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Can membrane-bound carotenoid pigment zeaxanthin carry out a transmembrane proton transfer?☆

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ABSTRACT

Polar carotenoid pigment zeaxanthin (β,β-carotene-3,3′-diol) incorporated into planar lipid membranes formed with diphytanoyl phosphatidylcholine increases the specific electric resistance of the membrane from ca. 4 to 13×10^7 Ω cm^2 (at 5 mol% zeaxanthin with respect to lipid). Such an observation is consistent with the well known effect of polar carotenoids in decreasing fluidity and structural stabilization of lipid bilayers. Zeaxanthin incorporated into the lipid membrane at 1 mol% has very small effect on the overall membrane resistance but facilitates equilibration of the transmembrane proton gradient, as demonstrated with the application of the H^+-sensitive antimony electrodes. Relatively low changes in the electrical potential suggest that the equilibration process may be associated with a symport/antiport activity or with a transmembrane transfer of the molecules of acid. UV–Vis linear dichroism analysis of multibilayer formed with the same lipid–carotenoid system shows that the transition dipole moment of the pigment molecules forms a mean angle of 21° with respect to the axis normal to the plane of the membrane. This means that zeaxanthin spans the membrane and tends to have its two hydroxyl groups anchored in the opposite polar zones of the membrane. Detailed FTIR analysis of β-carotene and zeaxanthin indicates that the polyene chain of carotenoids is able to form weak hydrogen bonds with water molecules. Possible molecular mechanisms responsible for proton transport by polyenes are discussed, including direct involvement of the polyene chain in proton transfer and indirect effect of the pigment on physical properties of the membrane.

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1. Introduction

Carotenoids are ubiquitous pigments present both in the plant and animal kingdoms, playing important physiological roles [1]. Among diverse biological functions of carotenoids protection against oxidative damage [2–6] and light harvesting in the photosynthetic apparatus [7–9] are the most frequently reported. The photoprotection of carotenoids is realized via quenching of the triplet states of photosensitizers, quenching of singlet oxygen and scavenging free radicals. These mechanisms are essential for maintaining integrity of both the functional membrane proteins and the lipid phase. Protection of lipid membrane by carotenoid pigments is also realized via decreasing fluidity of the membrane, which increases the barrier for penetration of the singlet oxygen [10]. The polyene chain of carotenoid pigments incorporated into lipid membranes is localized in the hydrophobic core of the bilayer [11–14]. Polar carotenoids tend to adopt an orientation within the membrane, which enables the polar groups, located at the ends of the long, bar-shaped hydrophobic molecules, to be anchored in the opposite polar zones of the membrane, formed with the lipid headgroups [11–14]. The polar carotenoid pigments (xanthophylls) are reported to influence the physical properties of the membranes [11–14]. One aspect of such a modification is the increase of the barrier for transmembrane transport of ions and small molecules. For example, the 2 mol% zeaxanthin (the polar derivative of β-carotene, see Fig. 1) incorporated into the membranes of liposomes formed with digalactosyldiacylglycerol, reduced the permeability for protons [15]. In the present work we analyze the effect of zeaxanthin on transmembrane proton transfer at a low concentration (1 mol%) at which its effect on physical properties of the lipid membrane is very small.

2. Materials and methods

2.1. Chemicals

Diphytanoyl phosphatidylcholine (DPhPC), used to form model lipid membranes, was purchased from Avanti Polar Lipids, Inc (Alabama, USA).

☆ Dedicated to Professor Stanisław Przestalski on the occasion of his eightieth birthday.

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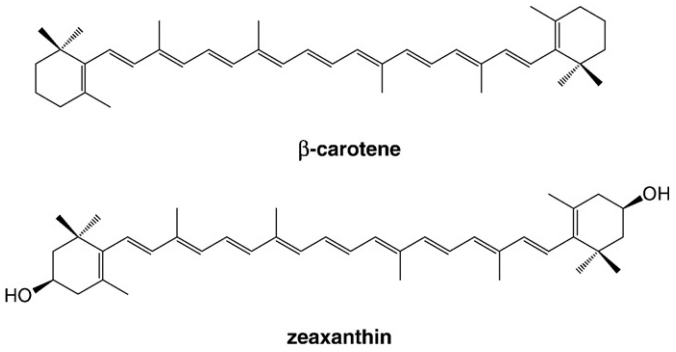

Fig. 1. Chemical structure of β-carotene and zeaxanthin.

Zeaxanthin ((3*R*,3′*R*) β,β-carotene-3,3′-diol) was isolated from the fruits of *Lycium barbarum* and purified chromatographically by HPLC technique (the YMC GmBH C-30 column: 250 mm×4.6 mm, flow velocity: 1 ml/min, mobile phase: acetonitrile:CH_3OH:H_20 (72:8:3, by vol.)) as described in detail previously [16].

Synthetic β-carotene (β,β-carotene) was purchased from Sigma-Aldrich Chem. Co. The pigment was re-crystallized twice from the hexan:CH_3OH solvent mixture (4:1, v:v) before use. The pigment purity was tested chromatographically with the same HPLC column as in the case of zeaxanthin purification and with the mobile phase the same as the solvent mixture applied for re-crystallization.

