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Spectroscopic study on formation of aggregated structures by carotenoids: Role of water

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HIGHLIGHTS

- We examine formation of aggregated structures of β-carotene and zeaxanthin.
- Spectroscopic analysis was based on UV–Vis absorption, FTIR and Raman scattering.
- Carotenoid aggregated structures formed by evaporation from hydrated chloroform bind water.
- Carotenoid aggregates bind water via hydrogen bonds to polar groups but also to polyene chains.

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ABSTRACT

Formation of molecular aggregate structures of β-carotene and zeaxanthin, by means of evaporation from pigment solutions in organic solvent was studied with UV–Vis absorption, FTIR and resonance Raman techniques. Pigment solutions were prepared in $CHCl_3$ and CCl_4 both dehydrated and hydrated with trace amounts of water. Formation of the aggregated structures characterized by either strong-coupling (e.g. zeaxanthin in hydrated $CHCl_3$) or weak-coupling (e.g. β-carotene in hydrated $CHCl_3$) has been observed. FTIR analysis and molecular modeling showed that H_2O molecules can be bound to the aggregated structures formed by zeaxanthin in the form of molecular bridges, predominantly between the terminal hydroxyl groups of adjacent molecules (stabilized by strong hydrogen bonds), but also between the polyene chains (by means of the π-type weak hydrogen bonds). Resonance Raman analysis revealed that the structures formed with the presence of H_2O molecules are characterized by twisting of the polyene backbone. The effect of twisting is observed particularly in the case of zeaxanthin structures deposited from hydrated $CHCl_3$ and is not observed in the case of the structures of β-carotene deposited from dehydrated CCl_4. Involvement of water molecules in stabilizing aggregated structures of carotenoids is postulated.

1. Introduction

Carotenoids are ubiquitous pigments present both in the plant and animal kingdom, playing important physiological roles [1]. Among the 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 membranes by carotenoid pigments is also realized via decreasing fluidity of the membrane, which increases the barrier for penetration of the singlet oxygen [10]. Interaction of carotenoids with lipids and their effect on the structural and dynamic properties of lipid membranes depends on molecular organization of carotenoid molecules, in particular formation of aggregated structures [11–13]. According to a general understanding, the van der Waals interactions between the polyene chains and hydrogen bond formation between the polar xanthophyll groups are principally responsible for stabilization of molecular aggregates of carotenoids [14–17]. Interestingly, the pronounced effect of the pH of the medium and percentage of water on a level and pattern of zeaxanthin aggregation in the hydrated organic solvent solutions has been observed [18]. This interesting effect stimulated us to address question regarding a role of proton binding and water molecules in mediation of the formation of aggregated structures by carotenoids. In the present work we have studied formation of molecular aggregates by two carotenoid pigments: nonpolar β-carotene and its polar derivative zeaxanthin

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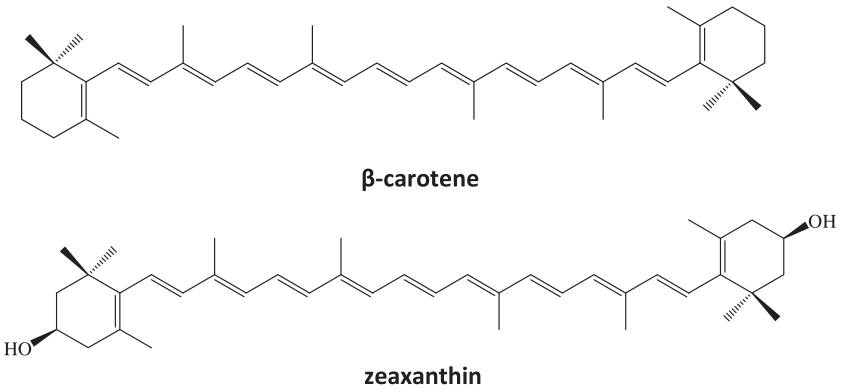

Fig. 1. Chemical structure of β-carotene and zeaxanthin.

