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Structure related aggregation behavior of carotenoids and carotenoid esters

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ABSTRACT

Carotenoids are lipophilic natural pigments inherently aggregating in hydrophilic environments. Such molecular self-assembly is crucial for the proper functioning of biological systems. Although several carotenoid aggregates have been investigated in recent years, our study provided further insights into the influence of specific structural modifications. For this purpose, the aggregation of 12 structurally-related carotenoids was studied in hydrated polar solvents, thin films, and precipitates from saturated solutions by UV/vis and circular dichroism spectroscopy, light microscopy, point-dipole approximation and computational modeling. Regarding the acyclic ψ,ψ-carotene (lycopene), the monocyclic β,ψ-carotene (γ-carotene), and the bicyclic β,β-carotene (β-carotene), the replacement of acyclic ψ-end groups by β-rings was demonstrated to consecutively modulate spectroscopic properties and the intermolecular distance within the aggregate. The presence of at least one open chain ψ-end, as found in γ-carotene and lycopene, fostered the formation of strongly coupled H-aggregates, whereas β,β-carotene prevailed as J-aggregate. While the insertion of one hydroxyl function to the β,β-carotenoid molecule (β-cryptoxanthin) similarly yielded J-aggregates, the presence of two hydroxyl functions (zeaxanthin) led to tightly-packed H-aggregates due to the formation of intermolecular hydrogen bonds. When blocking at least one of these hydrogen bonding sites, as studied with short-, middle-, and long-chain acyl zeaxanthins, H-type switched to J-type aggregation, irrespective of the chain length of the acyl moiety. Moreover, (*Z*)-isomerization was shown to prevent an ordered aggregation of carotenoid molecules. In brief, the optical properties and the aggregate structure of several most frequently occurring carotenoids are highly influenced by typical biosynthetic reactions, such as cyclization, isomerization, hydroxylation and esterification.

1. Introduction

Carotenoids are natural, lipophilic pigments. They can be classified into the highly apolar 'carotenes', representing pure hydrocarbons, and the often more polar 'xanthophylls', representing oxygenated hydrocarbons [1]. A characteristic structural feature of all carotenoids is their conjugated polyene system with highly delocalized π-electrons, which is a prerequisite for their light absorption and photochemical properties [1,2]. As a function of their lipophilicity, carotenoids tend to aggregate in hydrophilic environments. The formation of carotenoid aggregates is a process of molecular self-assembly being largely driven by hydrophobic effects. In addition, weak and reversible intermolecular forces such as hydrogen bonds, dipole forces, and van der Waals interactions have an enormous effect on the type of aggregate being formed [3,4]. Two borderline cases of aggregation types have been characterized previously, i.e., strongly coupled aggregates (H-aggregates), and weakly coupled aggregates (J-aggregates). In an ideal H-aggregate, molecules are in tight association, and their conjugated chains are located more or less parallel to each other. H-aggregates are therefore called 'card-pack' aggregates. Carotenoids within ideal J-aggregates are believed to have their polyene chains set in a head-to-tail manner, forming a more loose association [4]. The aggregate type can be determined by optical analytical techniques such as UV/vis and circular dichroism (CD) spectroscopy, since the optical properties of carotenoids change due to the delocalization of excitation energy over chromophores of neighboring molecules. H-aggregation is characterized by a loss

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of vibrational structure of the electronic absorption spectra, and the resulting absorption band exerts a large hypsochromic shift as compared to the main band of the monomer. In contrast, J-aggregates are characterized by a red-shifted, bathochromic UV/vis absorption spectrum, often causing a visually clearly noticeable color deepening [3,4]. Besides changes in UV/vis absorption, aggregates of chiral carotenoids were shown to exhibit a strong supramolecular chirality as measured by CD spectroscopy in the wavelength range of 300–600 nm, where carotenoid monomers in solution do not show CD signals [4]. When investigating carotenoid aggregates, CD spectroscopy represents a powerful tool for the investigation of structural relationships between monomers in aggregates [3]. From the analytical data, the point-dipole approximation allows the estimation of the intermolecular distance within H-aggregates [5–7], and further models have been proposed for estimating distances between J-aggregated molecules [8]. Regarding such approximations, ideal H- and J-aggregates are assumed to represent one-dimensional arrangements. In fact, however, the formation of aggregates generally occurs in a three dimensional space including a large number of molecules. Although a simple classification of bulky carotenoid aggregates into pure H- or J-aggregates may be intricate and some authors currently prefer the denomination as strongly- or weakly coupled aggregates [8], carotenoid aggregates will be denoted as H- or J-type aggregates according to their predominant optical properties, following the recommendations of previous studies [3,4,9].

Aggregation of carotenoids not only occurs in artificial systems, but is a prerequisite for the proper functioning of important biological systems such as the photosynthetic apparatus [10]. Studying carotenoid aggregation is of utmost interest, as their aggregation form has an enormous influence on their photochemical properties, and possibly also on their stability and bioavailability. Previous studies about carotenoid aggregates included structural and environmental factors [4,5,11–16]. However, investigations on structure related aggregation behavior of carotenoids are only fragmentary. To provide a deeper understanding of the influence of specific structural features on the aggregation process, a variety of aggregates produced from 12 structurally related carotenoids, namely lycopene, γ-carotene, β-carotene, (9*Z*)-β-carotene, β-cryptoxanthin, zeaxanthin, zeaxanthin acetate, -decanoate, -palmitate, zeaxanthin diacetate, -didecanoate, and -dipalmitate was analyzed by UV/vis and CD spectroscopy as well as by light microscopy. Moreover, point-dipole approximation and computational modeling were performed to estimate intermolecular distances in aggregated carotenoid dimers. For the first time, the aggregation behavior of γ-carotene, (9*Z*)-β-carotene, β-cryptoxanthin and zeaxanthin mono- and di-esters is reported, while that of β-carotene, lycopene and zeaxanthin was included mostly for comparison.

