Comparative X-Ray Studies on the Interaction of Carotenoids with a Model Phosphatidylcholine Membrane

Mario Suwalsky[a,*], Paulina Hidalgo[a], Kazimierz Strzalka[b], and Anna Kostecka-Gugala[b]

[a] Faculty of Chemical Sciences, University of Concepción, Casilla 160-C, Concepcion, Chile. Fax: + 56-41 245974. E-mail: msuwalsk@udec.cl

[b] Institute of Molecular Biology, Jagiellonian University, Krakow, Poland

* Author for correspondence and reprint requests

Z. Naturforsch. **57c,** 129–134 (2002); received September 3/October 5, 2001

Carotenoids, Xanthophylls, Membrane

The interaction of structurally different carotenoids with a membrane molecular model was examined by X-ray diffraction. The selected compounds were β-carotene, lycopene, lutein, violaxanthin, zeaxanthin, and additionally carotane, a fully saturated derivative of β-carotene. They present similarities and differences in their rigidity, the presence of terminal ionone rings and hydroxy and epoxy groups bound to the rings. The membrane models were multibilayers of dipalmitoylphosphatidylcholine (DPPC), chosen for this investigation because the 3 nm thickness of the hydrophobic core of its bilayer coincides with the thickness of the hydrophobic core of thylakoid membranes and the length of the carotenoid molecules. Results indicate that the six compounds induced different types and degrees of structural perturbations to DPPC bilayers in aqueous media. They were interpreted in terms of the molecular characteristics of DPPC and the carotenoids. Lycopene and violaxanthin induced the highest structural damage to the acyl chain and polar headgroup regions of DPPC bilayers, respectively.

Introduction

According to the current view, in photosynthetic membranes physiologically active carotenoids are functionally attached to proteins. On the other hand, certain carotenoids are found in the lipidic moiety of membranes such as that of the retina, bacteria and also in photosynthetic membranes (Gruszecki, 1999). Among the most commonly occurring carotenoids three main groups differing in structure can be distinguished: cyclic, linear and xanthophylls containing oxygen in the form of hydroxy or epoxy groups. These structurally different groups have one feature in common: the conjugated double bond system that gives rise to rigid, rod-like structures. This property is lost upon hydrogenation; for instance, carotane, a fully saturated derivative of β-carotene is structurally similar to β-carotene but is a flexible molecule.

The aim of the present work was to obtain information about the significance of the different chemical elements present in these three carotenoid groups when they interact with biomembranes by comparing the structural perturbations induced by different carotenoids to multibilayers of dipalmitoylphosphatidylcholine (DPPC). The carotenoids were i) β-carotene; ii) its fully saturated form carotane; iii) lycopene, which lack the terminal rings; iv) lutein, with one hydroxy group in each of the β- and ε-rings; v) violaxanthin, containing one hydroxy and one epoxy group in each terminal β-ionone ring, and vi) zeaxanthin with one hydroxy group in each β-ionone ring. Their structural formulae are presented in Figures 1 and 2. These studies were performed by X-ray diffraction using a range of concentrations of each carotenoid.

Materials and Methods

Synthetic dipalmitoylphosphatidylcholine (DPPC) (lot 45H8383, 99+% purity, MW 734) and β-carotene (lot 72H0060, MW 537) were from Sigma (St. Louis, MO, USA); zeaxanthin (lot 706007, MW 568) and carotane (MW 558) were a gift from Hoffmann La Roche (Basle, Switzerland); Violaxanthin (MW 600) and lutein (MW 568) were isolated from daffodil (*Narcissus poeticus*) petals by extraction with acetone, cold saponification followed by column chromatography on

alkalized silica gel F254 (Merck) in hexane:acetone 4:1 v/v. Lycopene was isolated from tomato paste by extraction with chloroform:methanol 1:1, v/v, then water was added in a stepwise manner until a good phase separation was obtained; the tomato pigments from the chloroform fraction were separated by chromatography on MgO in hexane:benzene 9:1 v/v. DPPC was used without further purification; the carotenoids, except carotane were purified using HPLC performed on reverse Apex Prepsil SIN phase column (Jones Chromatography, Mid Glamorgan, U. K.), using a Jasco PU 980 isocratic pump and PU 970 UV/Vis detector (Jasco Corp., Tokyo, Japan) set on 440 nm. HPLC solvents were from Lab-Scan (Analytical Sciences, Dublin, Ireland); others were of the highest purity available. All carotenoids, except carotane, were purified using the following eluents: methanol:ethyl acetate 17:8 v/v for β-carotene, acetonitrile:hexane:methanol 5:2:2 v/v/v for lycopene, acetonitrile:methanol: deionized water 72:8:1 v/v/v for zeaxanthin and lutein, and acetonitrile:methanol:deionized water 72:8:8 v/v/v for violaxanthin, To check the purity of the carotenoids aliquots were separated in a Nucleosil 100 C18 analytical column (Teknokroma, Barcelona, Spain) with the detector set on 250 nm, and absorption spectra in the range of 300–550 nm were recorded using a SLM DW 2000 Aminco spectrophotometer (Urbana, IL, USA). Carotane purity, checked by thin layer chromatography on silica gel (Merck, Darmstadt, Germany) in acetone:benzene:water 81:30:4 v/v/v), revealed no impurities. The final carotenoid purity was estimated to be between 90 and 98%. The carotenoids were dark stored at −35 °C in a nitrogen atmosphere.

