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Journal of Photochemistry and Photobiology A: Chemistry 112 (1998) 127–133

Journal of Photochemistry and Photobiology A:Chemistry

Triplet–triplet extinction coefficients, rate constants of triplet decay and rate constants of anthracene triplet sensitization by laser flash photolysis of astaxanthin, β-carotene, canthaxanthin and zeaxanthin in deaerated toluene at 298 K

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Accepted 18 September 1997

Abstract

Triplet–triplet extinction coefficients were evaluated by laser flash photolysis for the all-*trans* C_{40} carotenoids astaxanthin (I), β-carotene (II), canthaxanthin (III) and zeaxanthin (IV) in deaerated toluene at 298 K in the spectral region from 450 to 600 nm by the energy transfer method combined with a nonlinear regression procedure, employing anthracene as sensitizer. The triplet–triplet extinction coefficients in toluene were more similar to the ground state coefficients than has previously been reported for C_{40} carotenoids in hexane or cyclohexane. The maximum triplet–triplet extinction coefficient was 1.0–1.2 × 10^5 dm^3 mol^{-1} cm^{-1}, depending on the carotenoid. Rate constants of triplet decay were I: 1.71 × 10^5 s^{-1}, II: 1.40 × 10^5 s^{-1}, III: 1.54 × 10^5 s^{-1}, IV:1.10 × 10^5 s^{-1}. For anthracene it was 1.31 × 10^5 s^{-1}. Bimolecular rate constants of energy transfer from triplet excited anthracene to the carotenoids were determined from (1) non-linear regression of time traces of carotenoid triplet, and (2) linear regression of the decay rate constant of anthracene triplet at varying carotenoid concentrations; the agreement between these measurements was good, except for canthaxanthin. The results indicated that triplet energy transfer was nearly diffusion-controlled, but faster to I and III than to II and IV. These findings imply that I and III offer better protection against photosensitized oxidation than do II and IV.

Keywords: Triplet–triplet extinction coefficients; Laser flash photolysis; Photosensitized oxidation

1. Introduction

Carotenoids are important constituents of many foods, e.g., vegetables, fruits, and some fish and shellfish [1]. In these products their bright colours are highly desirable, but carotenoids also function as antioxidants which makes them susceptible to light-induced oxidation [2–4] and radical-induced oxidation [5–7]. Important is also the ability of carotenoids to quench damaging singlet oxygen [8] and excited states of photosensitizers in vivo and in foods exposed to light. During this quenching, the triplet excited state of the carotenoid is formed, which then transfers the excess energy to the surroundings as heat by intersystem crossing to the ground state.

One way of monitoring the efficiency of carotenoids towards photosensitized oxidation in various model systems is to determine the concentration, the formation rate and the deactivation rate of carotenoid triplet in time-resolved experiments. To do so it is essential to have reliable estimates of relevant rate constants and triplet–triplet extinction coefficients of the involved carotenoids. We have previously reported the intersystem crossing yield of the C_{40} carotenoids astaxanthin (I), β-carotene (II), canthaxanthin (III) and zeaxanthin (IV), based on the triplet–triplet extinction coefficient and transient absorbance at a single wavelength [9]. Following this work, we now report the rate constants and triplet–triplet extinction coefficients over a broad wavelength range of carotenoids I–IV, determined by the energy transfer

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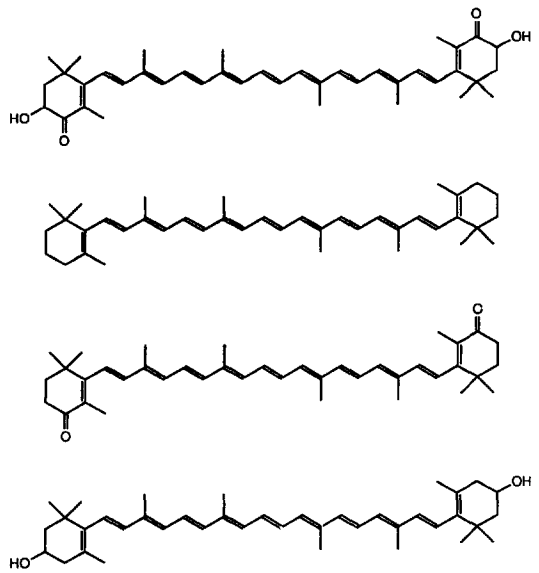
1010-6030/98/$19.00
PII S1010-6030(97)00285-2



Scheme 1. Structure of astaxanthin (I), β-carotene (II), canthaxanthin (III) and zeaxanthin (IV).

method. These carotenoids represent all combinations (presence or absence) of 3,3′-dihydroxy groups and 4,4′-dioxo groups on the basic β-carotene skeleton (see Scheme 1).

