ELSEVIER

Biochimica et Biophysica Acta 1194 (1994) 138–142

BBA Biochimica et Biophysica Acta

Effect of β-carotene on structural and dynamic properties of model phosphatidylcholine membranes. I. An EPR spin label study

Kazimierz Strzałka [a,*], Wiesław I. Gruszecki [b]

[a] *Department of Plant Physiology and Biochemistry, The Jan Zurzycki Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31–120 Kraków, Poland*

[b] *Institute of Physics, Maria Curie-Skłodowska University, Lublin, Poland*

Received 3 February 1994

Abstract

The influence of β-carotene on structural and dynamic properties of model membranes (multilamellar liposomes) prepared of dipalmitoylphosphatidylcholine was investigated. It was found that β-carotene: (1) decreases order within crystalline state of the membrane; the effect of β-carotene was more pronounced than in the case of the polar carotenoid, lutein, as revealed by means of spin label EPR; (2) increases penetration, stronger than lutein, of apolar molecules into the membrane as indicated by greater partition coefficient of 5-doxyldecane; (3) increases correlation times τ_B and τ_C stronger than lutein. In all cases the effect of β-carotene on a membrane was more pronounced at crystalline state than at fluid state. On this basis a hypothesis is proposed that β-carotene plays a physiological function in the fluidization of chloroplast membranes in a chilling stress to the photosynthetic apparatus.

Key words: Carotenoid pigment; EPR; Lecithin; Model membrane; Photosynthesis; Spin label

1. Introduction

Carotenoids are important constituents of the photosynthetic apparatus and their physiological meaning is mainly ascribed as light harvesting in the antenna system [1–5] and as protection against photodynamic damage to biomolecules [6–10]. These pigments are also widely distributed in the animal kingdom, including humans, where they normally occur in blood, adipose, liver and muscles [11,12]. Their involvement in anti-disease and in health promoting functions and associations constitutes rapidly expanding field of research [13,14].

Carotenoids are also postulated to play a physiological role in modifying structural and dynamic properties of biomembranes [15–17], model membranes [18–26] and in particular thylakoid membranes [27,28]. The effect of carotenoid pigments with respect to lipid membranes may be summarized as follows:

(1) Rigid, rod-like carotenoid molecules rigidify lipid bilayer in its fluid phase and this effect is about twice as strong as in the case of cholesterol.
(2) The rigidifying effect of xanthophylls is optimal when the thickness of the hydrophobic core of a membrane fits the length of a pigment molecule.

The above studies dealing with the effect of carotenoids on properties of model membranes as well as natural membranes focused either on xanthophylls (performed predominantly with model membranes) or on total carotenoids (carried out largely on natural membranes) and they are devoted primarily to the effects of these pigments on membranes in the fluid phase. However, systematic investigations on the influence of hydrocarbon carotenoids, belonging to group of carotenes, on molecular dynamics of membranes have not been performed. Absence of oxygen in the carotenes ionone rings may have a strong impact on the type of interactions occurring between these rings and membrane lipid headgroups when compared with

Abbreviations: CrOx, Tris(oxalato)chromium(III); 5-DD, 5-doxyldecane; 5-SASL, 5-doxylstearic acid; DPPC, DL-α-dipalmitoylphosphatidylcholine; EPR, electron paramagnetic resonance.

* Corresponding author. Fax: +48 12 336907. E-mail: strzalka@mol.uj.edu.pl.

SSDI 0005-2736(94)00135-C

xanthophyll type compounds, where polar interactions occur and the possibility exists of hydrogen bond formation.

Therefore investigation of the influence of carotenoids on structural and dynamic properties of model membranes is analyzed with special attention paid to β-carotene, a carotenoid commonly occurring in plants, and its modulating effect at low temperatures to which many plants of moderate climate zones are frequently exposed. The aim of this research is to test the hypothesis concerning the importance of β-carotene to maintain physiological fluidity of the thylakoid membrane under chilling stress conditions in the photosynthetic apparatus. Dipalmitoylphosphatidylcholine has been selected for this investigation since the thickness of the hydrophobic core of a membrane formed by this lipid corresponds to the length of the carotenoid molecule (about 30 Å) and the effect is expected to be pronounced [24]. On the other hand the thickness of a hydrophobic core of a thylakoid membrane is reported to be about 30 Å [29].

