Singlet *versus* Triplet Reactivity in Photodegradation of C_{40} Carotenoids

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Direct observations of triplet excited states of astaxanthin (**I**), β-carotene (**II**), canthaxanthin (**III**), and zeaxanthin (**IV**) in toluene at 25 °C following unsensitized laser flash photolysis and using transient absorption spectroscopy have yielded the singlet−triplet intersystem crossing yields 3.7×10^{-3} (**I**), 5.4×10^{-4} (**II**), 9.7×10^{-3} (**III**), and 1.8×10^{-3} (**IV**), based on triplet−triplet extinction coefficients obtained in anthracene-sensitized experiments. A carbonyl rather than a hydroxy group distinguished the carotenoids from each other, as further evidenced by rate constants for oxygen quenching of triplet carotenoids, 2.1×10^8 (**I**), 1.1×10^8 (**II**), 2.5×10^8 (**III**), and 0.95×10^8 M^{-1} s^{-1} (**IV**), determined under similar conditions and by the fluorescence quantum yields which depend on excitation wavelength (355 *versus* 430 nm) more significantly for **II** (factor of 13) and **IV** (factor of 21) than for **I** and **III** (common factor of 2). For **II** and **III**, using continuous-wave photolysis, competing oxygen-dependent and oxygen-independent photodegradation was demonstrated, and for the latter, **III** was shown to degrade both in the singlet manifold with a photodegradation quantum yield $\Phi_1 = 7 \times 10^{-6}$ and in the triplet with $\Phi_3 = 7 \times 10^{-6}$ for 366 nm excitation, while **II** almost exclusively degraded in the singlet with $\Phi_1 = 3.8 \times 10^{-5}$. The nature of the excited states of **III** (and **I**) with some n, π^* character yields (i) more facile transformation between excited states (higher intersystem crossing yield, less wavelength-dependent fluorescence) and (ii) less biradical character (less photolability) compared to excited states of **II** (and **IV**) with pure π, π^* character.

Keywords: *Astaxanthin; β-carotene; canthaxanthin; zeaxanthin; singlet−triplet intersystem crossing yields; oxygen quenching; fluorescence quantum yield; photodegradation quantum yield*

INTRODUCTION

Carotenoids have important protective functions against photooxidation in photosynthetic organisms, and there is increasing evidence that carotenoids also are scavengers of free radicals in biological systems (Krinsky, 1989; Palozza and Krinsky, 1993). In relation to the oxidative stability of foods, carotenoids have likewise been found to yield protection against light-induced processes as shown for light-exposed vegetable oils (Lee and Min, 1988; Jung and Min, 1991) and for frozen salmon steaks during retail display (Andersen *et al*., 1990). It has, however, been more difficult to obtain direct evidence for carotenoids as chain-breaking antioxidants during nonilluminated storage of foods (Haila and Heinonen, 1994) although such activity has been demonstrated in food-related model systems (Terao, 1989; Jørgensen and Skibsted, 1993). In relation to food systems, it seems to be important to realize (i) that the scavenging of lipid radicals most effectively results in chain breaking under conditions of low oxygen pressure (Burton and Ingold, 1984; Jørgensen and Skibsted, 1993) and (ii) that oxygenated carotenoids such as astaxanthin and canthaxanthin are more effective free-radical scavengers than β-carotene (Terao, 1989; Miki, 1991). In agreement herewith, more recent stability studies of sensitive foods like liquid eggs and salmon fillets seem to confirm that oxygenated carotenoids yield protection against oxidation during processing and especially during subsequent storage where oxygen may be depleted in the product (Lai and Gray, 1995; Clark *et al*., 1995; Bjerkend and Johnsen, 1995). Future use of carotenoids as additives to animal feed in order to increase the carotenoid level in foods of animal origin or as added antioxidants to processed foods will, however, clearly benefit from a deeper insight into the mechanisms by which carotenoids are interferring with lipid oxidation in foods. An important research goal in this context seems to be the establishment of structure−activity relationships for carotenoids as radical scavengers and as photoprotectors in foods. We have embarked on such studies using a variety of photochemical and spectroscopic techniques (Jørgensen *et al*., 1992), and now we report the result of a photophysical study in relation to photodegradation of the C_{40} carotenoids shown in Figure 1. These four carotenoids represent all combinations of the presence or absence of hydroxyl and carbonyl group in the 3,3′ and 4,4′ positions respectively, and they were studied under varying conditions of oxygen pressure using transient absorption spectroscopy for a direct observation of the triplet excited states and fluorescence spectroscopy for a characterization of singlet excited states.

MATERIALS AND METHODS

Materials. *all-trans*-Astaxanthin, *all-trans*-β-carotene, *all-trans*-canthaxanthin, and *all-trans*-zeaxanthin were obtained in ampules sealed under nitrogen or argon from Roche (Copenhagen, Denmark). Toluene and benzene were of analytical grade or HPLC grade from Merck (Darmstadt, FRG) or Aldrich (Steinheim, FRG). Anthracene (99%) and benzophenone (99+%, Gold Label) were from Aldrich. All were used as received. Fluorescence quartz cells (10 × 10 mm) with SubaSeal rubber stoppers from Hellma (Müllheim/Baden,

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S0021-8561(95)00800-4 CCC: $12.00