2. Experimental section

2.1. Materials

All-*trans*-astaxanthin, all-*trans*-β-carotene, all-*trans*-canthaxanthin and all-*trans*-zeaxanthin (hereafter referred to without the all-*trans* prefix) were obtained in dark ampoules sealed under nitrogen or argon from Roche (Copenhagen, Denmark) and kept at −18°C until use. Toluene was analytical grade from Merck (Darmstadt, Germany), and anthracene (99%) was from Aldrich (Steinheim, Germany). All were used as received. 10×10 mm fluorescence quartz cells with SubaSeal rubber stoppers from Hellma (Müllheim/Baden, Germany) were used for laser flash photolysis and ground state absorbance measurements. Magnetic stirring and a temperature of 298±0.5 K was used throughout.

2.2. Methods

The experiments were conducted with a complete LKS.50 laser photolysis spectrometer from Applied Photophysics (London, UK): The fundamental 1064 nm radiation of a pulsed, Q-switched Spectron SL 800 Nd:YAG-laser comprising an oscillator and an amplifier part was frequency tripled ($\lambda_{exc}=355$ nm) for optical sample excitation. The beam was circular with a diameter of 6±1 mm. The pulse duration was 8 ns according to specification. The pulse energy was about 20 mJ.

At right angles to the laser, a 150-W xenon arc lamp equipped with an arc lamp pulser and a UV-filter (cut-off wavelength about 400 nm) was used as the analyzing beam. A programmable shutter allowed the sample to be subjected to the analyzing light for only 10 ms per pulse. The diameter of the analyzing beam going through the sample and on to the monochromator was adjusted to be about 4 mm in diameter using iris apertures at the entrance and exit of the sample compartment. The distortion factor of the setup [10] was negligible because of the low transient absorbances observed. A symmetrical arrangement Czerny–Turner monochromator was used to disperse the analyzing light exiting from the sample. The entrance and exit slits were set to 0.5 mm, giving a spectral bandpass of 2.35 nm±5% over the wavelength range examined. This bandpass was small enough to confidently measure the sharp triplet–triplet peak of anthracene at 428 nm [11] and was similar to the bandpass used for the determination of the reference triplet–triplet extinction coefficient of anthracene [12]. The monochromator was checked by measuring the Raman emission line of water at 404 nm following excitation at 355 nm [13]; the discrepancy was less than 1 nm. The current from a Hamamatsu 1P28 photomultiplier was transferred through a terminal load of 1000 ohm (100 ns per point) or 50 ohm (2 ns per point) to a Philips PM3323 digital oscilloscope. Data were transferred to an Archimedes 420/I computer and converted to ΔA. Nonlinear regression was carried out using the Marquardt algorithm based on the routine Curfit [14]. Eq. (1) was fitted to the anthracene triplet decay at 428 nm with or without carotenoid to determine $\Delta A(t=0)$. Eq. (2) was fitted to the carotenoid triplet at 450–600 nm; in this region the anthracene triplet did not absorb appreciably.

$$\Delta A(t)=B\exp(-kt)+A_\infty \quad (1)$$

$$\Delta A(t)=K\{\exp(-(t-t_0)d)-\exp(-(t-t_0)u)\}+A_\infty \quad (2)$$

In Eq. (2), K is the common amplitude, and d and u are the rate constants responsible for the decay and rise of the sensitized signal, respectively. Incorporation of t_0 and A_∞ in the expression improves the quality of the fit in the absence of corrections for scattered light and fluorescence (see below). No data smoothing was performed. Representative time traces illustrating this procedure are shown in Fig. 1.

All solutions contained approximately 1×10^{-5} mol dm^{-3} carotenoid and 1×10^{-5} mol dm^{-3} anthracene. They were deaerated by bubbling with nitrogen for at least 30 min. During the acquisition of time traces the laser was fired at 0.5 Hz or less. Sixteen time traces were averaged at each wavelength to reduce noise. Time traces were not corrected for scattered light and fluorescence, since this had only a slight effect on estimates of the parameters in Eq. (2). A_{355}, the ground state absorbance at 355 nm, was between 0.16 and 0.20. An HP 8452A diode array spectrophotometer with 2 nm resolution was used for the measurements of ground state absorbance. The precision was ±0.002 absorbance units.