2. Materials and methods

β-Carotene and lutein were obtained as a gift from Hoffmann-La Roche (Basle, Switzerland). Spin probes: 5-doxylstearic acid (5-SASL) and 5-doxyldecane (5-DD) as well as DL-α-dipalmitoylphosphatidylcholine (DPPC) were purchased from Sigma. Dispersion of multilamellar liposomes of DPPC (20 mg/ml) or DPPC containing carotenoids was prepared by mixing chloroform solutions of respective compounds, evaporation of solvent, first in a stream of nitrogen and subsequently by vacuum. The dry residue was vortexed with 10 mM Hepes buffer (pH 7.6) containing 30 mM Tris(oxalato) chromium(III) (CrOx) and 1 mM potassium ferricyanide. The last two compounds were present in a spin labeled liposome suspension to broaden the EPR signal of probes not bound to membranes and to keep probes maximally oxidized. No CrOx was added in the experiments on partition coefficient of 5-DD. EPR measurements were performed with Varian E-109 spectrometer operating in an X band, using the parameters as described by Strzałka and Subczyński [30]. All presented data results from heating experiments. Prior to measurements, multilamellar vesicle suspension was adapted to 0°C.

3. Results

Temperature profiles of hyperfine splitting parameter $2A'_{\|}$ of the EPR spectra of 5-SASL doped into DPPC membranes and DPPC membranes containing 0.25 mol% or 2.5 mol% of β-carotene are shown in Fig. 1. As evident from this figure this parameter, in this particular case, is not sensitive to main phase transition $P'_{\beta} \Rightarrow L_{\alpha}$ ($\simeq$ 41°C,) nor to phase pretransition $L_{\beta'} \Rightarrow P_{\beta'}$ ($\simeq$ 35°C,) but rather to the $L_c \Rightarrow L_{\beta'}$ transition ($\simeq$ 25°C [31]). The main phase transition of DPPC membranes may be followed by some other spectral parameters of 5-SASL like height of a central line h_o [24] or low-field line width [32]. The hyperfine splitting parameter is directly related to order parameter [23] and is chosen in the present study to examine an effect of carotenoid pigments on structural properties of phospholipid bilayers particularly at low temperatures. As seen from Fig. 1, β-carotene decreases the value of maximum splitting especially below the $L_c \Rightarrow L_{\beta'}$ phase transition. The data presented in Fig. 2 clearly demonstrate that this effect is concentration dependent. The

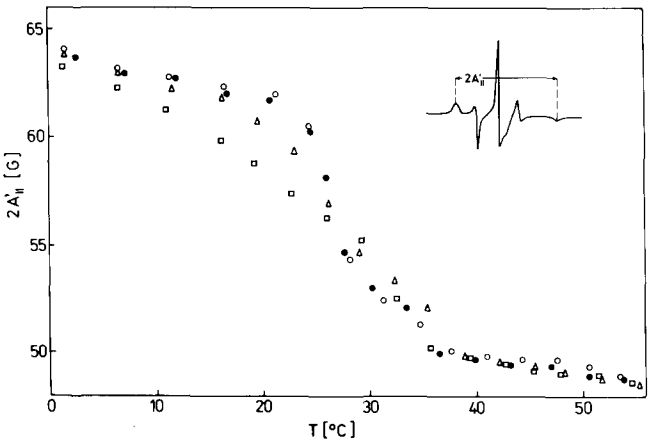

Fig. 1. Temperature dependence of hyperfine splitting of 5-SASL doped into DPPC multilamellar vesicles (●, ○, two independent experiments) and DPPC multilamellar vesicles containing 0.25 mol% (△) and 2.5 mol% (□) of β-carotene. Concentration of DPPC 20 mg/ml. (Inset) Typical EPR spectrum of 5-SASL with indicated hyperfine splitting parameter $2A'_{\|}$. Spectrum recorded at modulation amplitude 2 G.