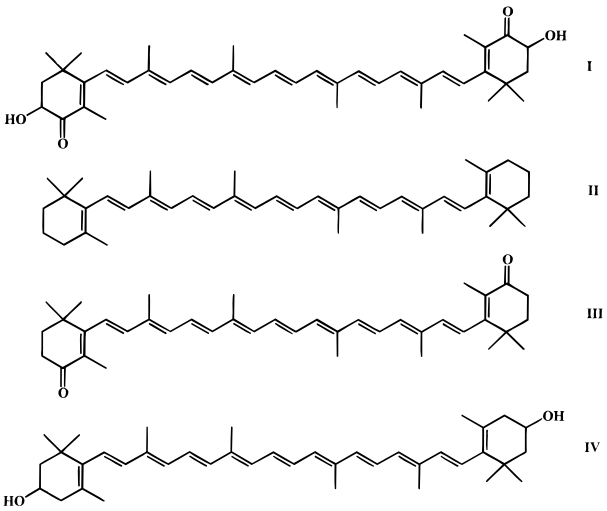

Figure 1. Chemical structures of astaxanthin (**I**), β-carotene (**II**), canthaxanthin (**III**), and zeaxanthin (**IV**).

FRG) were used for the photophysical investigations. For the photochemical investigation, cylindrical quartz cells (20 mm) were used.

Laser flash photolysis experiments were conducted with a complete LKS.50 laser photolysis spectrometer from Applied Photophysics Ltd. (London, UK): The fundamental 1064 nm radiation of a pulsed Q-switched Spectron SL 800 Nd:YAG laser was frequency tripled (355 nm) for optical sample excitation. The cross-sectional area of the beam was 0.29 cm^2, corresponding to an illuminated volume of 0.29 mL (path length 1 cm). At right angles to the laser, a 150 W xenon arc lamp equipped with an arc lamp pulser was used as the analyzing beam. A UV filter (cutoff wavelength ~400 nm) was placed between the xenon lamp and the sample to minimize photochemical degradation of the carotenoids by the flash lamp. The spectral bandpass was 2.35 nm ± 5% over the wavelength range examined.

Photodecomposition induced by the laser of the reactants in deaerated toluene was determined simply as the absorbance change between absorption spectra measured before and after photolysis at 500 (β-carotene, zeaxanthin) or 510 nm (astaxanthin, canthaxanthin). This was justified by the observation that none of the carotenoid degradation products absorb at wavelengths above ~500 nm. At 345−370 nm the absorbance of the solutions rose during laser photolysis, pointing toward *cis*-carotenoids or shorter polyenes as carotenoid decomposition products. In the sensitized experiments, astaxanthin, β-carotene, and canthaxanthin degradation was less than 1.5% whereas zeaxanthin showed up to 7% degradation. In the unsensitized experiments the carotenoid degradation was less than 5%.

All experiments were conducted at 25 ± 0.5 °C, the solutions being deaerated by bubbling with nitrogen for at least 30 min. Magnetic stirring was used in all experiments. An HP 8452A diode array spectrophotometer with 2 nm resolution was used for ground-state absorption measurements. ϵ_s values (ground-state extinction coefficients) were assessed for anthracene and the four carotenoids as the means of two to four determinations; standard deviations were less than 2%.

Fluorescence measurements were carried out with an SLM 48000S spectrofluorometer (450 W Xe arc lamp, SLM MC200 excitation and MC320 emission monochromators, SLM, Urbana, IL) mounted with a Hamamatsu R928P photomultiplier tube at 25 ± 0.5 °C in a thermostated cell holder. Emission spectra (4 nm bandpass on the excitation and 8 nm on the emission monchromator) were corrected for instrument response and were recorded for two excitation wavelengths (355 and 430 nm). Excitation spectra (bandpass as for emission spectra) were corrected for lamp spectral characteristics by the method of ratio recording using rhodamine 101 (3 g/L ethylene glycol, Exciton, Dayton, OH) as the quantum counter. The optically dilute concept (Demas and Crosby, 1971) was used for quantum yield determinations relative to rhodamine 6G (Molecular Probes, Eugene, OR): $\Phi_r = 0.95$ in ethanol for $\lambda_{ex} > 250$ nm (Kubin and Fletcher, 1982).

Steady-State Photolysis Experiments. Solutions of β-carotene and canthaxanthin (6.5×10^{-6} M in toluene) were irradiated with monochromatic light selected from an Osram HBO/2 high-pressure mercury lamp, mounted as part of an optical train (Spindler und Hoyer, Göttingen, FRG). The photolysis solutions were saturated with air or N_2/O_2 gas mixtures and kept under a blanket of the gas mixture during the experiments. Light intensities were determined by ferrioxalate actinometry (Hatchard and Parker, 1956). The extent of photodegradation was monitored at regular time intervals by spectrophotometric measurements employing a Cary Varian 219 (Mulgrave, Victoria, Australia) spectrophotometer. The photodegradation quantum yield was calculated from the degree of bleaching of the photolysis solution:

$$\Phi_{irr} = \frac{\text{moles of carotenoid molecules degraded}}{\text{moles of photons absorbed by carotenoid}} = \frac{D_{car}(t_i)}{Q_{car}(t_i)} \quad (1)$$

$$D_{car}(t_i) = C_0 V \frac{A(t_0) - A(t_i)}{A(t_0)} \quad (2)$$

$$Q_{car}(t_i) = \{I_0'A\} \sum_{i=1}^{N} (1 - 10^{-A_{irr}})(t_i - t_{i-1}) \quad (3)$$