Photodecomposition of the reactants in deaerated toluene was determined simply as the difference in absorbance before

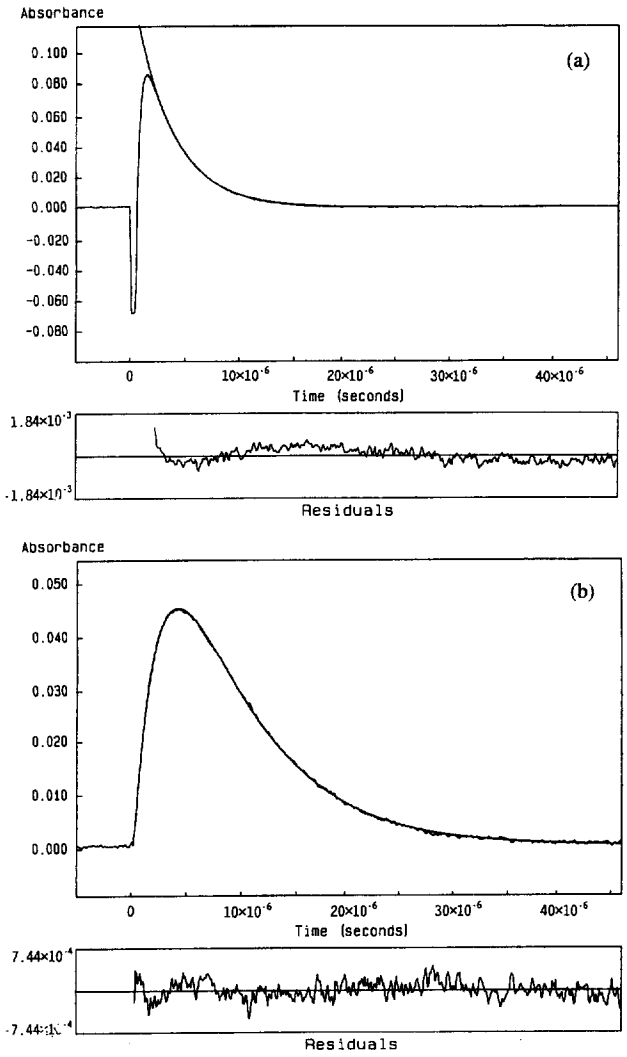


Fig. 1. Illustration of the non-linear regression procedure. Data from experiment with 0.94×10^{-5} mol dm^{-3} canthaxanthin + 2.0×10^{-5} mol dm^{-3} anthracene in deaerated toluene. (a) Time trace at 428 nm (anthracene triplet), including residuals. Parameters fitted to the trace: $\Delta A(t) = 0.134\times\exp(-2.81\times10^{5}t) - 4.59\times10^{-4}$; t in s. Range 2.0–45.7 μs. (b) Time trace at 560 nm (canthaxanthin triplet), including residuals. Parameters fitted to the trace: $\Delta A(t) = 0.124[-\exp(-3.87\times10^{5}(t+1.4\times10^{-7})) + \exp(-1.37\times10^{5}(t+1.4\times10^{-7}))]$; t in s. Range 0.0–45.7 μs.

and after laser photolysis, measured at 500 nm (β-carotene and zeaxanthin) or 510 nm (astaxanthin and canthaxanthin). The justification of this was the indication of the difference spectra (190–820 nm) that none of the carotenoid degradation products absorb at wavelengths above ca. 500 nm. At 345–370 nm the absorbance of the solutions increased during laser photolysis, pointing towards *cis*-carotenoids or shorter polyenes as carotenoid decomposition products. Astaxanthin, β-carotene and canthaxanthin degradation was less than 1.5%, whereas zeaxanthin showed up to 7% degradation. The concentration of anthracene decreased by 2–4% during laser flash photolysis, as estimated from the decrease of the 380 nm absorption peak of anthracene. 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%.

