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β-Carotene triplet state absorption in the near-IR range

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Abstract

The β-carotene absorption from the lowest triplet state was investigated over a wide spectral range. A new absorption transition in the near-IR region was found. The absorption band exhibited a 0–0 transition at about 8100 cm^{-1} with a low intensity, lower by a factor of approximately 300 than the intensity of the known triplet–triplet absorption in the visible range. The vibronic structure of the absorption band was similar to that of the steady state β-carotene absorption. The energy of the triplet state, resulting from this near-IR transition, was close to the β-carotene S_1 state energy. Correspondingly, the interconversion of the carotenoid should depend on the features of this triplet state. Moreover, this relatively low-lying triplet state could also take part in the quenching of molecular triplet states by carotenoids.

Keywords: Transient absorption; Phosphorescence; Energy transfer; Interconvesion; Retinoids; Carotenoids

1. Introduction

β-Carotene is one of the most widespread natural dyes. Because of the important photoprotective and light-harvesting roles of β-carotene and similar long-chain carotenoids [1,2], many investigations have been carried out for this group of polyenes. The lowest excited singlet state in polyenes has the same g symmetry as that of the ground state [3,4]. Therefore fluorescence from the S_1 state of carotenoids is forbidden, which hinders the investigation of the lowest excited singlet state by the sensitive fluorescence technique. The allowed fluorescence from the high singlet levels of carotenoids is easier to detect. According to Refs. [5–7], the quantum yield of the β-carotene S_2 fluorescence has been determined to be approximately 10^{-4} and the S_1 lifetime is known to be approximately 10^{-11} s. From the S_2 fluorescence quantum yield and S_2 radiation lifetime, the S_2 lifetime was estimated to be approximately 10^{-13} s [6]. A recent direct measurement has shown that the fluorescence lifetime of the β-carotene S_2 state is approximately 200 fs in *n*-hexane [8].

While the singlet states of carotenoids can take part in light-harvesting processes [2], the triplet states play the main role in their photoprotective function. It is well known that β-carotene and other carotenoids are extremely efficient quenchers of molecular triplet states and singlet oxygen [9,10]. The mechanism of photoprotection is usually described by photophysical energy transfer from the metastable states of the energy donor to the low-lying triplet states of the carotenoid [11]. Unfortunately, the lowest triplet level for long-chain carotenoids is not well identified. The lowest triplet level for β-carotene is usually estimated to be in the range 7400–7800 cm^{-1} [12,13]. These values are very close to the energy of singlet oxygen, and carotenoids can thus be regarded as photophysical quenchers of singlet oxygen.

A very intense triplet–triplet absorption band has been found for β-carotene and other carotenoids [14,15]. The spectral maxima are detected at slightly longer wavelengths than the 0–0 maxima of the ground state absorption. A high-energy triplet state, T_n, resulting from this triplet–triplet absorption cannot be realized in natural systems. Therefore the state is not interesting in itself, but due to its very high intensity it is very suitable in the optical testing of processes involving triplet states. For instance, the intense triplet absorption of β-carotene after triplet–triplet energy transfer from donors can be used as a quantitative indicator of the triplet quantum yields of the donors [16].

In this work, we present results on the transient absorption of β-carotene triplet states. A highly sensitive experimental system with detection over a broad spectral range was applied. The main emphasis is on the low-lying triplet states, which may be significant for the singlet–triplet internal conversion of β-carotene or take part in the triplet–triplet energy transfer from other molecules to carotenoids. The information obtained will be useful for theoretical modelling and calculation of the triplet states of carotenoids. In addition, we have attempted to detect the phosphorescence of β-carotene in order to estimate the upper limit of the phosphorescence quantum yield.

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2. Experimental details

Crystalline all-*trans*-β-carotene (β-carotene) was synthesized at the Vitamin Institute (Moscow). The sample purity was checked by different methods; β-carotene contained less than 1% impurities, mainly substances with shorter polyene chains [6]. The compound was stored in the dark at a temperature below 0 °C and at a residual pressure of less than 10^{-3} Torr. Anthracene was of scintillator grade; the solvents (toluene, *n*-hexane) were of spectroscopic grade purity. All measurements were carried out with fresh solutions.