$A(t_0)$ and $A(t_i)$ are the absorbances at the absorption maximum of the carotenoid prior to exposure to light and at time t_i, respectively. C_0 is the starting concentration of carotenoid (M), and V is the volume of the photolyzed solution. $\{I_0'A\}$ is the product of the light intensity (einstein cm^{-2} s^{-1}) as determined by actinometry and the cross-sectional area of the photolyzing beam (cm^2). A_{irr} is the absorbance at the wavelength of irradiation at a time $(t_i + t_{i-1})/2$. The light was absorbed by the solution in small but finite time intervals $t_i - t_{i-1}$. In order to avoid effects from secondary processes, calculations of quantum yields are based on photodegradations of less than 10% in single experiments in which at least five individual light exposures were made, corresponding to $N \geq 5$ in eq 3. Thermal reaction during the time span of photolysis was monitored spectrophotometrically for solutions prepared as the photolysis solutions but excluded from light. For the time span used for experiments with irradiation at the wavelengths 313, 334, and 366 nm, the thermal degradation was found to be insignificant compared to the photochemical degradation. However, for experiments with visible light (405 and 436 nm), longer irradiation times (up to 42 h) were required and with 436 nm irradiation the thermal reactions amounted to ~27% (canthaxanthin) and ~20% (β-carotene) of the total degradation, making the reported values of the photodegradation quantum yields for 405 and 436 nm excitation upper limits.

Intersystem Crossing Quantum Yield and Triplet Extinction Coefficients. Intersystem crossing quantum yields, Φ_{ISC}, were determined by relative actinometry (Carmichael and Hug, 1986) employing benzophenone in benzene using a value of 7220 M^{-1} cm^{-1} for the triplet extinction coefficient of benzophenone at 530 nm (Bensasson and Land, 1971; Hurley *et al.*, 1983) and an intersystem crossing quantum yield of 1.

$$\Phi_T^{Car} = \frac{\Delta A^{Car}(\lambda_1)\epsilon_T^{BP}(\lambda_2)}{\Delta A^{BP}(\lambda_2)\epsilon_T^{Car}(\lambda_1)} \Phi_T^{BP}$$

where Φ_T are the intersystem crossing quantum yields for carotenoid (Car) and benzophenone (BP), ϵ_T are the triplet−triplet extinction coefficients at λ_2 (= 530 nm) for benzophenone and λ_1 (= 520 or 560 nm, see below) for the carotenoids, and ΔA is the change in absorbance due to formation of triplet species extrapolated to time zero.

The triplet extinction coefficients, ϵ_T^{Car}, of the carotenoids (at a concentration of 1.0×10^{-5} M) were determined by the energy transfer method (Carmichael and Hug, 1986) employing anthracene (at a concentration of 1.0×10^{-5} M) as sensitizer using the previously (Darmanyan, 1982; Compton *et al.*, 1980) determined value 42 000 M^{-1} cm^{-1} for the triplet extinction coefficient of anthracene at 428 nm. In this method the transient absorbance of anthracene at time zero is found by extrapolation of the time trace. This value, together with the triplet extinction coefficient, is then used to calculate the concentration of triplet anthracene whereby the concentration of triplet carotenoids may be calculated (Carmichael and Hug, 1986). In order to get a reliable estimate of the intersystem crossing quantum yields, it is paramount to measure at the wavelength where the transient absorption is largest, which is where the difference between the triplet−triplet and singlet−singlet absorptions is a maximum (520 nm in the case of β-carotene and zeaxanthin; 560 nm in the case of astaxanthin and canthaxanthin).

Analysis of Time Traces. Nonlinear regression analysis was carried out using the Marquardt algorithm. Equation 4 was fitted to the time trace of the anthracene triplet at 428 nm whereas eq 5 was fitted to the time traces of the sensitized carotenoid triplets:

$$\Delta A(t) = B \exp(-kt) + A_\infty \quad (4)$$

$$\Delta A(t) = K\{-\exp(-(t - t_0)u) + \exp(-(t - t_0)d)\} + A_\infty \quad (5)$$

In eq 5, u and d are the rate constants responsible for the rise and decay of the sensitized signal, respectively. Incorporation of t_0 and A_∞ in the expression improves the quality of the fit.

RESULTS AND DISCUSSION

In Figure 1 is shown the structure of the four C_{40} carotenoids examined in this study. They represent all combinations of the presence or absence of hydroxy and carbonyl groups in the 3,3′ and 4,4′ positions, respectively. The investigation of the excited-state processes of the four carotenoids combines fluorescence spectroscopy, in order to characterize the singlet excited states, and transient absorption spectroscopy, in order to observe directly the triplet states of the carotenoids. Only two of the four carotenoids (β-carotene, canthaxanthin) were examined with respect to photodegradation because previous results (Jørgensen *et al.*, 1992) indicated that the carbonyl group rather than the hydroxy group distinguished the carotenoids from each other with respect to photophysical properties, and β-carotene and canthaxanthin were chosen as representatives of carotenoids without and with this group. Toluene was used as a common solvent for all investigations in order to facilitate a common discussion of the different types of results. The presentation of the results will be made with reference to Figure 2 in which the excited-state processes of relevance for light-induced processes in carotenoids may be identified.