3. Results

3.1. Triplet extinction coefficients

Uncorrected transient absorption spectra of carotenoids are easily obtained and may be found for astaxanthin, β-carotene, canthaxanthin and zeaxanthin in toluene in [9]. The corresponding wavelengths of maximum transient absorbance are shown in Table 1, together with previously published values from experiments in other non-polar solvents than toluene. We have estimated triplet extinction coefficients, $\epsilon_T^A(\lambda)$, of the carotenoids astaxanthin, β-carotene, canthaxanthin and zeaxanthin by the energy transfer method [15]. In the following, T means triplet, A means triplet acceptor (i.e. carotenoid), and D means triplet donor (anthracene). Eq. (2) was fitted to the time traces (Fig. 1b); this is the theoretical expression for a sensitized time trace, derived by considering only the following processes from the end of the pulse onwards [15,16]:

$$D \xrightarrow[h\nu]{k_{exc}} {}^1D^* \xrightarrow{ISC} {}^3D^* \text{ (very fast)} \quad (3)$$

$${}^3D^* \xrightarrow{k_D} {}^1D \quad (4)$$

$${}^3D^* + {}^1A \xrightarrow{k_{ET}} {}^1D + {}^3A^* \quad (5)$$

$${}^3A^* \xrightarrow{k_A} {}^1A \quad (6)$$

The available information from the parameters in Eq. (2) is:

$$K(\lambda) = \frac{(\epsilon_T^A(\lambda) - \epsilon_S^A(\lambda))\Delta A_D k_{ET}[A]}{(k_D + k_{ET}[A] - k_A)(\epsilon_{T,428}^D - \epsilon_{S,428}^D)} \quad (7)$$

$$u(\lambda) = k_{ET}[A] + k_D \quad (8)$$

$$d(\lambda) = k_A \quad (9)$$

Here $\epsilon_T^A(\lambda)$ and $\epsilon_S^A(\lambda)$ are the triplet–triplet and ground state extinction coefficients of the carotenoid at a given wavelength. ΔA_D is the absorbance of the anthracene triplet donor at $\lambda_{max} = 428$ nm, extrapolated to $t = 0$ by fitting Eq. (1) to the time trace (Fig. 1a). The triplet extinction coefficient of anthracene at 428 nm, $\epsilon_{T,428}^D$, is $42\,000 \pm 4000$ dm^3 mol^{-1} cm^{-1} [12,17], and we measured $\epsilon_{S,428}^D \leq 20$ dm^3 mol^{-1} cm^{-1}. ΔA_D was hardly affected by the underlying carotenoid absorption (see Fig. 1a). The extrapolation on this time scale was very reliable, as indicated by experiments conducted at a faster time scale (data not shown). [A] is the total concentration of carotenoid. k_D, k_{ET} and k_A are defined in Eqs. (4)–(6). Solving for ϵ_T^A yields at each wavelength:

$$\epsilon_T^A(\lambda) = \epsilon_S^A(\lambda) + \frac{(u(\lambda) - d(\lambda))K(\lambda)(\epsilon_{T,428}^D - \epsilon_{S,428}^D)}{(u(\lambda) - k_D)\Delta A_D} \quad (10)$$

Table 1
Overview of present and previously published parameters of uncorrected and corrected spectral triplet parameters in deaerated non-polar solvents at room temperature, and rate constants of carotenoid triplet decay for astaxanthin, β-carotene, canthaxanthin and zeaxanthin