The spectral and kinetic study of the transient β-carotene absorption was performed with the equipment described in more detail elsewhere [17,18]. Samples were excited with the doubled frequency of a ruby laser (347 nm). The pulse energy was usually in the 5–15 mJ range, the illuminated area in the sample was about 0.5 cm^2 and the pulse duration was 20 ns. A halogen filament lamp (microsecond timescale) and a flash high-pressure xenon lamp (submicrosecond range) were applied as probe lights. The intensity of the probe light was measured by a germanium photodiode with a grating monochromator. The spectral width of the monochromator slits was adjusted from 4 to 30 nm. The monochromator was complemented by red and IR cut-off filters. The photodiode had sufficient sensitivity in the 510–1660 nm spectral range. A vacuum photodetector was used for the 300–700 nm range. Since all solvents with C–H bonds in their molecules absorb probe light at greater than 1650 nm, this wavelength is the near-IR limit for investigations of electronic absorption in organic solvents.

The limit of the system time resolution was about 40 ns. An applied response time could be adjusted to obtain the best signal-to-noise ratio without noticeable distortion of the investigated kinetics. Kinetic traces were detected by oscilloscopes and, due to the high signal-to-noise ratio, we could use the measured kinetic traces without averaging. The noise values for a system response time of 0.5 μs corresponded to an optical density of transient absorption from 10^{-5} to 10^{-4}. Transient absorption intensities (ΔA) were determined from the ratio of the transient signal (ΔU) to the signal detected without excitation (U), i.e. $\Delta A = \ln(\Delta U/U)$. The absorption spectra were measured point by point in steps of 10 or 20 nm.

Since the β-carotene interconversion yield is very low [19], the main measurements were performed using the triplet–triplet energy transfer from a donor to β-carotene. The energy donor was anthracene, which can successfully be used to populate the β-carotene triplet state [20]. The concentrations of anthracene and β-carotene were chosen to provide an intense anthracene absorption at the excitation wavelength and sufficiently fast rates of triplet–triplet energy transfer from anthracene to β-carotene. The measurements were performed with deaerated solutions at a remaining air pressure of less than 10^{-3} Torr.

A similar detection system and anthracene as donor were used to examine the β-carotene phosphorescence and, in addition, the phosphorescence of 8,8′-apo-carotenal (Fluka).

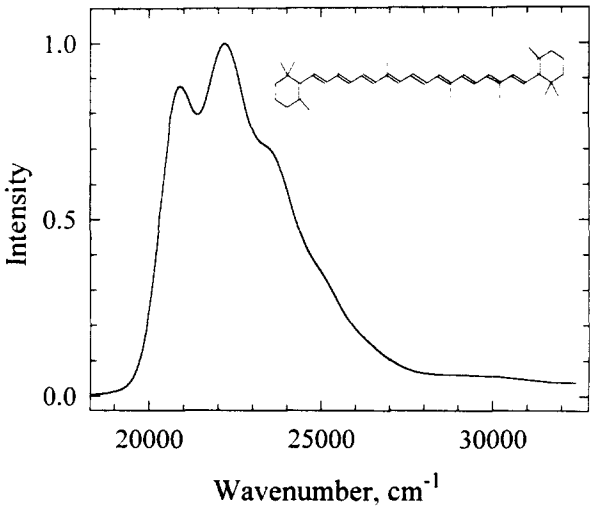

Fig. 1. Absorption spectrum of β-carotene in hexane.

The probe light was not used in this case. The excitation was provided by the third harmonic (354 nm) of a *Q*-switched Nd^{3+}:YAG laser with a pulse duration of 10–12 ns, an energy of about 1 mJ and a repetition rate of 3 Hz. Several porphyrins were applied as sensitizers and the doubled frequency (532 nm) was used for excitation. For the measurement of every kinetic trace, we used the averaging of up to 1024 kinetic signals transferred from a digital 20 MHz oscilloscope to a computer. The singlet oxygen luminescence produced in aerated anthracene solutions was applied as an intensity reference for the phosphorescence in the near-IR range.

3. Results and discussion

For pure anthracene solutions, no real transient absorption was observed at wavelengths above 600 nm with a duration of more than 1 μs. The anthracene S_1 state absorption was observed in the near-IR range [21]; the anthracene fluorescence and weak triplet absorption were recorded at shorter wavelengths. The intense signals from the singlet states of anthracene had lifetimes of a few nanoseconds and could not affect the signals on the microsecond timescale. In the case of pure β-carotene solutions, we observed a very weak absorption in the 520–550 nm range (in toluene) with a duration of a few microseconds. The intensity of this absorption was too low to measure the decay time accurately. It was probably due to absorption of the triplet state, and the β-carotene interconversion yield must be less than 3×10^{-4}.