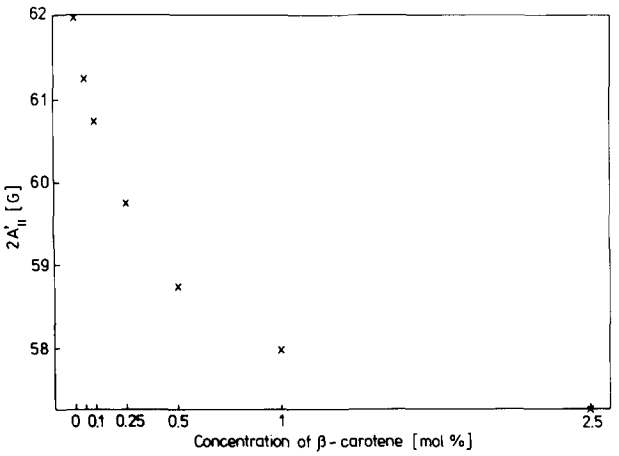

Fig. 2. β-Carotene concentration dependence of hyperfine splitting of 5-SASL doped into DPPC multilamellar vesicles. Temperature 22°C.

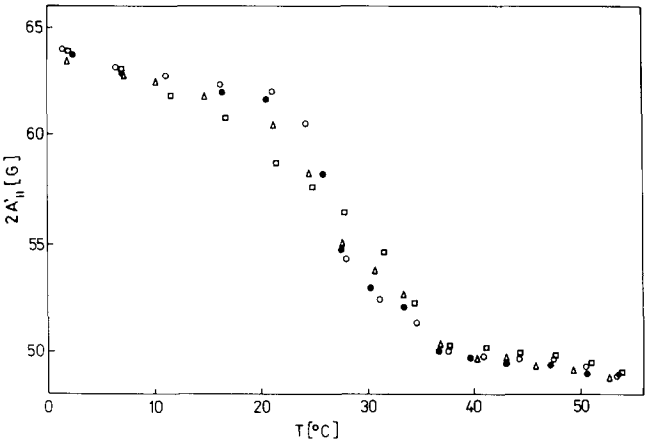

Fig. 3. Temperature dependence of hyperfine splitting of 5-SASL doped into DPPC multilamellar vesicles (●, ○, experiments the same as in Fig. 1) and DPPC multilamellar vesicles containing 0.25 mol% (△) and 2.5 mol% (□) of lutein.

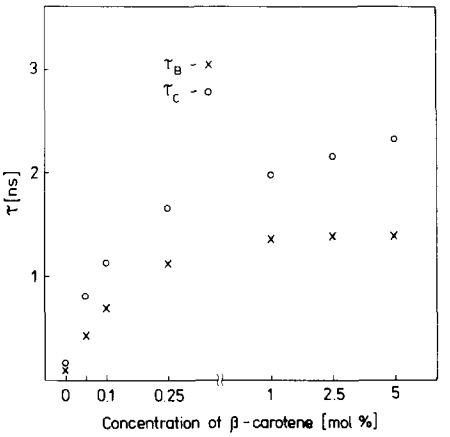

Fig. 5. β-Carotene concentration dependence of rotational correlation times τ_B (×) and τ_C (○) of 5-DD spin label doped into DPPC multilamellar vesicles. Temperature 22°C.

direction of the changes observed (the same as the increase of temperature) was interpreted as β-carotene incorporated into DPPC membranes decreases the order in the lipid core. A similar effect of a polar carotenoid lutein (see Fig. 3) is less pronounced than of β-carotene.

Fig. 4 indicates a temperature dependence of the partition coefficient of apolar spin probe 5-DD between water and lipid phases. The parameter was calculated on the central line basis of EPR spectrum as indicated by the inset of Fig. 4. The partition experiment results may be interpreted that β-carotene addition makes the membrane structure less compact and thus more spin probe molecules can enter directly the lipid phase. Two different phenomena may be responsible for this effect: fluidization of a membrane interior and/or disorder effect within the polar headgroup region. Again, polar carotenoid lutein which interacts by its hydroxy groups with phosphatidylcholine headgroups exerts a small effect on the measured partition coefficient compared with β-carotene.

