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Antioxidant synergism between carotenoids in membranes. Astaxanthin as a radical transfer bridge

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ABSTRACT

In phosphatidyl choline liposomes, carotenoids increased the lag phase for oxidation initiated by the water soluble azo-initiator AAPH (zeaxanthin > lycopene > astaxanthin > β-carotene) and more significantly for the oxidation initiated by the lipid soluble AMVN (zeaxanthin > astaxanthin > lycopene > β-carotene). For combination of astaxanthin with β-carotene or astaxanthin with lycopene, significant antioxidant synergism was observed for initiation of oxidation in the lipid phase. Astaxanthin anchored in the water/lipid interface is suggested to scavenge radicals in the interface and transfer an electron from a non-polar and more reducing carotenoid in the membrane interior. Spectroscopic detection of steady state concentration of carotenoid radical cations during electrochemical oxidation of carotenoid mixtures confirmed conversion of astaxanthin radical cations to radical cations of other carotenoids. Decrease in membrane fluidity as measured by fluorescence anisotropy was most significant for astaxanthin (and zeaxanthin), which may further hamper diffusion and bimolecular reactions of radicals, with an antioxidative effect.

1. Introduction

Carotenoids are only synthesised in the plant kingdom, but are transferred through the food chain to animals where some carotenoids are known to have specific functions or become important provitamins. In relation to human health, a high intake of carotenoids through fruits and vegetables is considered beneficial and health promoting effects are often assigned to the antioxidant activities of carotenoids (Bhosale, Serban, & Bernstein, in press; Hsu et al., 2008; Rodriguez & Rodriguez-Amaya, 2007; Velu & Munuswamy, 2007). Besides the well-documented role of lutein and zeaxanthin for protection of the eye during ageing, a possible protection of carotenoids against cardiovascular diseases and cancer are mainly based on epidemiological studies and the link to antioxidant activity is uncertain (Chen & Djuric, 2001; Gireesh & Sudhakaran, 2008; Kamath, Srikanta, Dharmesh, Sarada, & Ravishankar, 2008; Lockwood & Gross, 2005; Riccioni, Mancini, Di Ilio, Bucciarelli, & D'Orazio, 2008). Carotenoids are lipophilic but those with polar hydroxyl and keto functionalities have increased affinities for lipid/water interfaces. One such oxygen carotenoid, astaxanthin, found in many marine organisms, has been demonstrated to be of equal importance to α-tocopherol as an antioxidant in salmon muscles (Jensen, Birk, Jokumsen, Skibsted, & Bertelsen, 2007). Astaxanthin is among the least reducing carotenoids and in homogeneous solution moreover found to be a very poor radical scavenger (Han et al., 2006; Mortensen & Skibsted, 1997). Theoretical calculations likewise show that astaxanthin is placed very low in the antioxidant hierarchy of carotenoids (Galano, 2007). Still astaxanthin is found in liposomes, and other structured media as models for cell membranes, to be superior to other carotenoids as an antioxidant (Naguib, 2000). In such structured media, the distribution and orientation of carotenoids inside the membrane together with any changes in the physical properties of membrane owing to the incorporation of carotenoids will also affect the antioxidative activities of the carotenoids (Liebler, Stratton, & Kaysen, 1997; Stahl et al., 1998).

The orientation of carotenoids in model lipid membranes has been extensively studied in recent years by means of several experimental techniques including electron paramagnetic resonance spectroscopy, light scattering, differential scanning calorimetry and X-ray diffractometry (Gabrielska & Gruszecki, 1996; Goto et al., 2001). It may be summarised from such studies that the chromophore of carotenoid molecules, i.e. the polyene chain, is located in the hydrophobic core of membranes, while carotenoids with polar heads have their hydrophilic groups located in two opposite polar zones of the bilayer. It was further proved that