For the X-ray experiments about 3 mg of DPPC were mixed with the corresponding amount of each carotenoid (except carotane), dissolved in $CHCl_3$ and left to evaporate the solvent for about 24 h in the dark in a nitrogen atmosphere; each dry sample was placed in a 2 mm diameter special glass capillary and 200 μl of bidistilled water were added, into which nitrogen had previously been streamed. In the case of carotane, which is a viscous liquid at room temperature, the adequate amounts were first dissolved in $CHCl_3$ and then added to DPPC. The samples thus prepared were X-ray diffracted in flat-plate cameras with 0.25-mm-diameter glass collimators provided with rotating devices. The blanks, constituted by pure samples of DPPC, were recrystallized as described above and X-ray diffracted in Debye-Scherrer (Philips, The Netherlands) and flat-plate cameras (built by us). Specimen-to film distances were 8 and 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered CuKα radiation from a Philips PW 1140 X-ray generator was used. The relative reflection intensities on film were measured by peak integration using a Bio-Rad GS-700 densitometer (Richmond, CA, USA) and Molecular Analyst/PC image software; no correction factors were applied. The experiments were performed in the dark at 17 ± 2 °C.

Results

The molecular interactions of DPPC multibilayers with β-carotene, carotane, lycopene, lutein, violaxanthin and zeaxanthin were determined by X-ray diffraction in an aqueous media. Fig. 1 shows a comparison of the diffraction pattern of DPPC and of its mixtures with β-carotene (expressed as moles of carotenoid per 100 moles of DPPC), its fully saturated derivative carotane and the linear carotenoid lycopene. As expected, water altered the structure of DPPC: its bilayer width increased from about 5.8 nm in its dry form to 6.46 nm when immersed in water, and its reflections were reduced to only the first three, which are related to the bilayer width. On the other hand, a new and strong reflection of 0.42 nm showed up, whose appearance was indicative of the fluid state reached by DPPC bilayers and corresponded to the average distance between DPPC fully extended acyl chains organized with rotational disorder in hexagonal packing. It can be observed that 3% β-carotene induced a drastic reduction of DPPC low angle reflection intensities, which were further reduced with increasing carotenoid concentrations. This result implies a structural perturbation of the polarhead region of the lipid. On the other hand, no significant changes were noticed in the 0.42 nm reflection, which means that β-carotene did not affect DPPC hydrocarbon chain arrangement. Carotane (5% and 10%) induced a marked decrease of the low angle reflection intensities, while the intensity of the 0.42 nm reflection remained practically unchanged. However, a 7.5% concentration pro-