Photodegradation. Table 1 gives the photodegradation quantum yields, Φ_{irr}, of β-carotene and canthaxanthin at various irradiation wavelengths. The data are plotted in Figure 3. It is observed that the photodegradation quantum yield increases significantly with decreasing wavelength. This have been observed before (Jørgensen and Skibsted, 1990; Christophersen *et al.*, 1991) for a number of carotenoids in chloroform or solubilized in water. The strong dependence of Φ_{irr} on the wavelength indicates that closely spaced energy levels, i.e., vibrational levels, are involved and that the degradation takes place either from these unrelaxed vibrationally excited electronic singlet states, or that the degradation happens in the triplet manifold. The latter possibility would require a wavelength-dependent intersystem crossing quantum yield to explain the dependence of Φ_{irr} on wavelength. A discussion of the deactivation pathways of excited carotenoids will be deferred until the discussion of the intersystem crossing quantum yields.

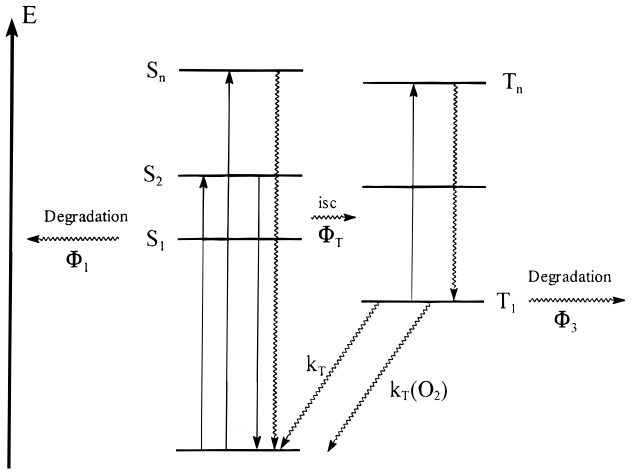

Figure 2. Excited-state diagram for symmetric C_{40} carotenoids. Fluorescence quantum yields are related to the emissive deactivation of the S_2 (and higher) singlet state to S_0. Φ_T is the quantum yield for intersystem crossing from singlet state(s) to triplet state(s). Triplet extinction coefficients, ϵ_T, are related to light absorption by T_1 to yield higher energy triplet states (T_n). Deactivation of T_1 occurs by an oxygen-dependent and an oxygen-independent path. Φ_1 and Φ_3 are quantum yields for oxygen-independent photodegradation in the singlet and triplet manifold, respectively. Wavy lines indicate radiationless transitions and straight lines transitions with the emission or absorption of radiation.

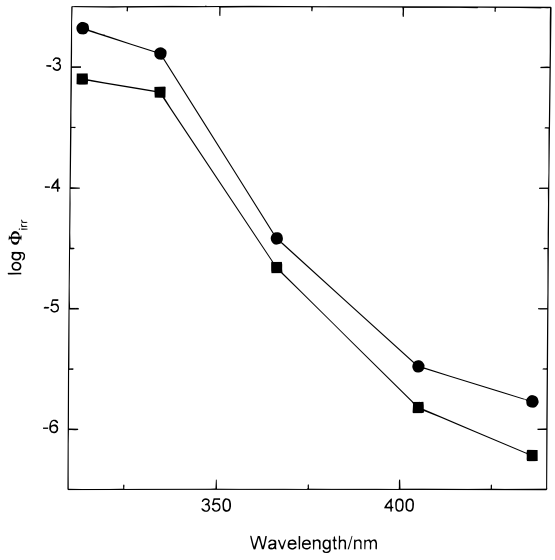

Figure 3. Photodegradation quantum yield of β-carotene (●) and canthaxanthin (■) as a function of irradiation wavelength.

Photodegradation of β-carotene and canthaxanthin at 366 nm as a function of oxygen partial pressure is depicted in Figure 4. It is observed that the photodegradation quantum yield of β-carotene is higher than that of canthaxanthin at all oxygen partial pressures. Furthermore, photodegradation takes place even in the absence of oxygen; i.e., there is an oxygen-dependent and an oxygen-independent degradation pathway.

Triplet Extinction Coefficients and Intersystem Crossing Quantum Yields. In Table 2 the triplet−triplet extinction coefficients (determined in sensitized experiments) and intersystem crossing quantum yields

Table 1. Photodegradation Quantum Yields for β-Carotene and Canthaxanthin in Air-Saturated Toluene (25 °C) at Various Wavelengths

	wavelength of irradiation[a] (nm)				
	313[b]	334[b]	366[b]	405[c]	436[c]
β-carotene	2.1×10^{-3}	1.3×10^{-3}	3.8×10^{-5}	3.3×10^{-6}	1.7×10^{-6}
canthaxanthin	7.9×10^{-4}	6.1×10^{-4}	2.2×10^{-5}	1.5×10^{-6}	0.6×10^{-6}

[a] Average light intensity in experiments (einstein L^{-1} s^{-1}): $^{313}I_0 = 3.8 \times 10^{-7}$, $^{334}I_0 = 2.5 \times 10^{-7}$, $^{366}I_0 = 1.0 \times 10^{-5}$, $^{405}I_0 = 8.7 \times 10^{-6}$, and $^{436}I_0 = 8.5 \times 10^{-6}$. [b] Reproducibility of quantum yields are 10% or better. [c] Thermal degradation contributes significantly to degradation under conditions for determination of quantum yields with 405 and 436 nm irradiation, and quantum yields are upper limits.