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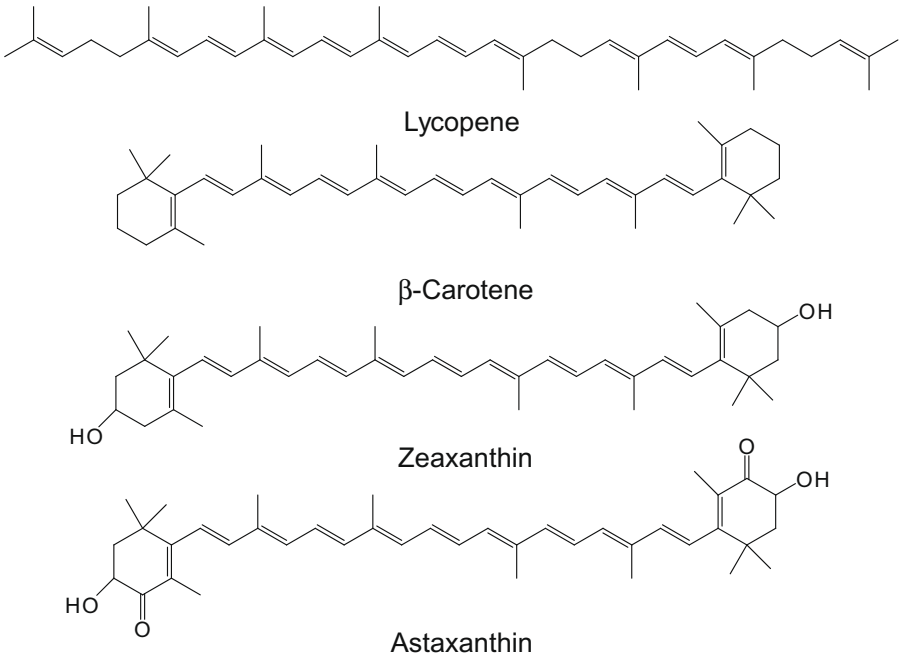


Scheme 1. Structures of four carotenoids examined in this study.

carotenoid phospholipids, the carotenoids connected with polar groups, can form stable micelles with the hydrophobic groups in the core and the polar heads at the interface to the aqueous phase (Foss et al., 2005). Confirming the importance of spatial orientation of carotenoids, it was further shown for inhibition of oxidation in liposomes, that the sequence of carotenoids with respect to efficiency as antioxidant was clearly different from that for homogeneous systems (Naguib, 2000).

Interaction of antioxidants is increasingly being recognised as important for antioxidant effects on food stability and for possible health effects of antioxidants (Becker, Nissen, & Skibsted, 2004). The antioxidant efficiency of mixtures of carotenoids and cooperative interactions of different carotenoids in relation to their distributions in membranes have however not been studied in any detail. The present study was designed to investigate the interaction in liposomes between the hydrophobic carotenes β-carotene and lycopene, and the more polar carotenoids zeaxanthin and astaxanthin (Scheme 1).

2. Materials and methods

2.1. Chemicals

Zeaxanthin (Zea) and astaxanthin (Ast) sealed in ampoules under argon were supplied by Roche A/S (Hvidovre, Denmark) and used as received. Soybean L-R-phosphatidyl choline (PC), β-carotene (β-Car, >99.0%), methylene chloride (HPLC grade), tetrabutylammonium hexafluorophosphate (TBAHFP, polarographic grade), 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma (St. Louis, MO, USA). Lycopene (Lyc, >99.0%) was a gift from Technical Institute of Physics and Chemistry of Chinese Academy of Science (Beijing, China). 2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) was purchased from Huichang Petrochemical Auxiliary Co. Ltd. (Zibo, Shandong, China). Chloroform (HPLC grade) and ethanol (>99%), were purchased from Beijing Chemical Plant (Beijing, China).

2.2. Evaluation of antioxidation in liposomes

Liposomes were prepared by extrusion (Roberts & Gordon, 2003). PC (0.68 mg) was dissolved in chloroform (3 ml), and carotenoids dissolved in absolute ethanol (45 μl, the concentration of each carotenoid stock solution was 500 μM) added to reach a carotenoid-to-PC molar ratio of 3% (molecular mass of soybean PC was taken as 900). In experiments with initiation of lipid oxidation in the lipid phase, the lipid-soluble radical generator AMVN in absolute ethanol (25 μl, 64 mM) was added and solvent removed under reduced pressure by a rotary evaporator (water-bath temperature of 30 °C). Nitrogen was introduced to re-establish atmospheric pressure, the flask covered with aluminium foil, and an oil-free vacuum pump used to maintain flask vacuum at <0.5 mm Hg for >1 h. The lipid residue was rehydrated with a sodium phosphate buffer (5.0 ml, 10 mM, pH 7.4), and the flask shaken while being sonicated for 1 min producing a homogeneous white suspension of multilamellar liposomes. Large unilamellar liposomes were obtained by pushing the multilamellar liposome solution through a polycarbonate membrane with 100-nm sieve pores (Whatman, Maidstone, England) five times and the water-soluble radical generator AAPH (25 μl, 150 mM) in sodium phosphate buffer (pH 7.4) added for experiments for the initiation of lipid oxidation from the aqueous phase. Lipid peroxidation was followed by monitoring formation of conjugated dienes using absorbance change at 234 nm (A_{234}). The unilamellar liposome suspension (3.5 ml) was pipetted into a quartz cuvette, thermostated at 43 °C with a RTE-110 thermostat (Neslab Instruments Inc., Newington, NH, USA), and change of A_{234} was monitored on a Cary 50 Spectrophotometer (Varian, Walnut Creek, CA, USA). The lag phase was determined as the evolution time to the point where a tangent to the propagation phase intercepted that of the initial phase with little or no oxidation (Roberts & Gordon, 2003).