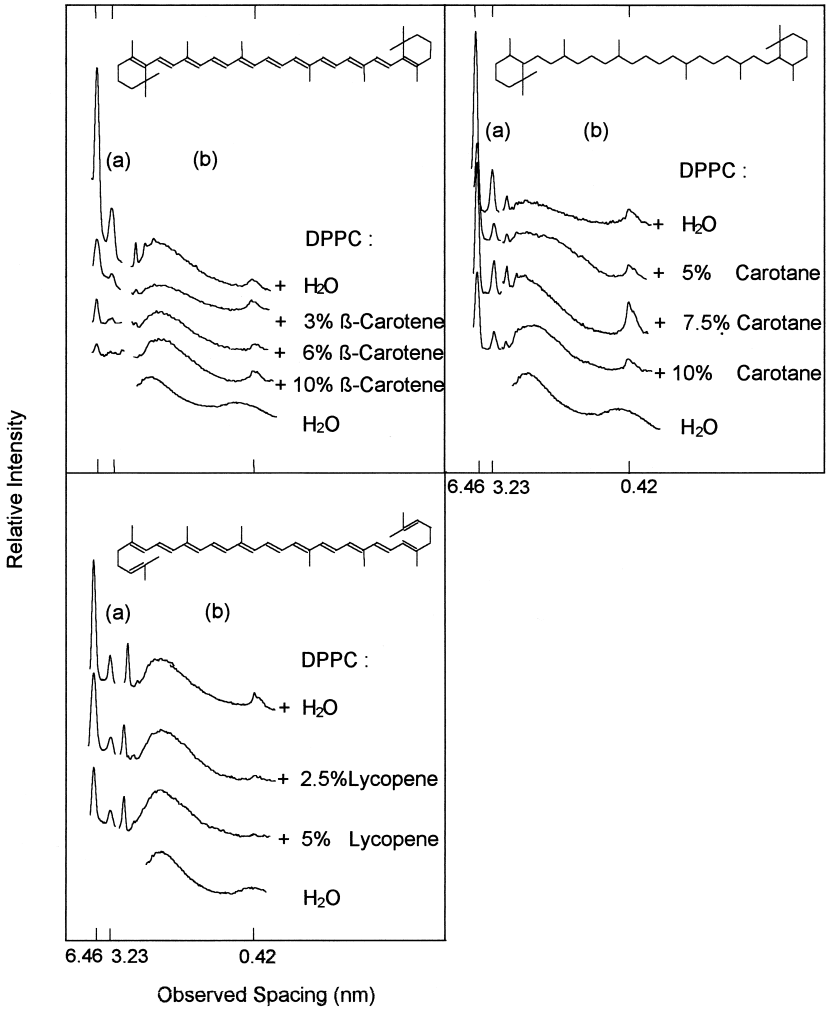

Fig. 1. Microdensitograms from X-ray diagrams of dipalmitoylphosphatidylcholine (DPPC) with water and the carotenoids β-carotene, carotane and lycopene; (a) low-angle and (b) high-angle reflections. Insets: structures of the corresponding carotenoids.

duced an increase of all the reflection intensities, particularly that of the 0.42 nm reflection, which can be interpreted as an ordering effect of DPPC acyl chains. The results of the interaction of lycopene with DPPC are also exhibited in Fig. 1. It may be noticed that this carotenoid (2.5%) had a moderate effect on the low angle reflections but ensured complete disappearance of the 0.42 nm reflection, a result that implies the total disorder of DPPC hydrophobic phase. Increasing lycopene concentration to 5% resulted in a further fainting of the low angle reflections arising from the lipid polar headgroups. In fact, of the six assayed compounds, lycopene induced the highest disorder in the acyl chain region. The results obtained from the interaction of the three xanthophylls (lutein, zeaxanthin and violaxanthin) are presented in Fig. 2. It may be observed that exposure to lutein gives results quite different from those exhibited by the action of lycopene. 2.5% lutein induced a significant decrease of the low angle reflection intensities and a moderate increase of the intensity of the 0.42 nm reflection. A lutein increase to 5% produced a slight decrease of DPPC reflection intensities. Fig. 2 also shows the effects of 5% violaxanthin (the only concentration to be obtained due