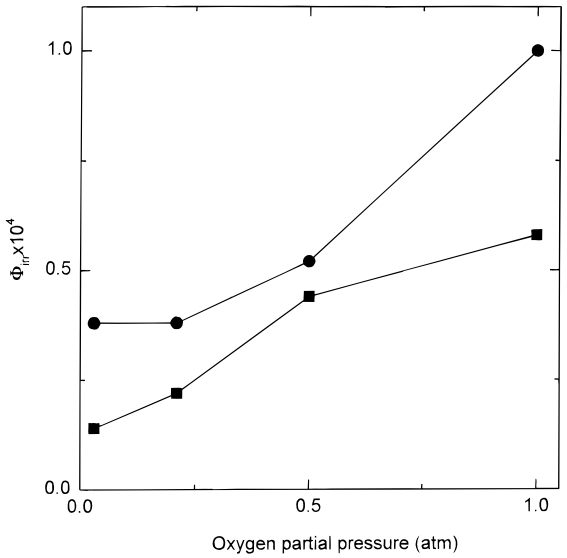

Figure 4. Photodegradation quantum yield of β-carotene (●) and canthaxanthin (■) as a function of oxygen partial pressure at 366 nm.

Table 2. Triplet Extinction Coefficients,[a] ϵ_T, Intersystem Crossing Quantum Yields, Φ_T, Fluorescence Quantum Yields, Φ_F, of Astaxanthin, β-Carotene, Canthaxanthin, and Zeaxanthin in Deaerated Solutions, and Second-Order Rate Constant, k_T, for Oxygen Quenching of Carotenoid Triplet State by Oxygen in Toluene at 25 °C

	asta-xanthin	β-carotene	cantha-xanthin	zea-xanthin
ϵ_T/M^{-1} cm^{-1}	3.43×10^4	10.50×10^4	3.59×10^4	7.24×10^4
Φ_T	3.7×10^{-3}	5.4×10^{-4}	9.7×10^{-3}	1.8×10^{-3}
$\Phi_F^{355\ nm}$	1.1×10^{-4}	9.6×10^{-4}	1.1×10^{-4}	1.8×10^{-3}
$\Phi_F^{430\ nm}$	5.4×10^{-5}	7.5×10^{-5}	6.6×10^{-5}	8.5×10^{-5}
$k_T(O_2)/M^{-1}$ s^{-1}	2.1×10^8	1.1×10^8	2.5×10^8	0.95×10^8

[a] Triplet extinction coefficients are determined at 520 nm for β-carotene and zeaxanthin and at 560 nm for astaxanthin and canthaxanthin.

(determined in unsensitized experiments) for β-carotene and zeaxanthin at 520 nm, and canthaxanthin and astaxanthin at 560 nm, the region of maximum transient absorption (Figure 5), after laser flash photolysis at 355 nm are given. The transient absorption spectra were obtained from the time traces (Figure 6) 12 μs after the laser pulse. The regions of negative transient absorption (Figure 5) correspond to higher singlet−singlet extinction coefficients than triplet−triplet extinction coefficients, whereas the reverse is true when the transient absorption is positive. In Figure 6 are shown the time traces obtained in unsensitized experiments. It is seen qualitatively from these time traces that astaxanthin and canthaxanthin have a higher population of the triplet state, provided that the triplet extinction coefficients, ϵ_T, are comparable for the four carotenoids. From the time traces, it is possible to deduce the concentration of triplet excited molecules and, hence, the intersystem crossing quantum yield, by extrapolation to time zero, if the triplet extinction coefficients are known.

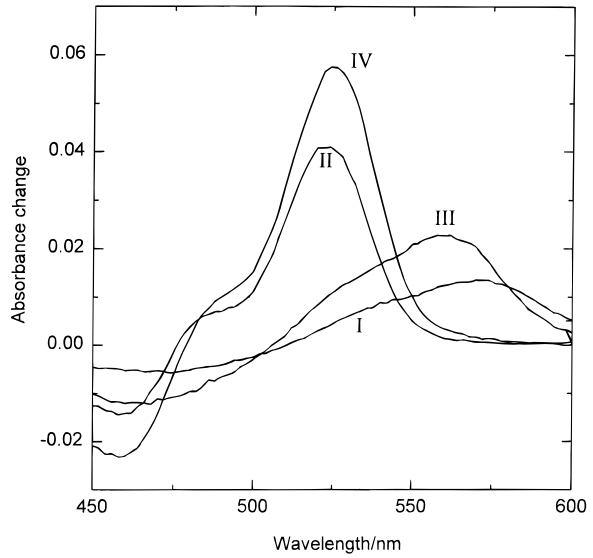

Figure 5. Uncorrected transient absorption spectra at 12 μs of the four carotenoids (sensitized experiments) in toluene: **I**, 9.9 μM astaxanthin + 21 μM anthracene; **II**, 10.9 μM β-carotene + 18 μM anthracene; **III**, 9.3 μM canthaxanthin +20 μM anthracene; **IV**, 9.7 μM zeaxanthin + 17 μM anthracene.