Since the β-carotene extinction coefficient is small at 347 nm (Fig. 1), the excitation light was mainly absorbed by anthracene in solutions containing both β-carotene and anthracene. Correspondingly, the photophysical processes in the system can be described as

$$^1A + {}^1C \xrightarrow{h\nu} {}^1A^* + {}^1C \xrightarrow{k_{ic}} {}^3A^* + {}^1C \xrightarrow{k_d} {}^1A + {}^3C^* \xrightarrow{k_t} {}^1A + {}^1C \quad (1)$$

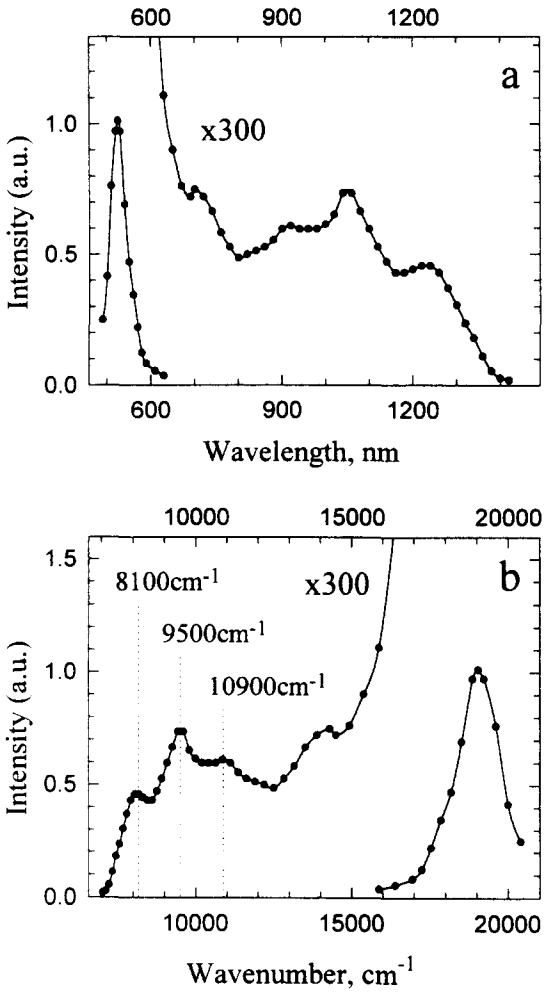

Fig. 2. Transient absorption spectrum of the β-carotene triplet state; (b) also shows the positions of the near-IR vibronic maxima.

where A is anthracene, C is β-carotene, k_{ic} and k_d are the intersystem crossing in anthracene and the triplet–triplet energy transfer from A to C respectively and k_t is the non-radiative interconversion in C. The excited singlet states of β-carotene decay with a lifetime of 10 ps and cannot produce triplet states. Photochemical processes resulting in β-carotene or anthracene radicals are negligible. This is shown below from the analysis of the kinetic traces. The absence of radicals can briefly be explained by the very fast photophysical quenching of the anthracene triplet states by β-carotene and by the low energy of the β-carotene T_1 state. Such a small triplet energy is probably not sufficient for charge separation in the β-carotene–anthracene system.

The well-known intense absorption band [14,20] from the β-carotene triplet state was detected. This absorption exhibited a maximum at about 525 nm in toluene and about 515 nm in hexane. An additional absorption in the near-IR range from 0.8 to 1.3 μm was also observed (Fig. 2). The maximum optical density of the near-IR absorption was about 300 times lower than the optical density of the triplet–triplet absorption at 525 nm. The near-IR transient spectrum was significantly different from the diffuse β-carotene radical absorption spectrum [22]. It was similar to the long-wavelength triplet absorption spectrum of retinoids [18], but the corresponding absorption bands of these shorter chain polyenes were detected at shorter wavelengths (860–940 nm) and exhibited a slightly higher intensity [18]. The vibronic structure (Fig. 2(b)) of the broad near-IR absorption is similar to the usual structure in polyene absorption spectra, and very similar to the β-carotene absorption spectrum (Fig. 1). The 0–0 maximum for the β-carotene triplet absorption was approximately 8100 cm^{-1}.