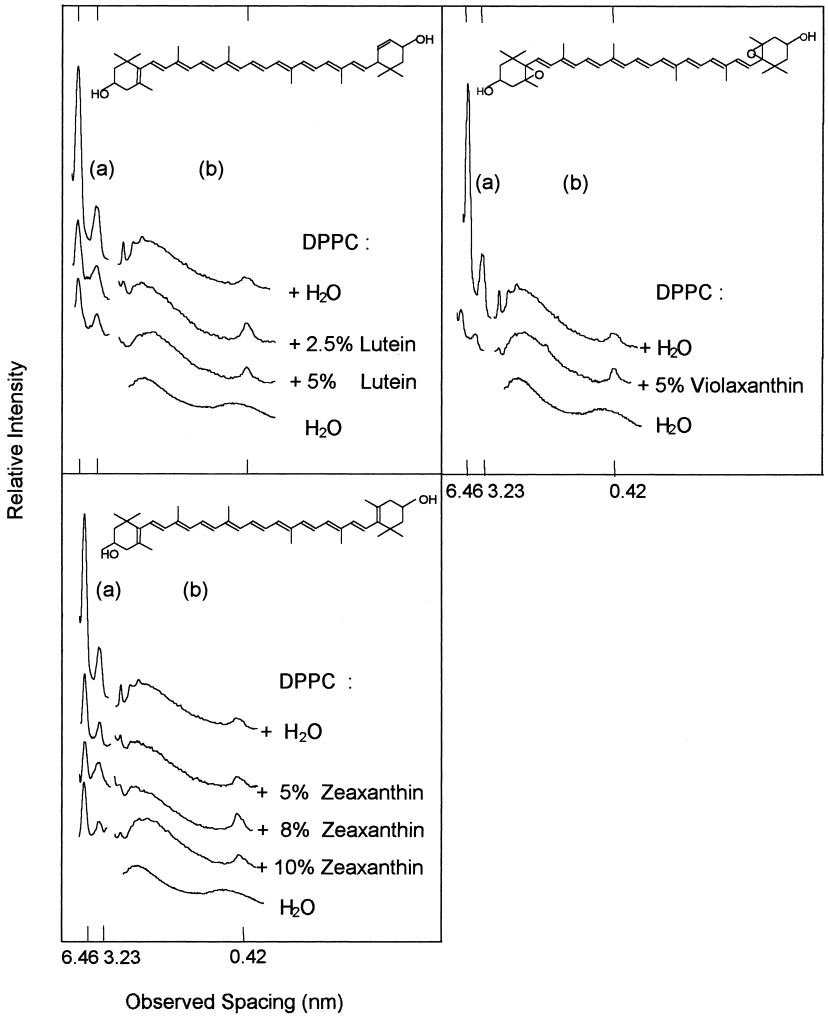

Fig. 2: Microdensitograms from X-ray diagrams of dipalmitoylphosphatidylcholine (DPPC) with water and the xanthophylls lutein, violaxanthin and zeaxanthin; (a) low-angle and (b) high-angle reflections. Insets: structures of the corresponding xanthophylls.

to the scanty amount available) upon DPPC. Of all the assayed compounds, this xanthophyll induced the highest degree of disorder in the polar head region, and was the only one to change DPPC bilayer width, which increased by about 0.2 nm. It was also observed that 5% zeaxanthin had a perturbing effect in the low angle reflection intensities of DPPC, and an almost negligible effect on the reflection coming from the acyl chain region. Increasing its concentrations to 8% and 10% did not show significant changes with respect to those observed with 5% zeaxanthin.

Discussion

In spite of a large volume of accumulated data, the interaction between carotenoids and biological membranes, specially at the molecular level, remains poorly understood. This article describes the interaction of chemically different carotenoids with a membrane molecular model. The latter consisted in multibilayers of dipalmitoylphosphatidylcholine (DPPC), chosen for this investigation because the 3 nm thickness of the hydrophobic core of its bilayer coincides with the thickness of the hydrophobic core of thylakoid membranes and the length of the carotenoid molecules (Strzalka

and Gruszecki, 1994). The assayed carotenoids, which had small chemical differences, induced different types and degrees of structural perturbations to DPPC bilayers. Under the experimental conditions applied three different zones can be distinguished in DPPC: the hydrophobic core formed by the hexagonally packed and fluid acyl chains, the highly polar headgroup region and the layer of water located between headgroups of neighboring bilayers (Suwalsky, 1996). Lycopene induced the highest structural damage to the acyl chain region. In fact, at a 2.5% concentration this carotenoid caused the complete disappearance of the 0.42 nm reflection. This result can be attributed to total perturbation of the hexagonal packing of the hydrocarbon chains. This is not surprising because lycopene, due to its lack of polar groups, is a highly hydrophobic molecule; this means that this rigid, rod-like and linear molecule can be completely immersed into the acyl chains leading to perturbation of DPPC hydrophobic region. The other five cyclic compounds did not significantly damaged this region. It is noteworthy that lycopene produced the least disorder at the polar headgroup region, a result that is consistent with the previously mentioned effect as this carotenoid did not interact with DPPC polar headgroups. On the other hand, violaxanthin induced the highest perturbation to the polar region of DPPC. This was the only molecule with hydroxy and epoxy groups; thus, the possibility existed of strong polar interactions and hydrogen bond formation between its rings and DPPC headgroups breaking the stabilizing interpolar groups interactions with the resulting perturbation of this region of the lipid. In general, the xanthophylls induced higher perturbation than did the other carotenoids in this region. The only unexpected result was the high degree of perturbation induced by β-carotene to the polar region of DPPC, which was a little lower than that produced by violaxanthin. A possible explanation might be that this cyclic carotenoid is less flexible than the saturated carotane and shorter than the linear lycopene, which can accommodate better in DPPC bilayers than β-carotene.

