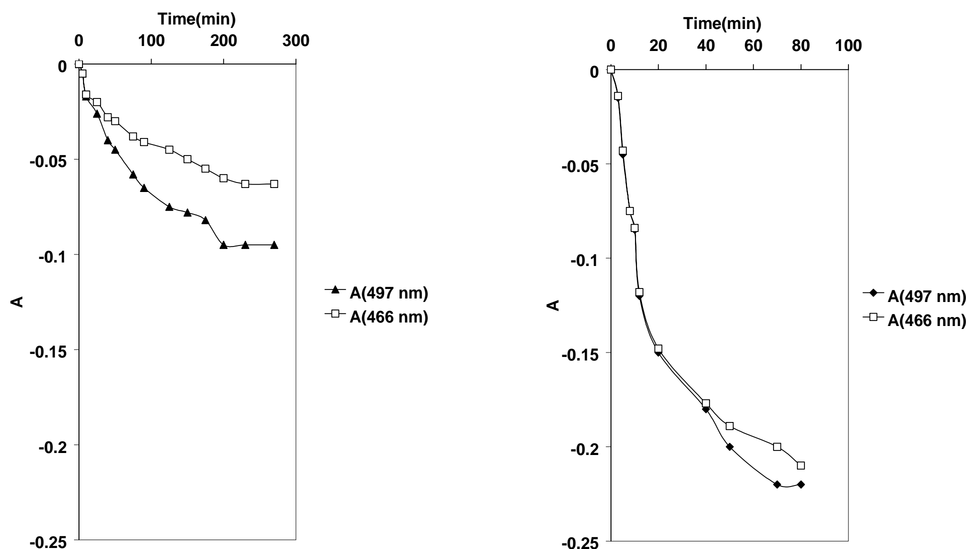


Figure 7. Consumption of β-carotene present in a) liposomes made from erythrocyte phospholipids, and b) liposomes made from egg yolk phosphatidylcholine, in the presence of 150 μM $FeSO_4$.

phospholipids, lacking or containing beta-carotene, in 150μM $FeSO_4$ solution. Although the curves differ to some extent, the overall variation seem to be similar in both cases, at least up to 220 min, and a clear difference in behavior seems to arise after this long period (31).

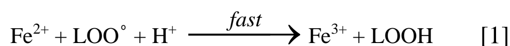
Fig. 6 shows the appearance of conjugated dienes as a function of time in liposomes prepared from egg yolk phosphatidylcholine lacking or containing beta-carotene bathed in 150 μM $FeSO_4$ solution (31). It is seen that in the absence of beta-carotene the amount of conjugated dienes rise sharply in a pattern similar to those previously seen. While liposomes containing beta-carotene show an entirely different behavior, i.e. there is a clear lag time of about 10 minutes before the onset of peroxidation.

Fig. 7 shows the consumption of beta-carotene (absorbance in λ_{max} of beta-carotene: 466 and 497 nm) in liposomes prepared either from egg yolk phosphatidylcholines or erythrocyte phospholipids in the presence of Fe^{2+} (32). It is seen that in both cases there is an exponential trend in beta-carotene consumption, being somewhat more intense in the case of liposomes made of egg yolk phosphatidylcholine, a difference which may be attributed to their different lipid compositions.

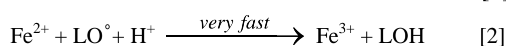
DISCUSSION

The antioxidant potency is determined by several factors such as intrinsic chemical reactivity of the antioxidant towards the specified radical, site of generation and reactivity of the radicals, antioxidant-radical microenvironment, stability and fate of antioxidant-derived radicals, and the interaction with other possible reactants. The precise location of β-carotene in the membrane is not yet fully known. Some propose that it is aligned parallel to the hydrocarbon tails such that the central 15, 15ί-double bond is located in the central hydrophobic region of the bilayer (33), while others present evidence that it is mainly located in the tail-end region at the center of the bilayer. Regarding the carotenoids action, it is proposed that the mechanism may involve peroxyl radical addition to and electron capture by the carotenoid polyene chain. It is also suggested that hydrogen abstraction from the allylic positions may occur (34). Thus, the operating mechanism depends on the oxygen tension (34). Upon comparing Figs. 1-3 with Fig. 4 it becomes evident that perhaps the reason for such a dispute lies in the difference between the membrane compositions studied by different groups. It has been found that the antioxidant potency is determined not only by the chemical reactivity of the antioxidant towards radicals but also by other physical factors (33).

Borg and Schaich (35, 36), suggested that at high concentrations Fe^{2+} can be expected to inhibit peroxidation by the following radical scavenging reactions:



$$Fe^{2+} + LOO^{\circ} + H^{+} \xrightarrow{fast} Fe^{3+} + LOOH \quad [1]$$



$$Fe^{2+} + LO^{\circ} + H^{+} \xrightarrow{very\ fast} Fe^{3+} + LOH \quad [2]$$

Yoshida (37), Vile and Winterbourn (38), have

shown that iron ions binds to liposomal membrane and causes a lag period to appear.

Driomina, et al. (39), have suggested that there may be a massive lipid peroxidation after a time lag at a critical concentration of Fe^{2+} at the membrane surface. However, as can be seen, Figs. (1-3) clearly show that when Fe^{2+} is chelated by EDTA or ascorbic acid, the lag period is not affected. On the other hand the data regarding the formation of conjugated dienes in liposomes made of erythrocyte phospholipids exposed to Fe^{2+} ions in the presence or absence of β-carotene do not differ significantly (i.e. neither an antioxidant nor a pro-oxidant role), Fig. (5). Whereas, in the case of liposomes made of egg yolk phosphatidylcholine the antioxidant behavior of β-carotene in the presence of Fe^{2+} ions can be clearly discerned by the 10 min time lag observed, Fig. (6). This is confirmed by spectroscopic studies that show β-carotene present in liposomes made of egg yolk phosphatidylcholine is consumed faster (in presence of Fe^{2+}) than that present in liposomes made from erythrocyte phospholipids under the same conditions.

CONCLUSIONS

Although some contend that the antioxidant activity depends on partial pressure of the oxygen (pro-oxidant at high oxygen pressures and anti-oxidant at low oxygen pressures) our data show that antioxidant activity of β–carotene is also dependent on the membrane lipid composition and even in atmospheric oxygen pressures it can act as an anti-oxidant. This may provide an explanation about the conflicting reports on the anti-/pro-oxidant functioning of β–carotene in ordinary or promoted oxidation experiments.

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