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Excited state dynamics of the astaxanthin radical cation

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ABSTRACT

Femtosecond transient absorption spectroscopy in the visible and NIR and ultrafast fluorescence spectroscopy were used to examine the excited state dynamics of astaxanthin and its radical cation. For neutral astaxanthin, two kinetic components corresponding to time constants of 130 fs (decay of the S_2 excited state) and 5.2 ps (nonradiative decay of the S_1 excited state) were sufficient to describe the data.

The dynamics of the radical cation proved to be more complex. The main absorption band was shifted to 880 nm ($D_0 \rightarrow D_3$ transition), showing a weak additional band at 1320 nm ($D_0 \rightarrow D_1$ transition). We found, that D_3 decays to the lower-lying D_2 within 100 fs, followed by a decay to D_1 with a time constant of 0.9 ps. The D_1 state itself exhibited a dual behavior, the majority of the population is transferred to the ground state in 4.9 ps, while a small population decays on a longer timescale of 40 ps. Both transitions from D_1 were found to be fluorescent.

1. Introduction

Carotenoids are found in all kinds of living organisms and have various functions depending on their specific properties [1]. In photosynthetic organisms they fulfill important roles for both light harvesting and photoprotection [2,3]. These opposed functions result from the varying light conditions that plants, algae and photosynthetic bacteria are exposed to and requires the regulation of the energy transfer in light harvesting complexes (LHC) [4]. In their photoprotective function carotenoids scavenge potentially dangerous singlet oxygen molecules and quench singlet and triplet excited states of chlorophylls [5–8]. An important strategy of protection against a surplus of light is the non-photochemical quenching (NPQ) of chlorophyll fluorescence [9–11]. The formation of carotenoid radical cations ($Car^{\bullet+}$) was found to be correlated with the energy-dependent quenching (qE) that is one component of NPQ [12,13]. There are recent studies concerning the regulation of photosynthesis and the involvement of carotenoid radical cations [14,15]. Carotenoid radical cations were found to appear in several photosynthetic complexes, including bacterial LH2 [16,17] and isolated plant LHCs [18–20].

In addition to their role in photosynthesis, carotenoids are transferred to animals in the food chain, where they can act as scavenger of free radicals and antioxidants [21,22]. They have been found to effectively quench free radical species in liposomes [23], lipoproteins [24], membranes [25], cells [26], and animal models [27–30]. During these processes, radical cations are created and therefore can be found in plants and higher organisms alike, possessing a variety of different functions.

The optical properties of carotenoids and their radical cations are mainly determined by the extent of π-electron conjugation along their C_{40} carbon–carbon bond skeleton [31]. They are further fine-tuned by the presence of functional groups, configurational and conformational twisting as well as by the polarity and polarizability of the environment [32]. However, the photophysics of radical cations is much less examined. Their formation has been examined in some detail [33–38], yet little is known about the properties of the carotenoid cations. In a previous paper we described experiments performed on β-carotene and lutein radical cations which revealed the existence of an additional excited state between the well-known excited states D_1 and D_3 (previously known as D_2) absorbing in the NIR region [39].

Astaxanthin is a carotenoid which can be found in microalgae [40] but which is also present in salmon, shrimp or crab, and even in the feathers of some birds. It is a ten times stronger antioxidant than most other carotenoids [41]. It has therefore been widely used in biological and medical application in the context of damage protection [28,30].

Abbreviations: axt, astaxanthin; $axt^{\bullet+}$, astaxanthin radical cation; β-car, β-carotin; ESA, excited state absorption; GSB, ground state bleach; NIR, near infrared; R2P2CI, resonant-2-photon-2-color-ionization.

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In this paper we will present a comprehensive approach combining femtosecond time resolved pump–probe absorption and fluorescence spectroscopy together with steady state absorption spectroscopy allowing us to unravel the excited state dynamics of astaxanthin radical cations in solution. In the first step transient absorption measurements of neutral astaxanthin in chloroform are presented. Subsequently, the optical properties of the astaxanthin radical cation generated by resonant two-photon two-color ionization and chemical oxidation are discussed together with the solvent dependence of the primary absorption bands. Finally, pump–probe absorption and fluorescence upconversion experiments are used to elucidate the excited state dynamics of the astaxanthin radical cation.

2. Materials and methods

2.1. Sample preparation

Astaxanthin was purchased from Sigma and stored at −20 °C. Chloroform, acetone, dichloromethane, and CS_2 were also purchased from Sigma and used without further purification. Following the same procedure as described in Amarie et al. [39], astaxanthin radical cations were produced by chemical oxidation, i.e. carotenoid radical cations were generated by addition of a 0.5 M $FeCl_3$ solution to a 1 M carotenoid solution. For the time resolved experiments sample concentration was adjusted to an optical density of 0.6/mm at 890 nm. Sample stability was monitored by measuring the absorption spectra before and after the time resolved measurements.