2.2. Electrical measurements

Planar bilayer lipid membranes, BLM [17] were formed from DPhPC or the lipid containing 1, 3 or 5 mol% zeaxanthin dissolved in *n*-decane:2-propanol mixture 3:1, v:v (lipid concentration 10 mg/ml). Composition of deposition solvents used to form lipid membranes with and without zeaxanthin was kept identical, in order to avoid possible effect of a solvent on the membrane resistance and ion transport properties. A small amount of forming solution was applied on a hole (1 mm in diameter) in a Teflon cup located in a Plexiglas chamber with two glass windows. The membrane separated two compartments, each of them containing 10 ml of water containing 0.1 M KCl. During the experiment, solutions were stirred in both compartments with magnetic microstirrers. The process of membrane thinning to a bilayer stage was monitored by visual inspection through a microscope and by measuring electrical resistance in a set-up described below. Measurements started when the membrane became optically black. The resistance of the membranes in their steady-state was about 20 GΩ. The set-up for the BLM formation was placed in a Faraday cage on a vibration-isolating table. Some other details of measurements were described previously [18].

The first part of the experiments involved the measurements of voltage across lipid membrane at various concentrations of zeaxanthin, evoked by the passing current. Current–voltage characteristics were obtained by using a four-electrode method. The electrodes of Ag/AgCl wires were placed in pairs in two compartments separated by the membrane. One pair of them, connected to the amplifier (*Elektrometer Duo 773*) represented the voltage registration circuit. The other (cathode and anode) connected to a regulated DC source represented the current circuit. Selected range of current intensities (50–700 pA) was applied in order to obtain a relation between the voltage and the membrane resistance.

The next part of experiments was focused on the examination of H^+-ion conductivity across the lipid membrane, pure and modified with zeaxanthin. The measuring arrangement consisted of antimony filled H^+-selective microelectrode, prepared according to the method described previously [19], and Ag/AgCl reference electrode placed in a separate compartment and connected to the amplifier. 0.5 ml of 10 mM oxalic acid added on one side of the membrane, to the compartment with the reference electrode was used as a source of H^+ ions. The transmembrane proton transfer was registered as a change of the pH in the other compartment with the pH-selective electrode. To analyze changes of the voltage the BIOWYK program was used. All the experimental values were displayed as mean±S.E.

2.3. UV–Vis linear dichroism measurements

The linear dichroism measurements of orientation of zeaxanthin molecules in the lipid membranes were performed on the oriented lipid multilayers deposited to glass support [20,21], composed of 100 DPhPC bilayers containing 1 mol% of the pigment. Polarized light absorption spectra were recorded with UV–Vis Shimadzu 160A-PC spectrophotometer equipped with the Shimadzu polarizing attachment. The dichroic ratio was determined at 460 nm and the mean orientation of the dipole transition moment of zeaxanthin with respect to the axis normal to the plane of the membrane (v) was calculated on the basis of the absorption spectra recorded with the tilt angle of 45°, between the axis normal to the plane of the membrane and the direction of the measuring light beam (the refraction on the lipid phase border has not been considered):

$$A_{\parallel}/A_{\perp} = \mathrm{ctg}^2 v + 0.5. \quad (1)$$

2.4. FTIR measurements

Infrared absorption spectra of carotenoid pigments were recorded with the Fourier-transform infrared absorption spectrometer equipped with the attenuated total reflection set-up (ATR-FTIR). Samples were deposited on the ATR crystal element by evaporation from CCl_4. The solvent was distilled before use and kept under metallic Na in order to remove possible traces of water. The spectra were recorded with a Vector 33 spectrometer (Bruker, Germany). The internal reflection element was a ZnSe crystal (45° cut) yielding 10 internal reflections. Typically, 10 scans were collected, Fourier transformed and averaged for each measurement. Absorption spectra at a resolution of one data point every 2 cm^{-1} were obtained in the region between 4000 and 400 cm^{-1} using a clean crystal as the background. The instrument was purged with argon for 40 min before and continuously during measurements. The ATR crystals were

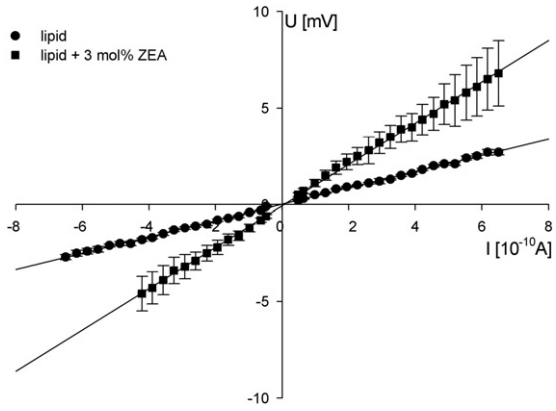

Fig. 2. Current–voltage characteristics of planar lipid bilayer membrane formed with DPhPC and DPhPC containing 3 mol% of zeaxanthin. Each experimental point represents mean from 6 experiments±S.E. The experimental points are presented along with the linear regression fits.

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