Carotenoid	Solvent	$\lambda(\Delta A_{A,max})$ (nm)	$\lambda(\epsilon_{T,max})$ (nm)	$\epsilon_{T,max}$ ($\times 10^5$ dm^3 mol^{-1} cm^{-1})	Method [a]	k_A ($\times 10^5$ s^{-1})	Ref.
Astaxanthin	Benzene	580	–	–	PR/ET (N) [b]	1.6	[32]
Astaxanthin	Toluene	570	486–488	1.02 ± 0.15	LP/ET (A)	1.71 ± 0.03	This work [c]
β-carotene	Hexane	514	–	–	FP (A, 1,2-BA, 2,3-BA, DBA, Chl a)	0.023	[16]
β-carotene	Hexane	515	–	–	LP [d]/ET (Chl a)	0.65 [e]	[33]
β-carotene	Hexane	515	–	~2	?	1.4	[21]
β-carotene	Hexane	515	–	2.3 [f] 1.7 [g]	PR, PR/ET PR/ET (N)	1.1	[22]
β-carotene	Hexane	515	–	2.5 [h] 0.7 [i] 1.3 [g]	PR/ET (N,A) PR PR/ET (N)	1.07	[23]
β-carotene	Hexane	515	–	2.42 [j]	PR/ET (BP)	1.7	[24]
β-carotene	Cyclohexane	–	–	1.95 [j]	PR/ET (BP)	2.12	[25]
β-carotene	1% ethanol in benzene	526	–	–	FP/ET (Chl a)	1.7	[34]
β-carotene	Benzene	540	–	–	PR/ET (NB)	–	[35]
β-carotene	Benzene	520	–	–	LP/ET (A)	1.25	[36]
β-carotene	Benzene	515	–	–	PR/ET (N)	–	[37]
β-carotene	Benzene	530	–	–	PR/ET (N)	–	[38]
β-carotene	Benzene	530	–	–	PR/ET (N, BP)	2.9	[39]
β-carotene	Benzene	530	–	–	PR/ET (N)	–	[40]
β-carotene	Benzene	515	–	–	LP/ET (Bchl a)	–	[41]
β-carotene	Toluene	524	494	1.19 ± 0.18	LP/ET (A)	1.40 ± 0.15	This work [c]
β-carotene	CS_2	550	–	–	LP [d]/ET (Chl a)	0.65 [e]	[33]
Canthaxanthin	Hexane [k]	541 [l]	–	–	FP/ET (A)	1.4 [m]	[42]
Canthaxanthin	Benzene	555	–	–	PR/ET (N)	2.6	[43]
Canthaxanthin	Benzene	565	–	–	PR/ET (N)	–	[40]
Canthaxanthin	Benzene	555	–	–	PR/ET (N) [b]	2.6	[32]
Canthaxanthin	Toluene	558	486	1.05 ± 0.16	LP/ET (A)	1.54 ± 0.07	This work [c]
Zeaxanthin	Hexane [k]	505 [l]	–	–	FP/ET (A)	–	[42]
Zeaxanthin	Benzene	520	–	–	PR/ET (N)	1.1	[43]
Zeaxanthin	Benzene	520	–	–	PR/ET (N) [b]	1.5	[32]
Zeaxanthin	Toluene	524	492	1.05 ± 0.16	LP/ET (A)	1.10 ± 0.10	This work [c]

[a] LP: laser flash photolysis; ET: energy transfer; PR: puls radiolysis; FP: flash photolysis. Sensitizer in parentheses: A, anthracene; BA, benzanthracene; Bchl a, bacteriochlorophyll a; BP, biphenyl; Chl a, chlorophyll a; DBA, 1,2:5,6-dibenzanthracene; N, naphthalene; NB, norbornene.
[b] Most probably, otherwise LP/ET (A).
[c] From nonlinear regression on entire time trace. Concerning error limits, see text.
[d] 'Rapid flash' photolysis.
[e] Value measured in either CS_2 or hexane.
[f] Assuming that the triplet does not absorb between 380 and 500 nm. Sensitizer(s) not stated.
[g] Following Ref. [44] (curve fitting procedure at different carotenoid concentrations). Not corrected for self-decay of donor.
[h] Assuming that the triplet does not absorb at 450 nm.
[i] Direct population of the triplet without sensitizer.
[j] Linear regression method relating $\epsilon_{T,max}$ to transient absorbance measurements at different carotenoid concentrations.
[k] Most probably, otherwise benzene.
[l] As stated in Ref. [15].
[m] From pulse radiolysis experiment without sensitizer. The rate constant was somewhat dose dependent.

k_D was determined separately from 14 independent anthracene experiments; it was $(1.31 \pm 0.13) \times 10^5$ s^{-1}, corresponding to a lifetime $\tau_D = 7.6$ μs. The rate constants u and d should, of course, be independent of the monitoring wavelengths, but they were calculated at each wavelength in the spectral range investigated—hence the notation $u(\lambda)$ and $d(\lambda)$. The resulting triplet extinction coefficients are shown in Fig. 2. Values of $\epsilon_{T,max}{}^A$ are shown in Table 1 together with previous estimates of this parameter. Standard deviations of $\epsilon_T{}^A$ of the four carotenoids changed with wavelength but never exceeded 15% [18]; this is the error limit used in Table 1.