2.3. Spectroelectrochemistry

Absorption spectra (600 nm < λ < 1100 nm) of each carotenoid (β-Car, Lyc, Zea, Ast) dissolved in dichloromethane with 0.10 M

tetrabutylammonium hexafluorophosphate as supporting electrolyte were measured at room temperature in a spectroelectrochemical cell with an optical path of 8 mm in a Cary 50 Spectrophotometer (Varian, USA). Conductive glass (Leybold, Alzenau, Germany) was used as working and counter electrodes and the reference electrode was a platinum wire. The voltage was fixed at 1.0 V and the spectra recorded when a maximal absorption value was achieved, which occurred within a few seconds after the potential was applied. Spectra were recorded for 1×10^{-3} M solution of β-Car, Lyc and Zea and for each of these carotenoids for solutions with successive addition of Ast with increment of 1×10^{-3} M. All solutions were degassed with nitrogen prior to measurements. The conductive glass was renewed prior to each measurement.

2.4. Evaluation of membrane fluidity

The influence of added carotenoids on the fluidity of the hydrophobic region of membranes was measured by using the of fluorescence probe DPH (Socaciu, Lausch, & Diehl, 1999). The carotenoid was added to the liposome solution to yield a final concentration of 4.5 μM, while DPH was added to the PC solution during the preparation of the liposome (final concentration of 0.5 μM; DPH-to-PC molar ratio of 1/300). Fluorescence polarisation (P) was determined at 425 nm (excited at 358 nm) using a LS-55 luminescence spectrophotometer (Perkin Elmer, Beaconsfield, England) according to the relation,

$$P = (I_{\parallel} - G \cdot I_{\perp})/(I_{\parallel} + G \cdot I_{\perp})$$

where $I_{\parallel}$ and $I_{\perp}$, respectively, represent the fluorescence intensities measured with the emission polarisation parallel and vertical to the excitation polarisation, and G is the instrumental correction factor.

3. Results and discussion

Lipid peroxidation of the PC liposomes suspended in an aqueous phosphate buffer was induced by thermolysis of azo-initiators and monitored spectrophotometrically through formation of conjugated dienes, and used for evaluation of carotenoids as antioxidants. Among the four carotenoids studied, β-Car and Lyc are hydrophobic and will concentrate in the interior of the membrane, while Zea and especially Ast will be anchored at the lipid/water interface. In order to obtain optimal experimental conditions, the lipophilic initiator AMVN was used in a concentration of 320 μM at a temperature of 43 °C and the hydrophilic initiator for AAPH in a concentration of 750 μM. Addition of β-Car or Lyc in the relatively high concentration of 4.5 μM resulted in a small but significant increase in the lag phase as may be seen from Fig. 1 for initiation with AMVN. Ast and Zea both had larger effects. The relative effect of carotenoids as antioxidants was more significant for initiation from the lipid phase (Zea > Ast > Lyc > β-Car) than from the aqueous phase (Zea > Lyc > Ast > β-Car). The nature of the radical initiator has also previously been found to be important for efficiency of the same antioxidant and linked to the spatial distribution of the initiating radical (Sliwka et al., 2007). It was recently demonstrated by us using quantum chemical calculations that the dipolar moment of the peroxyl radical generated by the lipophilic initiator AMVN is higher than the dipolar moment of the peroxyl radical generated by hydrophilic AAPH (Liang et al., 2008). The AMVN derived radical is accordingly expected to concentrate at the lipid/water surface in competition with biomolecular reactions in the membrane interior. Lyc and especially β-Car, both efficient as radical scavengers (Mortensen & Skibsted, 1997), were seen to have only small effects on the length of the lag phase, while Zea and Ast, both less efficient radical scavengers and less reducing than Lyc and β-Car (Han et al., 2004, 2006), more than increased