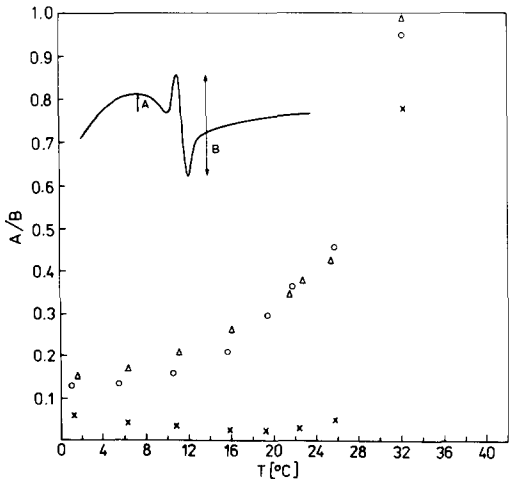

Fig. 4. Temperature dependence of partition coefficient A/B (calculated as indicated in the inset, on the basis of a central line of EPR spectrum) of 5-DD spin label between lipid and water phases. ×, suspension of DPPC multilamellar vesicles; ○, suspension of multilamellar vesicles of DPPC containing 2.5 mol% of lutein and △, 2.5 mol% of β-carotene.

Figs. 5 and 6 illustrate the β-carotene effect on rotational correlation times τ_B and τ_C of spin label 5-DD which molecules are predominantly localized parallel to the membrane surface within the membrane's hydrophobic interior. This spin probe was chosen since its mobility in the temperature range selected was high enough to permit calculation of correlation time parameters for rotational diffusion. Fig. 5 illustrates a substantial increase in both correlation times in function of β-carotene concentration as measured at 22°C, below $L_c \Rightarrow L_{\beta'}$ phase transition temperature of DPPC. The difference between values of τ_B and τ_C both in pure DPPC as well as in the membranes containing various amounts of β-carotene indicates an anisotropy of the spin label movement. The degree of anisotropy, measured as the ratio of both correlation time values does not appear to be dependent upon the amount of β-carotene at its low concentration range but becomes more pronounced at higher concentrations in the membrane (Fig. 5). On the other hand the effect of investigated carotenoids on both correlation time parameters depends greatly on temperature (Fig. 6). Both β-carotene as well as lutein increase greatly values of τ_B and τ_C with the rise of temperature, β-carotene being again more effective than lutein. τ_C was found to be more sensitive to temperature increase than τ_B which is clearly manifested, specifically at lower temperature range. This indicates an increased anisotropy of 5-DD molecules movement at these temperatures. One should note that control DPPC membranes show a slight decrease in the value of both correlation times in the temperature ranges studied.

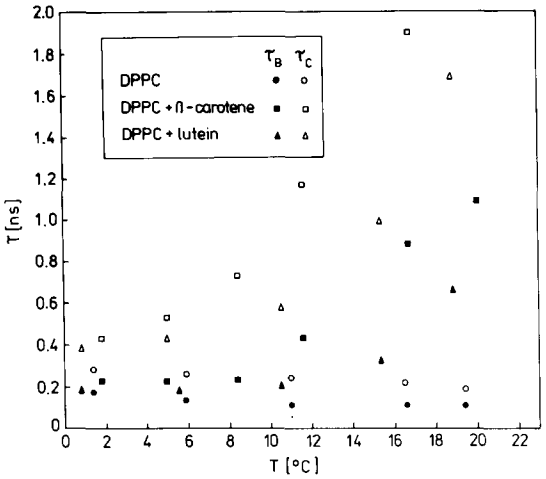

Fig. 6. Temperature dependence of rotational correlation times τ_B (full symbols) and τ_C (open symbols) of 5-DD spin label doped into DPPC multilamellar vesicles (●, ○) and such vesicles containing additionally 2.5 mol% of β-carotene (■, □) and 2.5 mol% of lutein (▲, △).

The rise in values of the measured correlation times of 5-DD spin label in carotenoid-containing membranes as a function of increasing temperature and as a function of β-carotene concentration (in contrast to their decrease in pure DPPC vesicles) indicates that both investigated carotenoids create a disorder in the regular crystalline structure of membrane lipids. As a consequence, an increased penetration of 5-DD molecules to slits and cavities generated due this disorder occurs. 5-DD molecules entering such spaces exhibit a higher motion hindrance than when localized in a more regular way in the hydrophobic interior between two well ordered membrane leaflets, parallel to the surface.