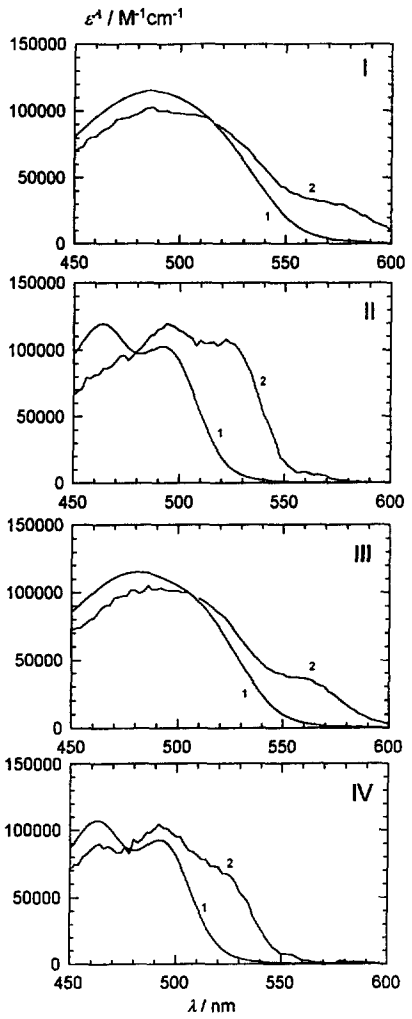


Fig. 2. Comparison of $\epsilon_T^A(\lambda)$ with $\epsilon_S^A(\lambda)$. Experimental conditions as in Fig. 1. (1) $\epsilon_S^A(\lambda)$, (2) $\epsilon_T^A(\lambda)$. (I) Astaxanthin, (II) β-Carotene, (III) Canthaxanthin, (IV) Zeaxanthin.

3.2. Rate constants

Values of the rate constant k_A of the four carotenoids, calculated according to Eq. (9), may be found in Table 1, together with some previously published values. Values of the rate constant k_{ET} of the four carotenoids, calculated according to Eq. (8), may be found in Table 2. For both k_{ET} and k_A, we arrived at the reported values in the following way: In each of n independent experiments ($j=1,2,\ldots,n$), the value $k_P^j(\lambda_i)$ of the rate constant of the relevant process P (P=A (acceptor decay) or ET (energy transfer)) was evaluated at N different wavelengths ($i=1,2,\ldots,N$), covering regions of both positive and negative ΔA. In all experiments n was between 2 and 13, and N was between 13 and 74. Outliers were rejected on the assumption of approximately normally distributed data, and the remaining values were averaged to give the quantity $\overline{k_P^j}$ according to Eq. (11).

Table 2
Bimolecular rate constants of energy transfer from triplet excited anthracene to astaxanthin, β-carotene, canthaxanthin and zeaxanthin in toluene at 298 K [a]

Carotenoid	k_{ET} ($\times 10^{10}$ dm^3 mol^{-1} s^{-1}) [b]	k_{ET}' ($\times 10^{10}$ dm^3 mol^{-1} s^{-1}) [c]
Astaxanthin	2.59 ± 0.07	2.81 ± 0.69
β-Carotene	1.20 ± 0.08	1.35 ± 0.72
Canthaxanthin	5.44 ± 1.18	1.60 ± 0.73
Zeaxanthin	2.05 ± 0.62	0.92 ± 0.73

[a] All on the basis of $k_D = 1.31 \times 10^5$ s^{-1} (see text).
[b] Calculated from Eq. (8), i.e., non-linear regression of time trace of carotenoid triplet.
[c] Calculated from the formula $k_{obs} = k_D + k_{ET}' \times [A]$, by measuring the decay rate constant of anthracene triplet in the presence or absence of acceptor.

$$\overline{k_P^j} = (1/N) \sum_{i=1}^{N} k_P^j(\lambda_i) \quad (11)$$

Standard deviations of the $\overline{k_P^j}$ were less than 2.5% for k_A and less than 8% for k_{ET}. The $\overline{k_P^j}$ were averaged with equal weights to yield the rate constants k_P according to Eq. (12).

$$k_P = (1/n) \sum_{j=1}^{n} \overline{k_P^j};$$

$$\text{S.D.}(k_P) = \left\{ [1/(n(n-1))] \sum_{j=1}^{n} (\overline{k_P^j} - k_P)^2 \right\}^{1/2} \quad (12)$$

The values of k_A and k_{ET} reported in Tables 1 and 2 are the $k_P \pm$ S.D.(k_P) and do not include the contribution from standard deviations of the $\overline{k_P^j}$.