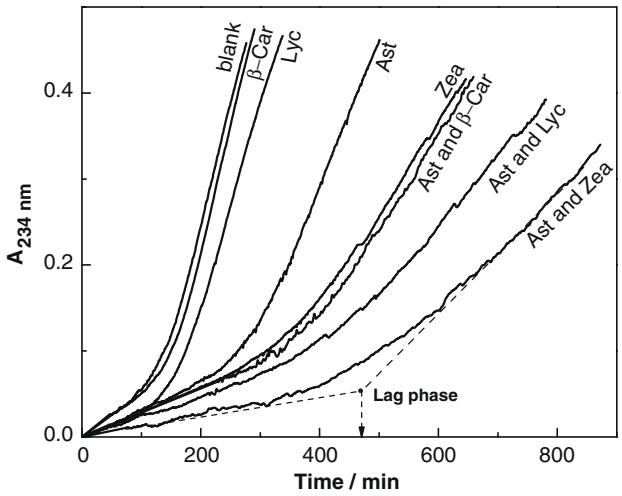


Fig. 1. Formation of conjugated dienes at 43 °C as monitored spectrophotometrically at 234 nm for liposomes with 4.5 μM β-Car, Lyc, Zea or Ast, or with mixtures of 4.5 μM Ast and 4.5 μM Lyc, β-Car or Zea. Lipid oxidation was initiated by AMVN. The determination of lag phase is shown, as an example, for the case of Ast combined with Zea.

the length of the lag phase by a factor of two for initiation by AMVN. These observations taken together add further support to the suggested importance of the polarity of the AMVN derived peroxyl radical (Liang et al., 2008).The AMVN derived radical will react with Zea and Ast at the lipid/water interface rather than with Lyc and β-Car in the interior of the membrane, and Zea and Ast will appear as more efficient antioxidants despite their lower efficiencies as radical scavengers. Zea is more reducing (0.63 V) than Ast (0.75 V), a difference of importance for the higher over-all efficiency of Zea compared with Ast. A similar difference is noted for the two carotenoids concentrating in the membrane interior of which Lyc (0.59 V) is more reducing than β-Car (0.62 V) and accordingly the more efficient antioxidant (Han et al., 2004, 2006). Carotenoids had less effect on the lag phase, when oxidation was induced by the initiator AAPH, located in the water phase most likely due to less efficient interaction between the AAPH derived radical and the lipid and carotenoids. Combination of each of two less efficient but highly reducing antioxidants β-Car and Lyc with Ast gave a clear synergistic effect for initiation of oxidation in the lipid phase with AMVN as may be seen from Fig. 1 and the length of lag phase collected in Table 1. A similar effect was not seen for combination of Zea and Ast, and for β-Car or Lyc combined with Ast for initiation in the aqueous phase by AAPH.

In order to investigate the free radical interaction between Ast and β-Car, Lyc and Zea, absorption spectra were recorded in the spectral region where carotenoid radical cations show absorption. Oxidation conditions were established electrochemically (~1.0 V),

Table 1
Increase in lag phase for formation of conjugated dienes in PC liposomes as initiated by radical initiators as a result of addition of carotenoids (4.5 μM) or combination of carotenoids (9.0 μM).

Sample	Lag phase (min)	
	AAPH (water soluble)	AMVN (lipid soluble)
β-Car	2 ± 1	15 ± 4
Lyc	9 ± 2	47 ± 6
Ast	6 ± 2	143 ± 8
Zea	13 ± 3	226 ± 6
Ast + β-Car	10 ± 3	232 ± 8
Ast + Lyc	14 ± 2	305 ± 11
Ast + Zea	23 ± 3	346 ± 12

and the spectrum of each carotenoid in dichloromethane in Fig. 2 is seen to display an absorption maximum in the near-infrared region which may be assigned to the radical cation (Han et al., 2006). The energy of the near-infrared transition decreases with efficiency of the parent carotenoid as radical scavenger, confirming previously results (Mortensen & Skibsted, 1997), and the more efficient radical scavengers among the carotenoids are seen to absorb also at the wavelengths longer than 1000 nm in contrast to the radical cation of Ast (Fig. 2). Under the conditions used, the steady state concentration of the radical cation was found proportional to the carotenoid concentration as shown for Lyc in Fig. 2B (inset). Adding increasing concentration of Ast to each of β-Car, Lyc or Zea had a similar effect as the steady state concentration of the radical cation of β-Car, Lyc or Zea increased proportional to total carotenoid concentration. An efficient conversion of the radical cation of Ast initially formed electrochemically to radicals of the more reducing carotenoid β-Car, Lyc or Zea seems accordingly to have been demonstrated.