2.2. Steady state absorption

Absorption spectra of the astaxanthin radical cations were recorded at room temperature using a Specord S100 photodiode array spectrometer (Analytic Jena) for measurements up to 1020 nm, and a Jasco V 670 spectrometer to cover the spectral range above 1000 nm.

2.3. Time resolved absorption spectroscopy

Femtosecond transient absorption measurements were carried out using a well-described pump–probe setup [19]. Excitation pulses were generated using a noncollinear optical parametric amplifier (NOPA) tuned to 500 nm for the neutral astaxanthin and to 890 nm for its radical cation. The maximum excitation energy was kept below 30 nJ per pulse (corresponding to a photon flux of 1.2×10^{14} and 2.1×10^{14} Photons/(pulse cm^2), respectively) to avoid photo-degradation. For the probe pulses a small part of the 775 nm laser fundamental was focused into a 5 mm sapphire window for white light generation. Femtosecond time delays between pump and probe were controlled by a translation stage covering delay times up to 1.5 ns. To minimize accumulation of photoproducts, the sample was moved continuously both horizontally and vertically in a direction normal to the bisector of the pump and probe beams at ~10 cm/s. The time resolution depends on the excitation wavelength and was 80 fs for the astaxanthin experiment and 120 fs for the radical cation experiment.

2.4. Time resolved fluorescence spectroscopy

Fluorescence upconversion measurements [42] were carried out using a previously described setup [43]. In brief, excitation was carried out as described for the absorption setup. A maximum energy of 50 nJ per pulse was used, corresponding to a photon flux of 5.9×10^{14} Photons/(pulse cm^2). Fluorescence was then overlapped with a gate pulse (800 nm, 110 μJ) originating from the laser fundamental in a 0.2 mm BBO crystal (angle of 51°) for sum frequency generation. The crystal was tilted for maximum fluorescence upconversion signal. Detection was carried out at an upconversion wavelength of 533 nm corresponding to a fluorescence wavelength of 1600 nm. The cross correlation was determined to be 170 fs.

2.5. Resonant-2-photon-2-color-ionization

The method of resonant-2-photon-2-color-ionization (R2P2CI) was introduced by Amarie et al. to optically generate radical cations of carotenoids [19]. It is related to other multi-pulse techniques like pump–dump–probe and pump–repump–probe spectroscopy [44–47] and uses the combined effects of two ultrashort laser pulses tuned to different colors to selectively excite the neutral carotenoid into the desired state. In this study, the first pump pulse was centered at 500 nm to excite astaxanthin to the S_2 state. The second pulse (resonant with the $S_2 \rightarrow S_N$ transition) induces further excitation of the transiently populated S_2 state into higher electronically unbound excited states which decay by ionization forming the astaxanthin radical cation. Absorption spectra were then taken at a delay time of 50 ps to identify the radical cation species.

2.6. Data analysis

For the transient absorption experiment several corrections of the raw data set were carried out prior to the application of a fitting procedure to the data. Solvent signals were subtracted and the transients were corrected for group velocity dispersion using a procedure introduced by Kovalenko et al. [48], which takes the temporal evolution of the coherent signal of pure buffer solution into account.

For the quantitative analysis of the absorption data we used a kinetic model that describes the data as a sum of n exponential decays described by their lifetimes τ_i, folded with the system response function. The model assumes Gaussian pump and probe pulses with a 1/e cross correlation width t_{cc}. A Levenberg–Marquardt algorithm optimizes a number of time constants simultaneously to the whole spectrum, yielding wavelength-dependent amplitudes $A_i(\lambda)$ (decay associated spectra) corresponding to the different time constants applied. For the fluorescence upconversion data, a Levenberg–Marquardt algorithm was used to deconvolute the previously determined cross correlation from the transient.

3. Results and discussion

3.1. Optical properties of neutral astaxanthin and its radical cation

Fig. 1A shows the absorption spectra of β-carotene (β-car) (dotted) and astaxanthin (axt) (solid) in chloroform. Both absorption bands with their maxima at 450 nm for β-car and at 490 nm for axt are associated with the corresponding strongly allowed $S_0 \rightarrow S_2$ transition, since the $S_0 \rightarrow S_1$ transition is symmetry forbidden for carotenoids [32,49,50]. While the envelope of the transition is generally similar for both axt and β-car, the former shows a broader and generally featureless spectrum that is red-shifted by 40 nm. This is rather unusual for xanthophylls, which usually exhibit vibrationally structured absorption spectra [51]. Other works show that the vibrational structure of the $S_0 \rightarrow S_2$ absorption of axt becomes visible only at a lower temperatures [52]. The resolution of vibrational peaks in the absorption spectrum usually disappears for carotenoids, where the conjugation of the double bonds