4. Discussion

As it is concluded on the basis of EPR spin label studies, β-carotene affects structural and dynamic properties of model DPPC membranes. The effect of β-carotene with respect to examined membranes may be termed bilayer fluidization and summarized to consist of:

(1) Decrease of an order of alkyl chains in crystalline phase as demonstrated by the decrease of a maximum splitting parameter of 5-SASL.
(2) Decrease of a diffusional barrier and solubility increase of apolar molecules within a lipid phase demonstrated by an increased partition coefficient of 5-DD between water and lipid phases.
(3) Increase of correlation times τ_B and τ_C of 5-DD as a function of β-carotene concentration in a membrane.

The membrane fluidizing effect of lutein which is a polar carotenoid was found not to be as pronounced as the effect of β-carotene. On the other hand the presence of polar carotenoids in phospholipid membranes results in ordering of acyl chains particularly in the membrane fluid state [22–24]. This ordering effect is explained by hydrophobic interactions between hydrocarbon lipid chains and a rigid xanthophyll molecule anchored by its polar groups in two opposite polar regions of a lipid bilayer [25,26]. Fluidizing effect of β-carotene with respect to the gel state of DPPC membranes reported in this paper might be explained in terms of 'an impurity'-introduced disorder effect in the network of well-ordered acyl chains in a crystalline state of the membrane. β-Carotene does not possess in its structure any polar groups which may interact with opposite polar surfaces of the bilayer. As a consequence, an orientation of β-carotene with respect to lipid bilayer is not as well defined as xanthophyll pigments oriented almost perpendicularly to the plane of the membrane [25,26,33].

The fluidizing effect of β-carotene with respect to well ordered lipid membranes (at low temperatures) may have physiological significance in chilling temperatures and in cold stress of the photosynthetic apparatus. Physiological functioning of thylakoid membranes requires their highly fluid state, realized mainly by relatively high unsaturation of chloroplast lipids [34]. This requirement is related to lateral diffusion of photosynthetic, mobile electron carriers like plastoquinone [35] or a membrane fluidity-dependence of membrane-bound enzymes. All these processes may be obviously affected after the low temperature-related drop of a physiological membrane fluidity in cold stress. All photosynthetic pigments are deemed bound in situ to functional proteins, however, the repair processes expected to be particularly active in stress conditions require constant pigment synthesis and migration through the membrane. It means that a certain amount of carotenoid pigments may also be present directly in the lipid phase (see also Discussion in Gruszecki and Strzałka [27]). In fact, as it was reported, cold hardening of pine [36] and rye [37] results in the increased synthesis of carotenoids, particularly β-carotene. This model system work should now be followed by study of the effect of β-carotene on properties of natural membranes, and such research is in progress.

The membrane fluidizing effect of β-carotene may be also important for its antioxidant activity, which is believed to be a basis of its anti-disease properties [13,14,38]. As reported by Conn et al. [10] the efficiency of singlet oxygen quenching by β-carotene was inversely proportional to solvent viscosity. Extrapolating these results to the membrane system one may conclude that the degree of fluidity should also affect the antioxidative efficiency of carotenoids as well as other membrane localized antioxidants by modulating their diffusion rate within the membrane.

Acknowledgements

This study was financially supported by the Committee of Scientific Research of Poland under project No. 6 6075 92 03 as well as by Danish Natural Sciences Research Council. We would like to express our gratitude to Drs. M. Miller and R.P. Cox for their assistance and use of EPR instrumentation at Institute of Biochemistry, Odense University, Denmark.