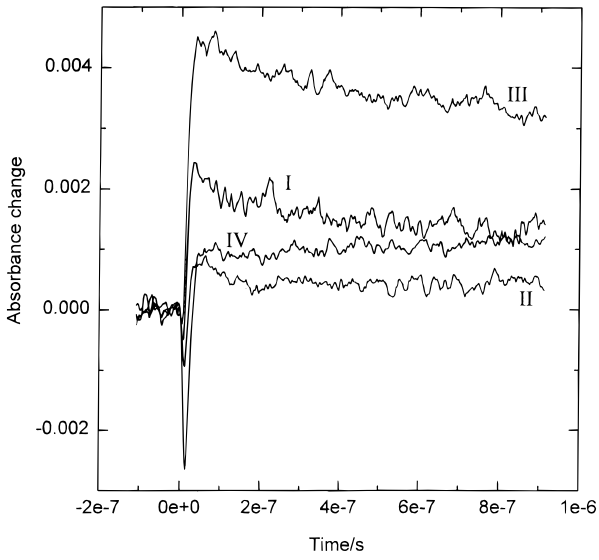

Figure 6. Time traces at 560 (**I**, **III**) or 520 nm (**II**, **IV**) from unsensitized experiments in toluene: **I**, 15.8 μM astaxanthin; **II**, 13.3 μM β-carotene; **III**, 12.7 μM canthaxanthin; **IV**, 12.4 μM zeaxanthin.

Intersystem crossing yields have been estimated earlier for β-carotene only and by indirect methods (Hashimoto *et al.*, 1991; Kuki *et al.*, 1991; Bensasson *et al.*, 1977). A value on the order of 10^{-3} was reported. Our value for β-carotene (Table 2) is in agreement with these earlier estimates. The intersystem crossing quan-

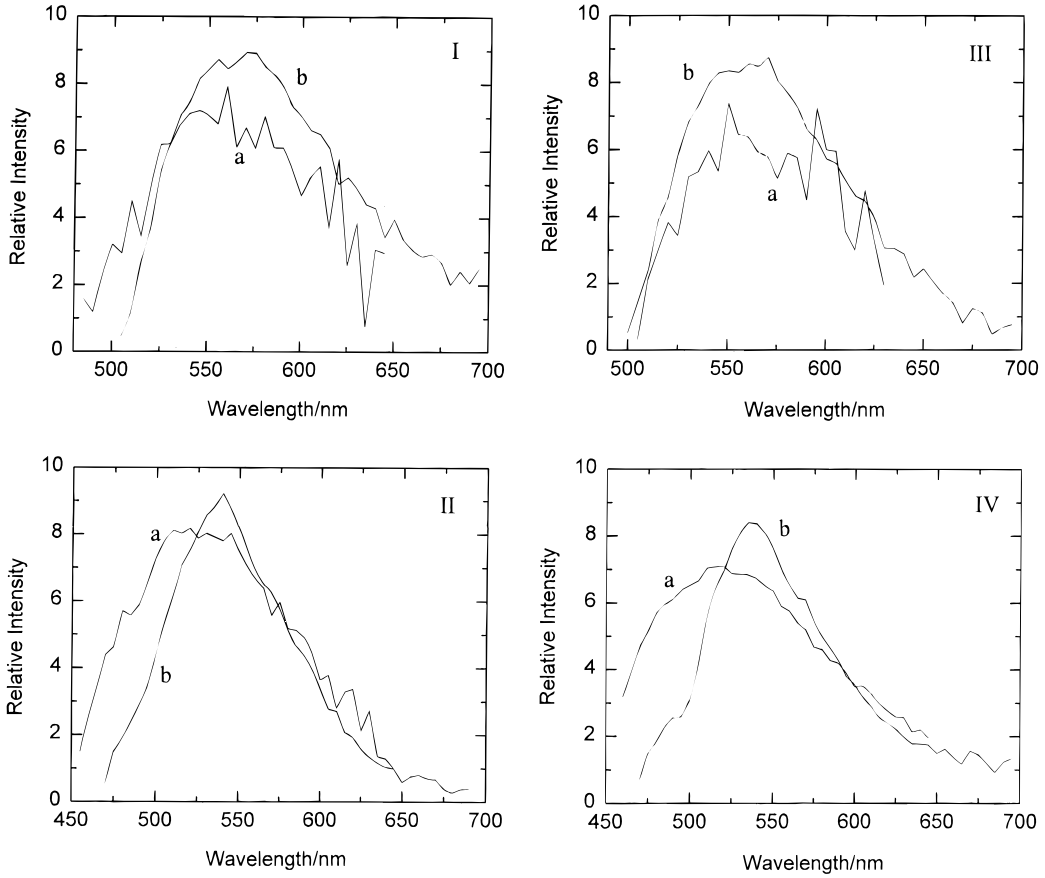

Figure 7. Fluorescence spectra of astaxanthin (**I**), β-carotene (**II**), canthaxanthin (**III**), and zeaxanthin (**IV**). Excitation wavelength: (a) 355 nm; (b) 430 nm.

tum yield is highest for the carotenoids containing a carbonyl group, indicating that the carbonyl group facilitates intersystem crossing.

Triplet extinction coefficients have previously only been determined for β-carotene, but in solvents other than toluene. Our value is determined at the wavelength (520 nm) where the difference between the triplet−triplet and singlet−singlet absorption is largest (Figure 5), whereas others have estimated the extinction coefficient at the maximum of the triplet absorption (486−488 nm).

