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Research paper

Spectroscopic properties of the triple bond carotenoid alloxanthin

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ABSTRACT

Alloxanthin, which has two triple bonds within its backbone, was studied by steady-state and femtosecond transient absorption spectroscopies. Alloxanthin demonstrates an S_2 energy comparable to its non-triple bond homolog, zeaxanthin, while the S_1 lifetime of 19 ps is markedly longer than that of zeaxanthin (9 ps). Along with corroborating quantum chemistry calculations, the results show that the long-lived S_1 state of alloxanthin, which typically corresponds to the dynamic of a shorter carotenoid backbone, implies the triple bond isolates the conjugation of the backbone, increasing the S_1 state energy and diminishing the S_1–S_2 energy gap.

1. Introduction

Carotenoids are known for their rich excited-state dynamics, the elucidation of which is key to understanding the various functions the molecules perform in many biological systems [1–3]. Basic spectroscopic properties of carotenoids can be explained using a three-state model consisting of the ground state (S_0), and two excited states denoted S_1 and S_2 [4]. Assuming carotenoids belong to the idealized C_{2h} symmetry group, the S_0–S_1 transition is symmetry-forbidden for one-photon processes, while the characteristic visible coloration of carotenoids is due the S_0–S_2 transition that gives rise to strong absorption in the blue–green spectral region. Therefore, the symmetry of the carotenoid conjugated backbone is the key property determining the spectroscopic properties.

Another key parameter determining the spectroscopic properties is the conjugation length, N, defined as a number of conjugated C=C bonds in the carotenoid structure. For carotenoids with conjugation extended to various functional groups the effective conjugation length, N_{eff}, is often used. It makes use of the fact that some spectroscopic parameters, such as S_1 lifetime, have a well-defined dependence on $1/N$ [5–7]. Therefore, knowing the value of e.g. S_1 lifetime of any carotenoid, and comparing the value to that of a linear carotenoid, allows one to obtain an effective value of N_{eff} [7]. For example, it has been demonstrated that the extension of conjugation to various types of terminal rings decreases N_{eff}, but it also provides opportunity for tuning the spectroscopic properties as the torsional angle of a terminal ring in respect to the main conjugation allows for fine tuning of N_{eff} [7]. Thus, in contrast to linear carotenoids whose properties can be only slightly altered by environment, carotenoids with conjugated terminal rings may have a wide range of spectroscopic properties, depending on their protein binding sites [8,9].

While there exist many carotenoids with conjugated terminal rings or keto groups, carotenoids having conjugated triple bonds are quite rare. Out of the nearly thousand carotenoids identified so far, only about 50 contain triple bonds [10] and only three triple bond carotenoids are commonly found in nature. Alloxanthin (Fig. 1) is a common carotenoid of cryptophytes [11], and diadinoxanthin and diatoxanthin (Supporting Information, Fig. S1) are found in diatoms and some marine algae [12]. Diadinoxanthin and diatoxanthin have one triple bond in their structure and due to their well-documented role in photoprotection in diatoms [12–14], their excited-state properties were studied in detail [15,16].

Alloxanthin is the only carotenoid with two triple bonds found in photosynthetic organisms. The role of carotenoid triple bonds in proper functioning of carotenoids in light-harvesting systems remains unknown. As demonstrated earlier for diadinoxanthin and diatoxanthin, their specific molecular structure with a triple bond may help in tuning the spectroscopic properties when bound to protein [17]. Alloxanthin, whose excited-state properties have not been reported so far, is the major antenna carotenoid in the cryptophyte *Rhodomonas salina* [18]. This organism, despite lack of any xanthophyll cycle, has developed a flexible and effective

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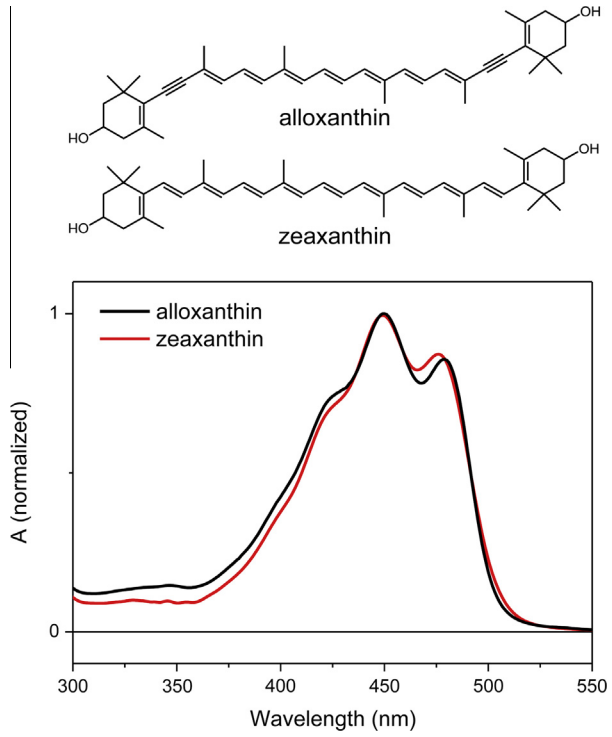

Fig. 1. Molecular structures (top) and absorption spectra (bottom) of alloxanthin and zeaxanthin in methanol.

photoprotective quenching [18], in which alloxanthin may play a role.