2. Experimental

2.1. Materials

All reagents or solvents used were of analytical or HPLC grade. Solvents used for spectroscopic analyses (acetone, ethanol) were of spectroscopic grade.

γ-Carotene ((all-*E*)-β,ψ-carotene) and β-cryptoxanthin ((all-*E*, 3*R*)-β,β-caroten-3-ol) were obtained from CaroteNature (Lupsingen, Switzerland). β-carotene ((all-*E*)-β,β-carotene) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Lycopene ((all-*E*)-ψ,ψ–carotene) was prepared according to a protocol described by Kopec et al. [17]. Zeaxanthin ((all-*E*, 3*R*, 3′*R*)-β,β-carotene-3,3′-diol) was extracted from a cold water dispersible powder containing at least 5% (w/w) of zeaxanthin, kindly provided by DSM Nutritional Products (Basel, Switzerland). Zeaxanthin acetate, zeaxanthin diacetate, zeaxanthin decanoate, and zeaxanthin didecanoate were prepared from zeaxanthin by acetyl chloride and decanoyl chloride according to a previously described protocol [18].

Zeaxanthin palmitate and dipalmitate were isolated from a partially saponified acetone/hexane (1:1, v/v) extract of dried goji berries (*Lycium barbarum* L.), kindly provided by PÄX Food (Magdeburg, Germany). For saponification, the dry extract was dissolved in hexane, and treated with methanolic KOH (10%, w/v) for 30 min. The progress of saponification was monitored by thin layer chromatography on silica gel plates (TLC Silica gel 60 F254, Merck, Darmstadt, Germany) using hexane/ethyl acetate (70:30, v/v) as mobile phase. (9*Z*)-β-carotene ((9*Z*)-β,β-carotene) was extracted from a powdered extract of the microalga *Dunaliella bardawil* kindly provided by Dr. Aviv Shaish (Bert W. Strassburger Lipid Center, Sheba Medical Center, Tel-Hashomer, Israel).

Carotenoids were purified from synthetic and natural extracts by HPLC on a semipreparative C30 reversed phase column (YMC30, 250 × 10 mm i.d., particle size 5 μm, YMC Europe, Dinslaken, Germany). The HPLC system (Bischoff, Leonberg, Germany) was equipped with a LC-CaDI 22–14 system controller, two 2250HPLC compact pump modules, and a SPD-10AVvp UV/vis detector (Shimadzu, Kyoto, Japan). Components were separated using a binary mixture of (A) methanol/water (90:10, v/v) and (B) *t* BME with gradient programs adapted for each individual compound.

Purity of compounds was determined using HPLC-PDA-MS^n analysis. The HPLC system (1100 series, Agilent, Waldbronn, Germany) was equipped with a G1315B photodiode array detector (PDA) coupled online to an ion trap mass spectrometer (Esquire 3000+, Bruker, Bremen, Germany) fitted with an APCI source. All parameters were adjusted as previously described by Schweiggert et al. [19]. Carotenoids were separated on a C30 reversed phase column (YMC C30, 150 × 3 mm i.d., particle size 3 μm, YMC Europe, Dinslaken, Germany) protected by a guard column (YMC C30, 10 × 3 mm i.d., particle size 3 μm, YMC Europe). HPLC solvents consisted of (A) methanol/water (90:10, v/v) and (B) methanol/*t* BME/water (20:78:2, v/v/v), both containing 0.4 g/L eluent of ammonium acetate. The elution gradient was as follows: from 100% to 80% A in 3 min, from 80% to 60% A in 5 min, from 60% to 40% A in 7 min, from 40% to 0% A in 2 min, isocratic at 0% A for 1 min, from 0% to 100% A in 2 min and isocratic at 100% A for 3 min. Total run time was 23 min at a flow rate of 0.8 mL/min and a column temperature of 40 °C. HPLC-MS purity was 93% for zeaxanthin, 97% for zeaxanthin palmitate, 98% for lycopene, 99% for (9*Z*)-β-carotene, zeaxanthin acetate, and zeaxanthin decanoate, and 100% for zeaxanthin diacetate, zeaxanthin didecanoate, and zeaxanthin dipalmitate. Purities of β-carotene, γ-carotene, and β-cryptoxanthin were 97%, 95%, and 97% according to the suppliers' specifications. For chemical structures see Fig. 1.

2.2. Preparation of lyotropic and thin-film aggregates for UV/vis and CD spectroscopy

Liquid samples for spectroscopic analyses were prepared from ethanolic or acetonic solutions. For preparation of aggregates, the respective carotenoid was dissolved in ethanol (zeaxanthin, zeaxanthin acetate, zeaxanthin diacetate, zeaxanthin decanoate, zeaxanthin didecanoate, zeaxanthin palmitate) or acetone (lycopene, γ-carotene, β-carotene, (9*Z*)-β-carotene, β-cryptoxanthin, zeaxanthin dipalmitate) and diluted with double distilled water. The water content in the ethanol/water or acetone/water mixtures was varied from 0 to 80%. The carotenoid concentration in the final solvent/water mixtures was 6×10^{-6} M. All samples remained clear without visible opalescence or precipitation. Immediately