Furthermore, in Table 2 we report values of k_{ET} obtained by considering the decay rate constant of anthracene at 428 nm in the absence of carotenoid ($k_{obs} = k_D$, see above) and in the presence of one concentration of carotenoid ($k_{obs} = k_D + k_{ET}' \times [A]$). The rate constant for energy transfer to the carotenoid determined by this indirect method is assigned k_{ET}' to distinguish it from the rate constant determined directly, although in principle it is the same quantity. No replicates of the latter k_{obs} were made. A crude estimate of the standard deviation of k_{ET}' can be made by assuming that the sampling variance, σ^2, of the k_{obs} used for the determination is equal to the sampling variance of k_D (see above), i.e., $\sigma^2 = 14 \times (0.13 \times 10^5)^2$. Then the standard deviation of k_{ET}', $\sigma(k_{ET}')$, is given by Eq. (13).

$$\sigma(k_{ET}') = (2\sigma^2/[A])^{1/2} \quad (13)$$

The values reported in Table 2 are the $k_{ET}' \pm \sigma(k_{ET}')$. For all carotenoids except canthaxanthin, k_{ET} is not significantly different from k_{ET}'. We have no ready explanation for the difference between k_{ET} and k_{ET}' for canthaxanthin.

4. Measurement of triplet extinction coefficients

The application of nonlinear regression to fit Eq. (2) to sensitized time traces, followed by insertion of the parameters in Eq. (10), is a very convenient way to obtain triplet extinction coefficients of acceptor molecules, $\epsilon_T{}^A$, in cases where neither the self decay of the triplet donor nor that of the acceptor can be disregarded on the time scale used. The only additional information needed is the ground state extinction coefficients of the donor ($\epsilon_S{}^D$) and acceptor ($\epsilon_S{}^A$) alone, the triplet extinction coefficient of the donor at a reference wavelength ($\epsilon_{T,ref}{}^D$), and the decay rate constant of the isolated donor triplet, k_D. To our knowledge this is the first time k_D has been determined for anthracene in toluene. Previous work has indicated lifetimes in the millisecond range of anthracene triplet in fluid phase (see for example the compilation in [15]), but these investigations were carried out with conventional flash photolysis equipment which, as has been recognized later [19,20], may have a substantial afterglow leading to artifactually long lifetimes. With this in mind our determination of the anthracene triplet lifetime of 7.6 μs is not unreasonable. It is required that the donor triplet does not absorb appreciably in the spectral region, where the $\epsilon_T{}^A$ are to be measured. Otherwise a correction must be made, in which case the method loses its appealing simplicity. Furthermore, the value of $\epsilon_T{}^A$ is only as good as the $\epsilon_{T,ref}{}^D$ used in Eq. (10).

Reports of λ_{max} of uncorrected transient spectra exist for all the carotenoids studied here. However, only for β-carotene have any triplet–triplet extinction coefficients been published previously, albeit at different experimental conditions [21–25]. These parameters are reproduced in Table 1. Our triplet–triplet extinction coefficients are very similar to the ground state extinction coefficients of the carotenoids. This strongly argues against the presence of a pure singlet depletion region (i.e., a wavelength region where the triplet does not absorb between 450 and 600 nm) for any of the four carotenoids examined (Fig. 2). Interestingly, the similarity between extinction coefficients of ground state and triplet state carotenoids is supported by the calculations of Gijzemann and Sykes [26], which predict the maximum extinction coefficient of the $T_1 \rightarrow T_n$ transition to be lower than the maximum $S_0 \rightarrow S_1$ extinction coefficient, although it was only concluded that the maximum extinction coefficient of $T_1 \rightarrow T_n$ and $S_0 \rightarrow S_1$ were of the same order of magnitude.

The present method obviates the need for studying the dependency of carotenoid triplet absorbance on carotenoid concentration in energy transfer experiments, as has been done in the past, when pulse radiolysis has been used for sample excitation. Our method gives the triplet decay constant k_A directly rather than as an approximated value from the late part of time traces from experiments with high carotenoid concentration. Cogdell et al. have used the expression 'correction factors 1 and 2' (K_1 and K_2 in the pertinent kinetic scheme, Eq. (14)), and their experimental strategy has been to near these to unity in order to get as good estimates of $\epsilon_T{}^A$ as possible [25].

$$\Delta\epsilon_T^A = \frac{\Delta\epsilon_T^D \Delta A_A}{\Delta A_D} \times \frac{k_D + k_{ET}[A]}{k_{ET}[A]} \Big/ \exp\left(- \frac{\ln((k_D + k_{ET}[A])/k_A)}{((k_D + k_{ET}[A])/k_A) - 1} \right) = \frac{\Delta\epsilon_T^D \Delta A_A}{\Delta A_D} \times K_1/K_2 \quad (14)$$