(Fig. 1). Zeaxanthin is a xanthophyll present both in the photosynthetic membranes of plants [19] and in the *macula lutea* of primates [20]. β-Carotene is probably the most abundant carotenoid in nature, plays multiple and diverse physiological functions [21] and the main objective and rationale to study molecular aggregates formed with β-carotene was to compare structures formed by carotenoids with and without polar oxygen groups. The present study is primarily based upon the vibrational spectroscopy techniques.

2. Materials and methods

2.1. Chemicals

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_2O (72:8:3, by vol.)) as described in detail previously [22].

Synthetic β-carotene (β,β-carotene) was purchased from Sigma–Aldrich Chem. Co. (USA). The pigment was re-crystallized twice from the hexane:CH_3OH solvent mixture (4:1, v:v). Directly before use the pigment was purified chromatographically, as in the case of zeaxanthin, except that the solvent mixture as used for re-crystallization was applied as a moving phase.

Synthetic dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma–Aldrich Chem. Co. (USA).

2.2. Sample preparation

Carotenoid samples were deposited either on the ZnSe-ATR crystal element (in the case of FTIR measurements) or on the surface of a quartz slide (in the case of UV–Vis absorption and Raman scattering measurements) by evaporation from organic solvents under the stream of gaseous Argon. Typically, a volume of 50 μl of a carotenoid solution was deposited at the crystal surface and subjected to evaporation under Argon, in darkness for 5 min. Such evaporation time has been found by ATR-FTIR monitoring, to be long enough to remove organic solvents from the sample. Generally, two types of carotenoid solution (initial concentration in the range 2.5–3.5 × 10^{-5} M) were prepared, strictly dehydrated and hydrated with trace amounts of H_2O. Dehydrated solutions were prepared in CCl_4 and $CHCl_3$, distilled before use and kept under molecular sieves (5 Å) in order to remove possible traces of water. Hydrated samples were prepared in the same dehydrated solvents mixed with water (1:1, v:v) and separated from the water phase on the extraction funnel. Water content in the hydrated $CHCl_3$ and CCl_4 was monitored by IR absorption analysis in the region corresponding to the O—H stretching vibrations (3000–3700 cm^{-1}, see Fig. S1 of the Supporting information) and evaluated on the basis of the IR extinction coefficients from the literature (ε^{3500} = 76 M^{-1} cm^{-1} [23]). The water concentration in the hydrated $CHCl_3$ used to prepare carotenoid solutions was 3.0 × 10^{-4} M and in the hydrated CCl_4 sample 1.1 × 10^{-4} M. Supporting information contains optical microscopy images of all the kind of samples formed (Figs. S2 and S3).

Zeaxanthin-containing lipid multibilayers were prepared with dipalmitoylphosphatidylcholine (DPPC) according to the procedure described in detail and tested previously [24–26].

2.3. UV–Vis absorption measurements

UV–Vis absorption spectra were recorded with Cary 50 spectrometer from Varian (Australia). Liquid samples were placed in the quartz cells (1-cm optical pathlength). Solid samples to be measured were deposited by evaporation to quartz glass slides. All measurements were taken at 21 ± 1 °C.

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). The IR absorption spectra were recorded with a Vector 33 spectrometer (Bruker, Germany) as previously described in detail [27]. 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 600 cm^{-1} using a clean crystal as the background. The instrument was purged with argon for 40 min, before and continuously during measurements, in order to remove water vapors and to protect samples against oxidative damage. The ATR crystals were cleaned with organic solvents (ethanol, chloroform and hexane). All experiments were done at 21 ± 1 °C. Spectral analysis was performed with OPUS (Bruker, Germany) and Grams AI software from Thermo Galactic (USA).

2.5. Resonance Raman measurements

The Raman scattering spectra were recorded with the in Via Reflex Raman Microscope from Renishaw (UK) equipped with two holographic ultra-high precision diffraction grating stages and high sensitivity ultra-low noise CCD detector [28]. A 514.5 nm Ar^+ laser has been applied to record Raman scattering. Laser power has been adjusted to 0.08 mW. The laser beam diameter was focused to