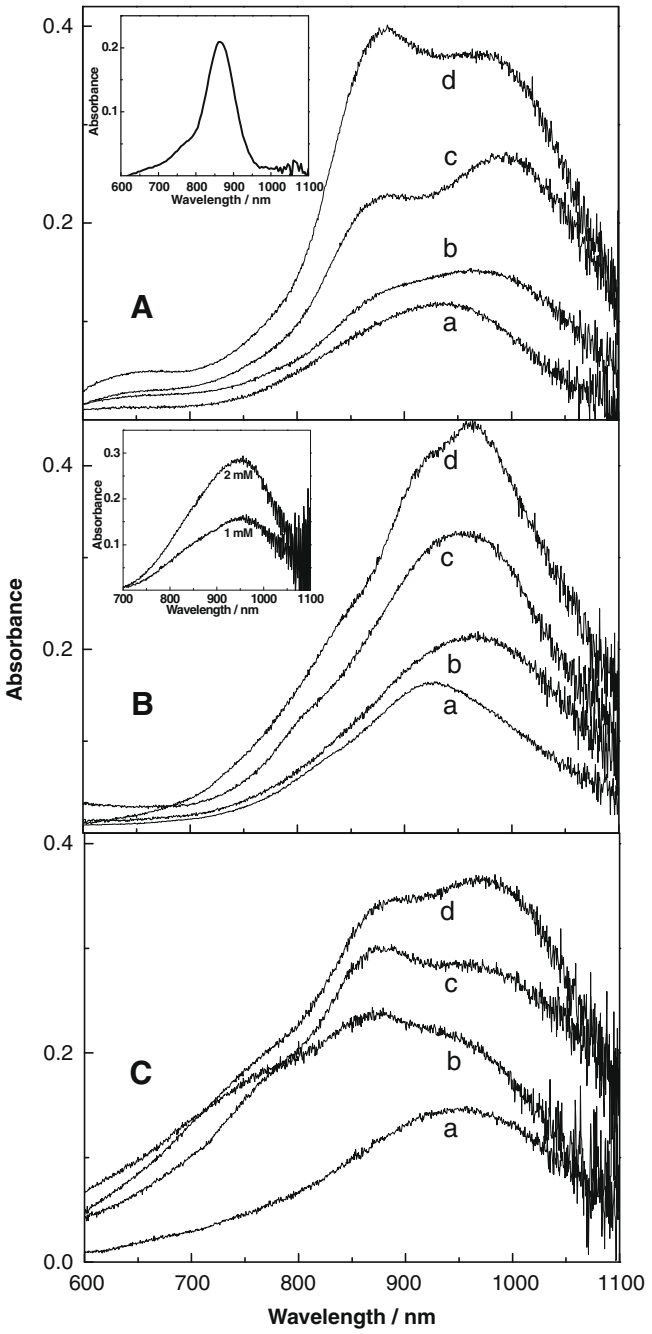


Fig. 2. Steady state spectra of carotenoids in dichloromethane measured in a spectroelectrochemical cell with at voltage of 1.0 V at room temperature: (A) β-Car, (B) Lyc and Zea, (C) at the concentration of 1×10^{-3} M (a), and in the presence of Ast in the concentration of 1×10^{-3} M (b), 2×10^{-3} M (c) and 3×10^{-3} M (d). Inset in A: steady state spectrum of Ast at 1×10^{-3} M. Inset in B: steady state spectra of Lyc at 1×10^{-3} M and 2×10^{-3} M.

The carotenoids also affected the fluidity of the membrane as was demonstrated by fluorescence spectroscopy. Addition of Ast and to a lesser degree addition of Zea and Lyc decrease the fluidity of the membrane as shown by an increase in fluorescence polarity of the probe DPH added to the liposomes (Fig. 3). The anchoring of Zea and especially Ast in the lipid/water interface at both sides of membrane makes the membrane more rigid through van der Waals interaction of the fixed polyene chains of Ast or Zea and the lipid acyl chains (Gabrielska & Gruszecki, 1996; Goto et al., 2001). The decreased fluidity hampers diffusion of free radicals in the membrane, in effect increasing the antioxidative efficiency of the carotenoid Zea or Ast, but clearly not β-Car or Lyc, both of which lack the polar anchoring groups. In the case of lipid oxidation triggered by AAPH, the ability of the antioxidants to stabilize the membrane is important to inhibit the diffusion of the radical into the membrane; this, together with the redox potential of the antioxidant lead to the sequence of antioxidant efficiency, Zea > Lyc > Ast > β-Car (Table 1).