The kinetic traces measured for the near-IR and visible bands were the same. The shape of the transient absorption kinetics is shown in Fig. 3. In this plot, we can see the decay as well as the increase in absorption. The absorption intensity $I(t)$ could be fitted by a bi-exponential law with a positive parameter A

$$I(t) = A[\exp(-t/\tau_1) - \exp(-t/\tau_2)] \quad (2)$$

The τ_1 and τ_2 durations were calculated by a least- squares fit according to Eq. (2). The rise time τ_1 was usually in the 0.8–2 μs range and was dependent on the β-carotene concentration. The decay time τ_2, calculated together with the rise time, was insensitive to changes in the β-carotene and anthracene concentrations. The lifetime τ_2 of the β-carotene T_1 state, 5.4 ± 0.2 μs in toluene, is similar to the known lifetime of the β-carotene triplet state [20]. The visible and near- IR triplet absorption vanished rapidly on flushing the experimental cell with air. These facts confirm the triplet origin of the near-IR transient absorption. Therefore the rise time is provided by energy transfer from anthracene triplet states (process k_d in Eq. (1)) and decay is a result of the $T_1 \rightarrow S_0$ conversion in β-carotene (k_t in Eq. (1)). Components of longer duration, ascribed to radicals, were not detected at $\lambda > 1000$ nm and were negligible in the 700–800 nm range.

Thus for β-carotene, as for retinoids [18], the relatively low-lying triplet state T_k can be detected and the $T_1 \rightarrow T_k$

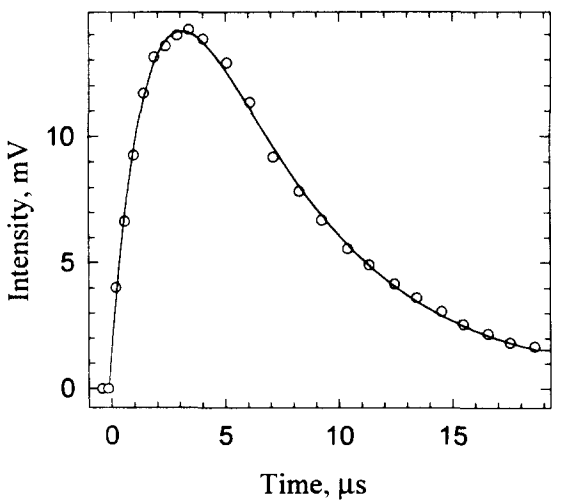

Fig. 3. Triplet absorption kinetics for β-carotene in toluene. Detection was at the intense maximum at 525 nm. The full line follows: $I(t) = -40.7 \exp(-t/2.0) + 39.4 \exp(-t/5.3)$.

transition is weak. The energy of the T_k triplet state can be calculated as the sum of the T_1 state energy and the $T_1 \rightarrow T_k$ transition energy. Since the T_1 energy is estimated to be approximately 7600 cm^{-1}, the T_k level energy is approximately 15 700 cm^{-1}. This value is interesting, because it is close to the S_1 energy supposed for β-carotene (Fig. 4). The corresponding T_k state level in retinoids is also close to the S_1 level of retinoids [18].

At a wavelength of about 700 nm, a third absorption band, overlapping with the intense band in the visible range, was observed (Fig. 2). Since it could not be correctly extracted from the total spectrum, we will not consider it in detail. It is quite possible that this band originates from a β-carotene triplet state, but the influence of anthracene and β-carotene radicals cannot be eliminated completely.

Emission measurements for solutions of β-carotene with anthracene and β-carotene with porphyrins do not show the detectable β-carotene phosphorescence. A signal-to-noise ratio for singlet oxygen luminescence from similar non-deaerated anthracene solutions in toluene was at least 300. Since the quantum yield of singlet oxygen phosphorescence in toluene is approximately 10^{-4} and the lifetime is about 3×10^{-5} s [23], the upper limit for the quantum yield of β-carotene phosphorescence was estimated to be 5×10^{-7}. The apo-carotenal phosphorescence was also absent. Emissions due to β-carotene S_1 fluorescence [24] and impurities in apo-carotenal were detected at wavelengths of less than 1200 nm.

The low-lying T_k state may be important in triplet–triplet energy transfer from organic molecules with relatively high-lying triplet states. For instance, since the anthracene T_1 energy is close to 15 000 cm^{-1}, triplet energy transfer from anthracene may probably occur to the β-carotene T_k state.

The present-day estimates of the β-carotene T_1 energy may exhibit large deviations from the correct value, and it is thus not possible to calculate the T_k energy exactly. Therefore both sequences of the T_k and S_1 levels may be considered to interpret the low interconversion yield and the absence of polyene phosphorescence.