Fluorescence Spectra and Fluorescence Quantum Yields. In the case of fluorescence, the carotenoids with carbonyl groups distinguish themselves from the carotenoids without carbonyl groups with respect to fluorescence quantum yield (Table 2) and fluorescence excitation spectra (Jørgensen *et al.*, 1992). The fluorescence quantum yield of canthaxanthin and astaxanthin is 1 order of magnitude lower than that of β-carotene and zeaxanthin when excited at 355 nm (Table 2) whereas the fluorescence quantum yield is comparable for all four carotenoids at an excitation wavelength of 430 nm, though it is still slightly higher for β-carotene and zeaxanthin than for canthaxanthin and astaxanthin (Table 2). The increase in fluorescence quantum yield when excited at 355 nm compared to excitation at 430 nm indicates that an excited state is involved at the shorter wavelength other than the S_2 state, which is usually involved in fluorescence (Jørgensen *et al.*, 1992) and which is excited directly at a wavelength of 430 nm. The state excited at 355 nm is often called the "cis" band (Jørgensen *et al.*, 1992).

In contrast to astaxanthin and canthaxanthin, a slight blue shift of the emission spectra of β-carotene

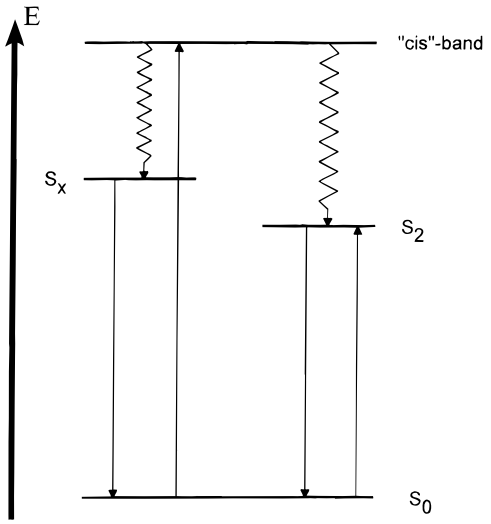

Figure 8. Energy levels involved in fluorescence from the four carotenoids. For non-carbonyl carotenoids, the fluorescence intensity decreases in the order $S_x > S_2 \gg S_1$; see text.

and zeaxanthin in toluene is observed when exciting at 355 nm compared to exciting at 430 nm (Figure 7). This emission band could be composed of two bands: one corresponding to emission from the S_2 state to which fluorescence is usually assigned and another corresponding to emission from a state at higher energy, S_x (Figure 8), but not as high as the cis band. That the cis band is involved (indirectly) in emission is corroborated by the observation that the cis band is more intense in the excitation spectra of β-carotene and zeaxanthin than in the absorption spectra (Jørgensen *et al.*, 1992). The same observations (blue shift of

emission and increased fluorescence quantum yield with decreasing excitation wavelength) have been reported before (Gillbro *et al*., 1990). Recently, it has been found in spheroidene, a non-carbonyl containing carotenoid present in certain photosynthetic algae (Watanabe *et al*., 1993), that there are two excited states close in energy corresponding to the observed absorption around 500 nm. If it is assumed (i) that such are also present in β-carotene and zeaxanthin, (ii) that these carotenoids when excited in the higher energy cis band (355 nm) relax to both of these states (S_x and S_2 in Figure 8), and (iii) that only one of these states is accessible upon direct excitation (430 nm), then a higher fluorescence quantum yield when excited at 355 nm will be the result if the state at higher energy (S_x) that is not populated by direct excitation has a higher fluorescence quantum yield than the state that is populated by direct excitation (Figure 8).

In the case of canthaxanthin and astaxanthin, the absorption and fluorescence−excitation spectra are very close; i.e., no prominent cis band is observed in the fluorescence−excitation spectra, indicating that fluorescence mainly originates from one level, irrespective of excitation wavelength, which is supported by the fluorescence quantum yields (Table 2) which increase only slightly with decreasing wavelength and to a much lesser degree than is observed in the case of β-carotene and zeaxanthin.

Quenching of the Triplet State by Oxygen. The second-order rate constant, $k_T(O_2)$, for quenching of the triplet state of the carotenoids by oxygen may be calculated from the observed triplet decay constant, K_T, for the triplet excited state in air-saturated toluene and k_T, the decay constant in the absence of oxygen, by

$$k_T(O_2)[O_2] = K_T(O_2 = 0.21 \text{ atm}) - k_T$$

$[O_2]$ is the oxygen concentration in air-saturated toluene at 25 °C [$= 2.1 \times 10^{-3}$ M; Battino (1981)]. The triplet quenching constants determined are given in Table 2. Again, the carotenoids with a carbonyl group distinguish themselves from the carotenoids without the carbonyl group. This could be due to different energies of the triplet excited states thereby making energy transfer to oxygen easier in the case of carotenoids with carbonyl groups, or it could be due to the nature of the excited triplet states; i.e., the excited state might contain some n, π^* character in the case of carbonyl-containing carotenoids (see below).

Mechanism of Photodegradation. Photodegradation may happen from either the singlet excited states or the triplet excited states. In the following, the photodegradation quantum yield from the excited singlet and triplet states in the absence of oxygen will be denoted Φ_1 and Φ_3, respectively. From Figure 4 it is possible to obtain the photodegradation quantum yield in the absence of oxygen ($\Phi_1 + \Phi_3$) by extrapolation to zero oxygen partial pressure. This value is 3.8×10^{-5} for β-carotene and 1.2×10^{-5} for canthaxanthin.