The observed synergism between Ast and β-car or Lyc may be understood on the basis of the spatial distribution of the carotenoids acting synergistically, a mechanism which is illustrated in Scheme 2. Ast is anchored in the lipid/water interface, where the initiating radicals from AMVN are oxidising Ast to yield the radical cation in competition with oxidation of PC. The single electron in the radical cation of Ast will relocate into the centre of the polyene chain of the carotenoid due to resonance stabilization (El-Agamey, Edge, Navaratnam, Land, & Truscott, 2006). The polyene chain may in the lipophilic centre of the membrane abstract an electron from the more reducing carotenoid Lyc or β-Car in effect regenerating Ast at the expenses of Lyc or β-Car as demonstrated spectroelectrochemically. Ast seems accordingly to act as a radical bridge channelling the electron from the more reducing carotenoid in the inner of the membrane to the interface, where the radicals with high dipole moment are concentrated. Notably, Zea and Ast will not act synergistically as they both have similar spatial distribution in the membrane. In this context, the recently reported dipolar Ast derivatives astaxanthin–lysine and cardax are more hydrophilic

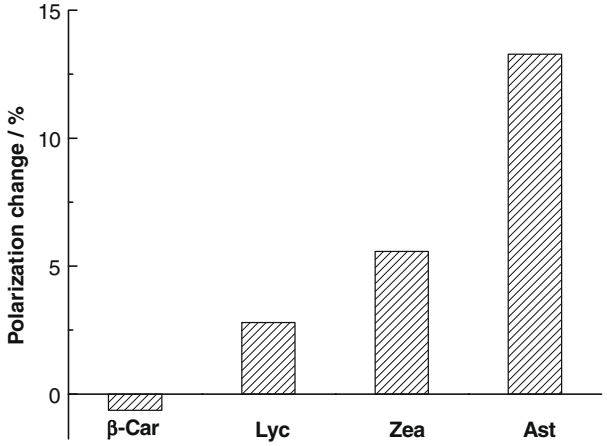
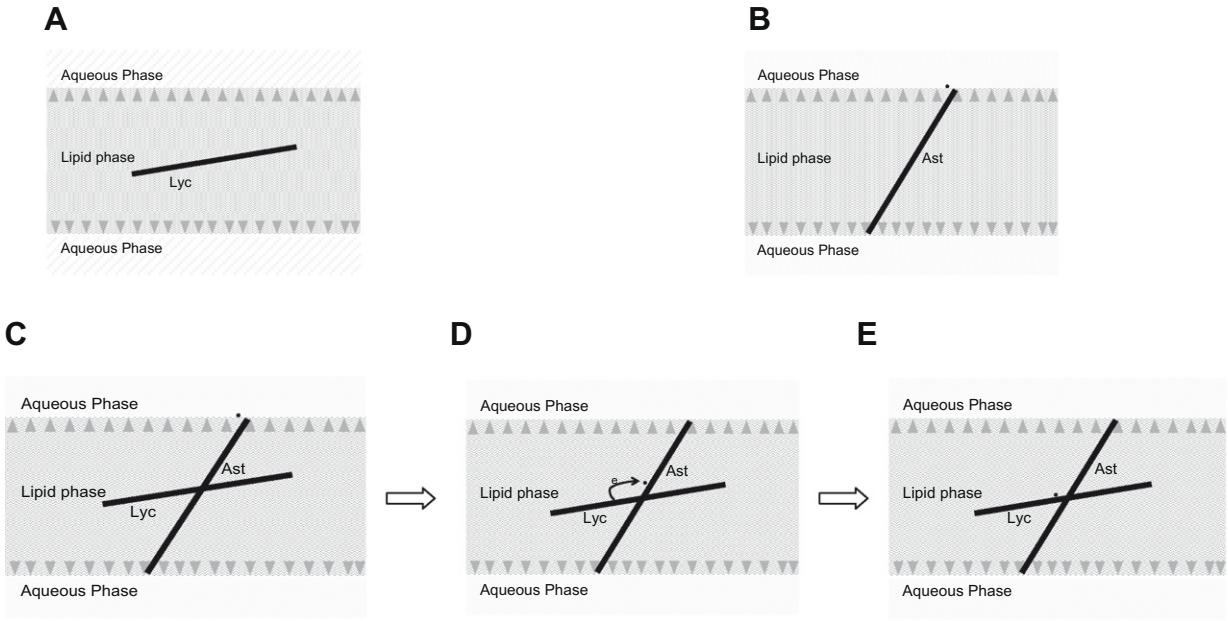


Fig. 3. Change in the fluorescence polarisation of DPH in liposomes (1/300 molar ratio to PC) at room temperature as a function of the addition of carotenoids (3/100 molar ratio to PC).