(1) T_k energy $> S_1$ energy. The T_{k-1} state may have an energy which is much lower than the S_1 energy. As a result of a large S–T energy gap, the interconversion rate will be slow. For retinoids, no absorption from T_1 could be detected in the range 1.00–1.65 μm. For β-carotene, the corresponding range is narrower, 1.30–1.65 μm. If some triplet states are present in these energy margins, absorption to them from T_1 must be forbidden. It is probable that this energy gap is empty of triplet states. For retinoids, this means an absence of triplet states below the S_1 state inside a range which is broader than 5000 cm^{-1}. This distance is sufficient to prevent S–T energy resonance and to decrease the S–T interconversion rates in the molecule. Since the "invisible" range of lower energies (below 6000 cm^{-1}) is rather small, it is also possible that $k = 2$, and T_k is the lowest excited triplet state above T_1. If $E(T_k) > E(S_1)$, the low probability of polyene $S_1 \rightarrow T_1$ conversion can simply be explained by the large energy gap between the S_1 state and the T_{k-1} triplet state, which is the closest state below the S_1 state.

(2) T_k energy $< S_1$ energy or T_k energy $\approx S_1$ energy. This case is more complicated. If a triplet level lies closely below the lowest singlet excited level and the interconversion process is almost absent, it must be provided by this triplet state feature. A possible explanation of the small interconversion is related to the T_k state symmetry. For example, the T_k state can have u symmetry. For this reason, spin–orbit interaction cannot mix the triplet state with the $S_1(A_g)$ state and, correspondingly, the interconversion rate should be slow. In this case, we may also suggest that the T_1 state is a g state, since we can detect the optical transition between T_1 and T_k. Because the $T_1 \rightarrow T_k$ absorption is weak, the last suggestion is not strongly confirmed. Such a weak transition can also be produced by transition between states with the same symmetry as a result of distortions of molecular symmetry. However, if the T_1 state is a g state, the phosphorescence is additionally forbidden by a symmetry selection rule (this means that spin–orbit interaction does not mix the T_1 state with all singlet excited u states of the molecule). Therefore this model can explain the absence of polyene phosphorescence. Our observations indicate that the quantum yield of β-carotene phosphorescence is less than 5×10^{-6} in toluene at room temperature and, correspondingly, the $T_1 \rightarrow S_0$ radiative lifetime is longer than 10 s.

Both of these considerations are not perfect and must be checked by additional experimental and theoretical investigations. Unfortunately, it is very difficult to compute the triplet state properties, and reliable theoretical calculations of the triplet states for carotenoids and other polyenes are still lacking. Determination of the S_1 level for β-carotene has been more successful in recent years. At present, the β-carotene S_1 energy is estimated to be in the 14 000–14 500 cm^{-1}

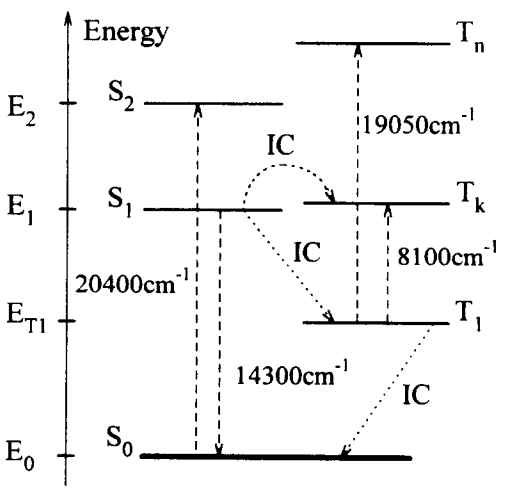

Fig. 4. Singlet and triplet level diagram for β-carotene. The labels IC indicate intersystem crossing. The frequencies of the detected singlet–singlet and triplet–triplet transitions are shown.

range. Recent direct fluorescence observation has resulted in an energy of $14\,400 \pm 120\ cm^{-1}$ for β-carotene in toluene [24]. This value is lower than the above estimation for the T_k energy and the model outlined in (1) is thus preferable (Fig. 4). In spite of this, the conclusive answer is still unknown because the T_1 level cannot be regarded as having been finally determined. The next step in understanding the triplet state sequence of carotenoids and retinoids should be a more exact determination of the T_1 energy. New semi-empirical calculations may also give important information on the wavefunctions and symmetry of the lowest triplet states.

4. Conclusions

In this investigation of the β-carotene triplet state absorption over a wide spectral range, a new band in the near-IR region has been found. This $T_1 \rightarrow T_k$ absorption has a low intensity and exhibits a vibronic structure similar to the vibronic structure of β-carotene absorption. The excited triplet state T_k is close to the S_1 state energy. It can thus influence the carotenoid $S \rightarrow T$ interconversion. The T_k level is probably higher than the S_1 level, and the low carotenoid interconversion is a result of a large S_1–T_{k-1} energy gap. Otherwise, T_k is not mixed with S_1 due to certain features of T_k, e.g. T_k may have a u-like wavefunction.

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