These results partially agree with comparative studies published in the literature. Thus, it was found by ^{31}P-NMR and ^{13}C-NMR that β-carotene increased the motional freedom within the lipid core and the headgroup region of DPPC multibilayer liposomes (Jezowska *et al*., 1994), and by spin label EPR that the fluidizing effect of β-carotene was more pronounced than that of the polar carotenoid lutein (Strzalka and Gruszecki, 1994). A comparison by X-ray diffraction and linear dichroism between violaxanthin and zeaxanthin upon DMPC oriented multibilayers concluded that both xanthophylls, located in the hydrophobic core of the lipid bilayer, were inclined by about 22° and 25°, respectively, with respect to the normal to the plane of the bilayer (Gruszecki and Sielewiesiuk, 1990). This is quite feasible as the 2.54 nm thickness of the hydrophobic region of DMPC (Gruszecki *et al*., 1992) is too short to fit the 3 nm length of both carotenoids, which is not the case with DPPC. However, applying different techniques it has been postulated that lutein and zeaxanthin have different orientations in DPPC and egg yolk lecithin: zeaxanthin would span the lipid bilayer with the hydroxy groups anchored in the opposite polar zones of the membrane, whereas roughly half the lutein molecules would orient the same way while the other half would locate parallel with respect to the plane of the membrane (Gruszecki, 1999; Gruszecki *et al*., 1999; Sujak *et al*., 1999; Sujak and Gruszecki, 2000; Sujak *et a*l., 2000). Our own results showed small but significant differences in the X-ray patterns of lutein and zeaxanthin with DPPC at the same molar concentrations (5%). The observation that lutein induced a somewhat higher perturbation at the polar headgroup region than zeaxanthin might be related to its double orientation in DPPC bilayers as postulated by Gruszecki *et al*. The presence of specific carotenoids in membranes, their location and different orientations they exhibit might be related to their physiological roles such as protection against oxidative damage (Sujak *et al*., 1999) and efficiency in the light absorption (Gruszecki *et al*., 1999).

Acknowledgements

This work was supported by grant 1990289 from FONDECYT (to M. S.) and by grant 6 P04A 028 19 from the Committee for Scientific Research of Poland (to K. S. and A. K.-G). Zeaxanthin and carotane were obtained from Hoffmann La Roche, Basel, as a free research gift.

Gruszecki W. I. (1999), Carotenoids in membranes, In: The Photochemistry of Carotenoids (Frank, H. A., Young, A. J., Britton, G. and Cogdell, R., eds), Kluwer, Dordrecht, Netherlands, pp. 363–379.

Gruszecki W. I. and Sielewiesiuk J. (1990), Orientation of xanthophylls in phosphatidylcholine multibilayers. Biochim. Biophys. Acta **1023**, 405–412.

Gruszecki W. I., Smal A. and Szymczuk D. (1992), The effect of zeaxanthin on the thickness of dimyristoylphosphatidylcholine bilayer: X-ray diffraction study. J. Biol. Phys. **18**, 271–280.

Gruszecki W. I. Sujak A., Strzalka K., Radunz A. and Schmid G. H. (1999), Organisation of xanthophyll-lipid membranes studied by means of specific pigment antisera, spectrophotometry and monomolecular layer technique. Lutein versus zeaxanthin. Z. Naturforsch. **54c**, 517–525.

Jezowska I., Wolak A., Gruszecki W. and Strzalka K. (1994), Effect of β-carotene on structural and dynamic properties of model phosphatidylcholine membranes. II. A ^{31}P-NMR and ^{13}C-NMR study. Biochim. Biophys. Acta **1194**, 143–148.

Strzalka K. and Gruszecki W. I. (1994), Effect of β-carotene on structural and dynamic properties of model phosphatidylcholine membranes. I. An EPR spin label study. Biochim. Biophys. Acta **1194**, 138–142.

Sujak A. and Gruszecki W. I. (2000), Organization of mixed monomolecular layers formed with the xanthophyll pigments lutein or zeaxanthin and dipalmitoylphosphatidylcholine at the argon-water interface. J. Photochem. Photobiol. B: Biol. **59**, 42–47.

Sujak A., Okulski W. and Gruszecki W. I. (2000). Organisation of xanthophyll pigments lutein and zeaxanthin in lipid membranes formed with dipalmitoylphosphatidylcholine. Biochim. Biophys. Acta **1509**, 255–263.

Sujak A., Gabrielska J., Grudzinski W., Borc R., Mazurek P. and Gruszecki I. (1999). Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: The structural aspects. Arch. Biochem. Biophys. **371**, 301–307.

Suwalsky M. (1996), Phospholipid bilayers, In: Polymeric Materials Encyclopedia (Salamone, J. C., ed.), **7,** CRC Press, Boca Raton, USA, pp. 5073–5078.