Scheme 2. Cooperation of Ast and Lyc as antioxidants in liposomal membrane: concentrating in the interface represents peroxyl radical formed by thermal cleavage of AMVN. A: Lyc alone has little contact with initially formed radicals. B: Ast alone is a poor scavenger. C, D, and E: Ast initially scavenge peroxyl radical, Lyc subsequently regenerate Ast by donating an electron.

(Foss, Nadolski, & Lockwood, 2006) and, therefore, may function as better bridge molecules than Ast.

For the lipid oxidation triggered from the aqueous phase, the radical derived from AAPH distribute more homogenously in the membrane due to its lower dipolar moment compared with that of radicals generated by AMVN. The carotenoids, irrespective to their orientation and deposition in the membrane, will accordingly come into contact with the AAPH radicals and no synergic effect will be evident.

The efficiency of carotenoids as antioxidants does not follow their capability as radical scavengers or the order of their oxidation potential (Han et al., 2006; Mortensen & Skibsted, 1997). The long standing controversy of the function of carotenoids as antioxidants may be related to extrapolation of properties determined in homogenous solution to more complex biological systems, where other factors such as spatial organisation and interaction between antioxidants become important.

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References

Becker, E. M., Nissen, L. R., & Skibsted, L. H. (2004). Antioxidant evaluation protocols: Food quality or health effects. *European Food Research and Technology, 219*, 561–571.

Bhosale, P., Serban, B., & Bernstein, P. S. (in press). Retinal carotenoids can attenuate formation of A2E in the retinal pigment epithelium. *Archives of Biochemistry and Biophysics*, doi:10.1016j/j.abb.2008.09.012.

Chen, G., & Djuric, Z. (2001). Carotenoids are degraded by free radicals but do not affect lipid peroxidation in unilamellar liposomes under different oxygen tensions. *FEBS Letters, 505*, 151–154.

El-Agamey, A., Edge, R., Navaratnam, S., Land, E. J., & Truscott, T. G. (2006). Carotenoids radical anions and their protonated derivatives. *Organic Letters, 8*, 4255–4258.

Foss, B. J., Nadolski, G., & Lockwood, S. F. (2006). Hydrophilic carotenoid amphiphiles: Methods of synthesis and biological applications. *Mini-Reviews of Medicinal Chemistry, 6*, 953–969.

Foss, B. J., Sliwka, H.-R., Partali, V., Naess, S. N., Elgsaeter, A., Melø, T.-B., et al. (2005). Hydrophilic carotenoids: Surface properties and aggregation behavior of a highly unsaturated carotenoid lysophospholipid. *Chemistry and Physics of Lipids, 134*, 85–96.

Gabrielska, J., & Gruszecki, W. I. (1996). Zeaxanthin (dihydroxy-β-carotene) but not β-carotene rigidifies lipid membrane: A ^{1}H NMR study of carotenoid-egg phosphatidylcholine liposomes. *Biochimica et Biophysica Acta, 1285*, 167–174.

Galano, A. (2007). Relative antioxidant efficiency of a large series of carotenoids in term of one electron transfer reaction. *Journal of Physical Chemistry B, 111*, 12898–12908.

Gireesh, T., & Sudhakaran, P. R. (2008). In vitro uptake of beta-carotene by human exfoliated colonic epithelial cells. *International Journal of Food Sciences and Nutrition, 12*, 1–10.

Goto, S., Kogure, K., Abe, K., Kimata, Y., Kitahama, K., Yamashita, E., et al. (2001). Efficient radical trapping at the surface and inside the phospholipids membrane is responsible for highly potent antiperoxidative activity of the carotenoids astaxanthin. *Biochimica et Biophysica Acta, 1512*, 251–258.