References

[1] Davidson, E. and Cogdell, R.J. (1981) Biochim. Biophys. Acta 635, 295–303.
[2] Larkum, A.W.D. and Barrett, J. (1983) Adv. Bot. Res. 10, 1–219.
[3] Siefermann-Harms, D. (1985) Biochim. Biophys. Acta 811, 325–355.
[4] Gillbro, T., Cogdell, R.J. and Sundström, V. (1988) FEBS Lett. 235, 169–172.
[5] Koyama Y. (1991) J. Photochem. Photobiol. B. Biol. 9, 265–280.
[6] Demmig, B., Winter, K., Krüger, A. and Czygan, F.-C. (1987) Plant Physiol. 84, 218–224.
[7] Krinsky, N.I. (1989) Free Radical Biol. Med. 7, 617–635.
[8] Terao, J. (1989) Lipids 24, 659–661.
[9] Bilger, W. and Björkman, O. (1990) Photosynth. Res. 25, 173–185.
[10] Conn, P.F., Schalch, W. and Truscott, T.G. (1991) J. Photochem. Photobiol. B. 11, 41–47.
[11] Rojas-Hidalgo, E. and Olmedilla, B. (1993) Int. J. Vit. Nutr. Res. 63, 265–269.
[12] Bendich, A. and Olson, J.A. (1989) FASEB J. 3, 1927–1932.
[13] Sies, H., Stahl, W. and Sundquist A. (1992) Ann. N.Y. Acad. Sci. 669, 7–20.
[14] Krinsky, N.J. (1993) Annu. Rev. Nutr. 13, 561–587.
[15] Huang, L. and Haug, A. (1974) Biochim. Biophys. Acta 352, 361–370.
[16] Rohmer, M., Bouvier, P. and Ourisson, G. (1979) Proc. Natl. Acad. Sci. USA 76, 847–851.
[17] Rottem, S. and Markowitz, O. (1979) J. Bacteriol. 140, 944–948.
[18] Milon, A., Wolff, G., Ourisson, G. and Nakatani, Y. (1986) Helv. Chim. Acta 69, 12–24.
[19] Milon, A., Lazrak, T., Albrecht, A.-M., Wolff, G., Weill, G., Ourisson, G. and Nakatani, Y. (1986) Biochim. Biophys. Acta 859, 1–9.
[20] Chaturvedi, V.K. and Kurup, C.K.R. (1986) Biochim. Biophys. Acta 860, 286–292.
[21] Lazrak, T., Milon, A., Wolff, G., Albrecht, A.-M., Mieh, M., Ourisson, G. and Nakatani, Y. (1987) Biochim. Biophys. Acta 903, 132–141.
[22] Subczyński, W.K., Markowska, E. and Sielewiesiuk, J. (1991) Biochim. Biophys. Acta 1068, 68–72.
[23] Subczyński, W.K., Markowska, E., Gruszecki, W.I. and Sielewiesiuk, J. (1992) Biochim. Biophys. Acta 1105, 97–108.
[24] Subczyński, W.K., Markowska, E. and Sielewiesiuk, J. (1993) Biochim. Biophys. Acta 1150, 173–181.
[25] Gruszecki, W.I. and Sielewiesiuk J. (1990) Biochim. Biophys. Acta 1023, 405–412.
[26] Gruszecki, W.I. and Sielewiesiuk, J. (1991) Biochim. Biophys. Acta 1069, 21–26.
[27] Gruszcki, W.I. and Strzałka, K. (1991) Biochim. Biophys. Acta 1060, 310–314.
[28] Havaux, M. and Gruszecki, W.I. (1993) Photochem. Photobiol. 58, 607–614.
[29] Deisenhofer, J. and Michel, H. (1989) Science 254, 1463–1473.
[30] Strzałka, K. and Subczyński, W.K. (1981) Photobiochem. Photobiophys. 2, 227–232.
[31] Small, D.M. (1986) The Physical Chemistry of Lipids, Plenum Press, New York.
[32] Marsh, D., Watts, A. and Knowles, P.F. (1977) Biochim. Biophys. Acta 465, 500–514.
[33] Van de Ven, M., Kattenberg, M., Van Ginkel, G. and Levine, Y.K. (1984) Biophys. J. 45, 1203–1210.
[34] Jordan, B.R., Chow, W.-S. and Baker, A.J. (1983) Biochim. Biophys. Acta 725, 77–86.
[35] Yamamoto, Y., Ford, R.C. and Barber, J. (1981) Plant Physiol. 67, 1069–1072.
[36] Oquist, G. (1986) J. Plant Physiol. 122, 159–168.
[37] Huner, N.P.A., Elfman, B., Król, M. and McIntosh, A. (1984) Can. J. Bot. 62, 53–60.
[38] Ames, B.N., Shigenaga, M.K. and Hagen, T.M. (1993) Proc. Natl. Acad. Sci. USA 90, 7915–7922.