An approximate value of Φ_3 can be calculated on the following basis: The maximum concentration of triplet carotenoid in a sensitized experiment was typically in excess of 2.5×10^{-6} M corresponding to 7.25×10^{-10} mol in the cylindrical volume of sample (0.29 mL) affected by the laser puls. With 1216 (76×16) pulses in each experiment this adds up to a total of 8.8×10^{-7} mol of triplet carotenoid being formed in one experiment. The degradation in 3 mL of 1.0×10^{-5} M carotenoid solution was typically less than 1.5% (except in the case of zeaxanthin), corresponding to 4.5×10^{-10} mol. That is, out of 2000 triplet excited carotenoid molecules only 1 is degraded. This gives for the quantum yield Φ_3 ($=\Phi_T/2000$) 2.7×10^{-7} and 4.9×10^{-6} for β-carotene and canthaxanthin, respectively. Comparing these results with the sums of $\Phi_1 + \Phi_3$ (see above) shows that, in the case of β-carotene, photodegradation takes place almost exclusively in the singlet excited states whereas in the case of canthaxanthin there is a significant degradation resulting from both triplet excited ($\Phi_3 = 7 \times 10^{-6}$) and singlet excited ($\Phi_1 = 4.9 \times 10^{-6}$) molecules. These results may be explained as follows.

In the absence of oxygen, carotenoids may be expected to degrade by a unimolecular process. Degradation of carotenoids may take place through breaking one of the central carbon−carbon bonds in one of the excited states leading to radical products. This hypothesis is corroborated by the finding (Wasielewski *et al*., 1989) that the central carbon−carbon double bonds of the singlet excited carotenoids are elongated compared to the ground-state bond lengths. This would facilitate breaking of the bonds. It also explains why the photodegradation yield increases with decreasing wavelength because in the vibrationally excited states the bonds are expected to be even more elongated and, hence, more susceptible to breaking. That is, photodegradation happens in the vibrationally unrelaxed molecules. Elongation of a carbon−carbon double bond increases the biradical character because the degree of bonding between the two π-electrons decreases. In the triplet states, true biradicals are formed. Hence, triplet carotenoids may, *a priori*, be expected to undergo defragmentation more easily than singlet excited carotenoids. However, due to the rather low intersystem crossing yield, photodegradation takes place in the singlet excited states as far as β-carotene is concerned. In the case of canthaxanthin, the intersystem crossing yield is higher and photodegradation takes place in both the triplet and singlet excited states. The two other carotenoids (astaxanthin, zeaxanthin) have lower intersystem crossing yields than canthaxanthin but higher than that of β-carotene. Therefore, they represent intermediate cases but, due to the generally higher intersystem crossing yields of the carotenoids with carbonyl groups, a larger proportion of photodegradation may be expected to happen from the triplet states of astaxanthin and canthaxanthin than from carotenoids without carbonyl groups.

In the presence of oxygen, the photodegradation quantum yield increases (Figure 4). However, from the present results no firm conclusion about whether a triplet or singlet excited state is involved can be made. The triplet excited state may be more prone to reaction with ground-state triplet oxygen due to alleviation of the spin restrictions. However, due to the low intersystem crossing quantum yield, photodegradation in the presence of oxygen may mainly take place from singlet excited carotenoids. If this assumption is true, the lower photodegradation quantum yield of canthaxanthin compared to β-carotene (Figure 3) may be explained according to the excited-state lifetimes. The lifetime of the S_1 state is 10 ps for β-carotene and 5 ps for canthaxanthin (Wasielewski and Kispert, 1986), leaving longer time for the excited β-carotene to react with oxygen than the excited canthaxanthin molecule and hence a higher photodegradation quantum yield. This, however, cannot explain the difference in photodegradation quantum

yield in the absence of oxygen since photodegradation happens on a much shorter time scale than these lifetimes, i.e., from vibrationally unrelaxed molecules (see above). The difference may be due to the nature of the excited states: in canthaxanthin the excited singlet states may contain some n, π^* character due to the carbonyl group in contrast to the excited singlet states of β-carotene which are pure π, π^* states. The excited states of carbonyl-containing carotenoids may hence be expected to show less diradical character in the central part of the excited molecule (the n, π^* state is "located" on the carbonyl group), and these carotenoids are therefore less susceptible to degradation.

CONCLUSION

The combined study of singlet excited states using fluorescence spectroscopy and of triplet excited states using transient absorption spectroscopy has provided an understanding of the higher stability of canthaxanthin (and carbonyl-containing carotenoids in general) toward photodegradation compared to β-carotene (and other carotenoids without carbonyl groups). Excited states of the carbonyl-containing carotenoids have less biradical character and more facile transformation between excited states, in effect resulting in less excitation wavelength-dependent fluorescence and in higher intersystem crossing quantum yields for these carotenoids compared to carotenoids without carbonyl groups. The more stable carotenoids may be more attractive as natural antioxidants in food. In this context it should be noted that astaxanthin in several investigations has been shown to have superior radical scavenging properties compared to other C_{40} carotenoids (Miki, 1991; Jørgensen and Skibsted, 1993), and our current research efforts are focused on a correlation between electronic properties and radical scavenging properties.

ACKNOWLEDGMENT

The continuing support of the Danish Research Councils, at present through LMC-Center for Advanced Food Studies as part of the FØTEK program, is greatly acknowledged.

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Received for review December 6, 1995. Accepted April 26, 1996.[⊗]

JF9508007

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1996.