Han, R. M., Wu, Y. S., Feng, J., Ai, X. C., Zhang, J. P., & Skibsted, L. H. (2004). Radical cation generation from singlet and triplet excited states of all-trans-lycopene in chloroform. *Photochemistry and Photobiology, 80*, 326–333.

Han, R. M., Tian, Y. X., Wu, Y. S., Wang, P., Ai, X. C., Zhang, J. P., et al. (2006). Radical cation generation from zeaxanthin and astaxanthin singlet and triplet exited states in chloroform with different mechanism. *Photochemistry and Photobiology, 82*, 538–546.

Hsu, Y. W., Tsai, C. F., Chang, W. H., Ho, Y. C., Chen, W. K., & Lu, F. J. (2008). Protective effects of *Dunaliella salina* – a carotenoids – rich alga, against carbon tetrachloride-induced hepatoxicity in mice. *Food and Chemical Toxicology, 46*, 3311–3317.

Jensen, C., Birk, E., Jokumsen, A., Skibsted, L. H., & Bertelsen, G. (2007). Effect of dietary levels of fat, α-tocopherol and astaxanthin on colour and lipid oxidation during storage of frozen rainbow trout (*Oncorhynchus mykiss*) and during chill storage of smoked trout. *Zeitschrift für Lebensmittel-Untersuchung und - Forschung, 3*, 189–196.

Kamath, B. S., Srikanta, B. M., Dharmesh, S. M., Sarada, R., & Ravishankar, G. A. (2008). U1cer preventive and antioxidative properties of astaxanthin from *Haematococcus pluvialis*. *European Journal of Pharmacology, 590*, 387–395.

Liang, J., Tian, Y. X., Fu, L. M., Wang, T. H., Li, H. J., Wang, P., et al. (2008). Daidzein as an antioxidant of lipid: Effects of the microenvironment in relation to chemical structure. *Journal of Agriculture and Food Chemistry, 56*, 10376–10383.

Liebler, D. C., Stratton, S. P., & Kaysen, K. L. (1997). Antioxidant actions of β-carotene in liposomal and microsomal membranes. *Role of carotenoid-membrane incorporation and α-tocopherol. Archives of Biochemistry and Biophysics, 15*, 244–250.

Lockwood, S. F., & Gross, G. J. (2005). Disodium disuccinate astaxanthin (Cardax): Antioxidant and antiinflammatory cardioprotection. *Cardiovascular Drug Review, 23*, 199–216.

Mortensen, A., & Skibsted, L. H. (1997). Importance of carotenoid structure in radical-scavenging reactions. *Journal of Agriculture and Food Chemistry, 45*, 2970–2977.

Naguib, Y. M. A. (2000). Antioxidant activities of astaxanthin and related carotenoids. *Journal of Agriculture and Food Chemistry, 48*, 1150–1154.

Riccioni, G., Mancini, B., Di Ilio, E., Bucciarelli, T., & D'Orazio, N. (2008). Protective effect of lycopene in cardiovascular disease. *European Review for Medical and Pharmacological Sciences, 12*, 183–190.

Roberts, W. G., & Gordon, M. H. (2003). Determination of the total antioxidant activity of fruits and vegetables by a liposome assay. *Journal of Agriculture and Food Chemistry, 51*, 1486–1493.

Rodriguez, E. B., & Rodriguez-Amaya, D. B. (2007). Formation of apocarotenals and epoxycarotenoids from β-carotene by chemical reactions and by autoxidation in model systems and processed foods. *Food Chemistry, 101*, 563–572.

Sliwka, H.-R., Melø, T.-B., Foss, B. J., Abdel-Hafez, S. H., Partali, V., Nadolski, G., Jackson, H., & Lockwood, S. F. (2007). Electron- and energy-transfer properties of hydrophilic carotenoids. *Chemistry a European Journal, 13*, 4458–4466.

Socaciu, C., Lausch, C., & Diehl, H. A. (1999). Carotenoids in DPPC vesicles: Membrane dynamics. *Spectrochimica Acta Part A, 9*, 2289–2297.

Stahl, W., Junghans, A., de Boer, B. D., Driomina, E. S., Briviba, K., & Sies, H. (1998). Carotenoids mixtures protect multilamellar liposomes against oxidative damage: Synergistic effects of lycopene and lutein. *FEBS Letters, 427*, 305–308.

Velu, C. S., & Munuswamy, N. (2007). Composition and nutritional efficacy of adult fairy shrimp *Streptocephalus dichotomus* as live feed. *Food Chemistry, 100*, 1435–1442.