The price of minimizing these correction factors is the need of using high carotenoid concentrations. This gives rise to high ground state absorbances and consequently to low analyzing light levels reaching the photomultiplier, resulting in lower signal-to-noise ratios. In the present method the 'correction factors' are an inherent part of the model, and in fact their presence has been turned from a nuisance to an advantage, since precise estimates of the involved rate constants can be obtained without varying the carotenoid concentration. The fact that our estimates of k_{ET} and k_{ET}' (Table 2) are not significantly different (except for canthaxanthin) is a good indication that the procedure is valid. Hence the method should be useful in obtaining triplet extinction coefficients in a variety of systems.

Strictly speaking, the $\epsilon_T{}^A$ obtained here could be regarded as a lower limit (in spectral regions of positive ΔA_A), because of the uncertainty of the efficiency of energy transfer. If ground state carotenoid deactivates the anthracene triplet in a spin-forbidden reaction without itself being excited to the triplet state, then at any given time too high a concentration of the carotenoid triplet is calculated. This is tantamount to saying that $k_Q[{}^1A]$ in Eq. (15) is comparable in size to k_D and $k_{ET}[{}^1A]$ defined in Eqs. (4) and (5):

$$^3D^* + {}^1A \xrightarrow{k_Q} {}^1D + {}^1A \quad (15)$$

If k_Q were large, then the observed k_{ET} would be smaller than the true k_{ET}, because the observed value of $u(\lambda)$ in Eq. (8) would diminish, as k_Q increased. Inspection of Table 2 reveals, however, that the observed values of k_{ET} are very close to or greater than the diffusion-limited rate constant, for which the approximate value $k_{dif} = 1.2 \times 10^{10}$ dm^3 mol^{-1} s^{-1} can be calculated for toluene at 298 K [27,28]. It is thus physically impossible that the true values of k_{ET} can be much larger than the observed values, and this constitutes strong evidence that k_Q must be small. Furthermore, the carotenoids studied here contain no atoms heavier than oxygen and should not passively enhance intersystem crossing from the excited triplet state of anthracene to the ground state [29]. This indicates that the triplet–triplet extinction coefficients reported here are true values.

5. Discussion

An important finding of the present study is that the triplet–triplet extinction coefficients of C_{40} carotenoids are more

similar to the ground state extinction coefficients in toluene than has previously been found in hexane or cyclohexane. It should be noted, however, that the triplet of astaxanthin and canthaxanthin has a large absorption in the wavelength region 550–600 nm, which is absent from the triplet spectrum of β-carotene and zeaxanthin. Hence the carbonyl groups of astaxanthin and canthaxanthin are part of the triplet conjugated system.

The triplet decay rate constants, k_A, and the rate constant of energy transfer from triplet excited anthracene, k_{ET}, are clearly lowest for β-carotene and zeaxanthin. Hence the two oxo groups of the conjugated system of astaxanthin and canthaxanthin help these molecules to accept triplet energy more efficiently and also to dissipate the triplet energy more efficiently than β-carotene and zeaxanthin. The presence or absence of the 3,3′-dihydroxy groups does not seem to have any clearcut influence on any of the determined parameters. The four carotenoids have been found to have very similar quenching constants of singlet oxygen in toluene or benzene [30], whereas astaxanthin and canthaxanthin are faster quenchers of singlet oxygen than β-carotene and zeaxanthin in ethanol/chloroform [31]. Hence astaxanthin and canthaxanthin may be better antioxidants against light-induced, singlet oxygen mediated oxidations than β-carotene and zeaxanthin, because:

1. They deactivate triplet sensitizers (precursors of singlet oxygen) faster.
2. They deactivate singlet oxygen equally fast or faster.
3. After deactivation of sensitizers or singlet oxygen they return to the ground state faster, so the efficiency per molecule per unit time is greater.

This opens up new perspectives for the use of carotenoids as antioxidants in light-exposed systems.

Acknowledgements

We would like to thank Dr. Niels-Henrik Jensen, Radiometer, for valuable discussions. The carotenoids were kindly provided by Roche, Denmark. The continuing support of the Danish Research Councils, at present through LMC-Center for Advanced Food Studies as part of the FØTEK programme, is greatly acknowledged.

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