Here we present ultrafast transient absorption study of alloxanthin complemented by quantum chemical calculations. We compare these results with those of zeaxanthin (Fig. 1) which has comparable structure but lacks the triple bonds. We show that the presence of triple bonds in the carotenoid structure has important consequences for excited-state dynamics of alloxanthin.

2. Materials and methods

Alloxanthin was purified by an adapted method described previously for violaxanthin [19]. *Rhodomonas salina* cells (strain CCAP 978/27) were grown in an artificial seawater medium supplemented with *f*/2 nutrient. Irradiation was provided by fluorescence tubes (40 μmol m^{-2} s^{-1}, day-night cycle 12/12 h) and cells were continuously bubbled by air. Harvested cells were re-suspended in 25 mM Hepes buffer, pH 7.8 and broken by glass beads using Mini-Bead-Beater (BioSpec, USA). Thylakoid membrane fraction was separated by centrifugation, resuspended in 1 ml of distilled water, mixed with 9 ml of methanol and incubated for one hour in dark at room temperature. The extract was clarified by centrifugation and the remaining pellet was extracted again by 10 ml of 90% methanol. Both supernatants were pooled and completely evaporated by a vacuum evaporator. Pigments were dissolved in methanol and separated by HPLC (Agilent 1200) on a semi-preparative reverse phase column (Reprosil 250 × 10 mm C8 5 μm, Watrex, Czech Republic) with 35% methanol and 15% acetonitrile in 0.25 M pyridine (solvent A) and 20% methanol and 20% acetone in acetonitrile as solvent B. Pigments were eluted with a linear gradient of solvent B (30–95% in 25 min) followed by 95% of solvent B at a flow rate of 0.8 mL min^{-1} at 40 °C.

The sample was maintained at room temperature during transient absorption measurements, constantly stirred with a magnetic stir bar within a 2 mm quartz cuvette. The pump and probe pulses were generated by the Spitfire Ace regenerative amplifier system (Spectra Physics), producing 4.2 mJ pulses about 800 nm wavelength at 1 kHz by chirped pulse amplification of a mode-locked Ti:Sapphire laser (MaiTai, Spectra Physics) pumped by a Nd:YLF pump laser (Empower, Spectra Physics). The excitation wavelength of 490 nm was achieved through parametric amplification (TOPAS, Light Conversion) and reduced to ~18 nJ per pulse at the sample location (4×10^{13} photons/pulse cm^{-2}) for an excitation spot size of approximately 400 μm. The probe pulse supercontinuum was generated in a 2-mm sapphire plate and focused to an area smaller than the spot size of the pump beam by a spherical mirror. The resulting probe absorbance was observed using a double-diode array detection system (Pascher Instruments) with a 300-groove grating spectrometer calibrated by the absorption bands of holmium oxide. Data were fitted globally using Glotaran global fitting analysis software (VU Amsterdam) under a sequential exponential decay scheme [20].

Optimized ground state geometries of carotenoids were created using standard ground-state density functional theory (DFT). All ground state conformers for each carotenoids were optimized with the B3LYP level of theory using the 6-31G (*d*,*p*) basis set in order to find stable conformers. This level of theory is suitable for calculations of structure geometry having C≡C bonds [21]. Stable conformers were re-optimized by means of Becke–Lee–Yang–Parr (BLYP) with the triple-ζ-quality (TZVP) basis set. These optimized structures were used as an initial guess to determine the equilibrium geometries of the excited states by means of time-dependent density functional theory within the Tamm−Dancoff approximation (TD-DFT/TDA) using either the BLYP functional, which provides the correct energy ordering for the two lowest excited states of carotenoids [7,22,23], or the hybrid exchange-correlation functional (Cam-B3LYP) with the TZVP basis set, which gives more reasonable data for the S_2 state energy [7]. All calculations were completed in the gas phase and performed with the Gaussian09 package.

3. Results and discussion

The absorption spectra of zeaxanthin and alloxanthin displayed in Fig. 1 show that adding two triple bonds to the carotenoid structure only marginally affects the S_0–S_2 transition. The absorption spectra are very similar, although alloxanthin has the lowest vibrational band slightly red-shifted, peaking at 479 nm while zeaxanthin's is at 477 nm. The resolution of vibrational bands, reflecting the conformational disorder in the ground state, is also comparable for both carotenoids. These observations imply that properties of the S_2 state are not much affected by the presence of triple bonds in alloxanthin.

Transient absorption spectra and dynamics monitoring spectroscopic properties of the S_1 state, however, demonstrate a more divergent spectral response among alloxanthin and zeaxanthin. The spectra in Fig. 2a represent the transient absorption profiles measured at 4 ps after excitation, corresponding to the S_1–S_n transition after all S_1 vibrational dynamics, which usually takes place at subpicosecond time scale [24], have subsided. In contrast to the S_0–S_2 transition shown in Fig. 1, the spectral profiles of the S_1–S_n transitions are clearly different for alloxanthin and zeaxanthin. Again, the peak wavelength is comparable, yielding 548 nm for alloxanthin and 550 nm for zeaxanthin, but it must be noted that while for the S_0–S_2 transition (Fig. 1) zeaxanthin has the slightly higher energy, it is opposite for the S_1–S_n transition. Further, the S_1–S_n profile of alloxanthin is narrower with a pronounced, blue shoulder, found to decay at the same rate as the peak's maximum (see below). In contrast, the blue shoulder is much less pronounced in zeaxanthin, but its main peak is broader than alloxanthin's and