Sub-μ-second Time-Resolved Absorption Spectroscopy of a Polar Carotenoid Analogue, 2-(All-*trans*-retinylidene)indan-1,3-dione; Formation of the Dication by Direct Triplet-Excited Sensitization

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Sub-μ-second time-resolved difference absorption spectra of a polar carotenoid analogue, 2-(all-*trans*-retinylidene)indan-1,3-dione (hereafter, we will call RetInd), were recorded in tetrahydrofuran at room temperature upon anthracene-sensitized triplet excitation. In addition to the typical $T_n \leftarrow T_1$ absorption spectrum of anthracene followed by that of RetInd, a novel transient species, which peaked at 670 nm, was detected. The lifetime and the population of the 670 nm species was not affected by the presence of oxygen but was quenched by the cation scavenger, triethylamine. Therefore, we have identified this species as a "cation". The transient 670 nm species was not generated by direct photoexcitation of RetInd in the absence of a triplet sensitizer. Therefore, this species was not generated via the T_1 species of RetInd but rather via an "invisible state" of RetInd, which is generated by direct energy or electron transfer from T_1 anthracene. This proposed pathway was confirmed by a singular-value decomposition followed by a global fitting analysis. The "cation" of RetInd shows vibrational structure in its absorption spectrum, and its lifetime was determined to be 15 μs. Chemical oxidation of RetInd in 2,2,2-trifluoroethanol (dichloromethane) produced a broad absorption band around 880 (1013) nm, which could be transformed into a shoulder around 640 (675) nm upon addition of increasing amounts of the oxidant, $FeCl_3$. The former absorption band can be assigned to a radical cation, while the latter to a dication. Because of the spectral similarity, the 670 nm species can be assigned to the dication, and the "invisible state" is ascribed to the radical cation of RetInd. This is the first direct evidence for the production of a dication of a biological polyene moiety generated in non-halogenated solution following anthracene-sensitized excitation.

Introduction

Carotenoids are abundant natural pigments. They are particularly important in photosynthesis where they are mainly components of the light-harvesting and reaction center pigment−protein complexes.[1] In these complexes, carotenoids have been shown to have two major functions. They act as accessory light-harvesting pigments, absorbing light between 450 and 550 nm and transferring that absorbed energy to the chlorophylls, thereby making it available to drive photosynthesis. Carotenoids are also essential photoprotective agents. If chlorophyll is illuminated in the presence of oxygen, triplet-excited chlorophyll can react with molecular oxygen to sensitize the formation of the very damaging singlet oxygen. Carotenoids prevent these harmful reactions by quenching the triplet-excited chlorophyll before it has time to react with molecular oxygen. The light-harvesting function of carotenoids reflects singlet−singlet energy transfer, whereas the photoprotective role reflects triplet−triplet energy transfer. Recently, another interesting reaction of carotenoids in photosynthesis has been described for carotenoids bound to photosystem II. It has been discovered that carotenoids in photosystem II can participate in secondary electron transfer involving the formation of carotenoid cations.[2,3] Although there have been many studies on these radicals,[4,5] there is rather little experimental data on biological electron transfer reactions involving carotenoids. Recently, the generation of cation radicals of carotenoids via the singlet excited state has been reported in the natural light-harvesting complexes[6] as well as in an artificial photosynthetic system.[7] Interestingly, carotenoids are also attracting considerable attention in the field of condensed matter photophysics.[8] They show sufficiently large third-order optical nonlinearity to be possible candidates for use in a variety of optoelectrical devices.[9]

In previous investigations,[9−11] we synthesized a novel carotenoid derivative, 2-(all-*trans*-retinylidene)indan-1,3-dione (RetInd), which has an electron-withdrawing group attached to the all-*trans*-retinal, in order to investigate its structural and electronic properties. Figure 1 compares the electronic absorption spectrum of RetInd (**1**) with those of retinal (**2**) and β-carotene (**3**). The main absorption band of RetInd shows a remarkable bathochromic shift in comparison with those of retinal and β-carotene. This has been explained in terms of an intramolecular charge transfer state based on a comparison of the electron distribution in the HOMO and the LUMO calculated using the MOPAC software.[11] The remarkable bathochromic shift of the absorption band could also contribute to the large

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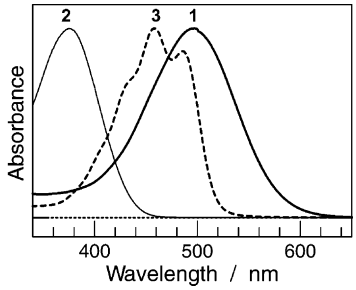

Figure 1. Electronic absorption spectra measured in THF of RetInd (**1**), retinal (**2**), and β-carotene (**3**).

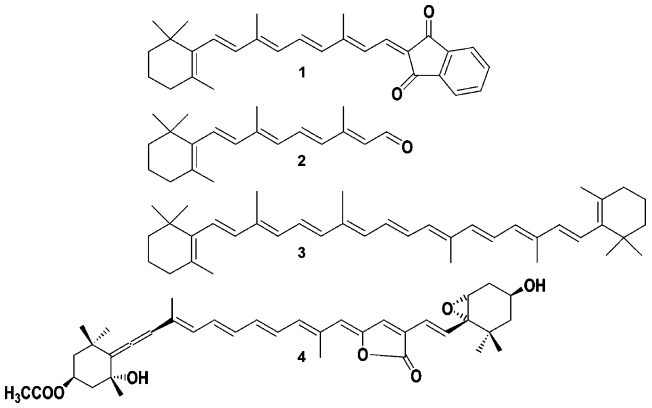

Figure 2. The chemical structures of RetInd (**1**), retinal (**2**), β-carotene (**3**), and peridinin (**4**).

third-order nonlinearity of the RetInd.[9,10] The conjugation of RetInd extended onto both the carbonyls but not onto the indan ring. It has six carbon−carbon conjugated double bonds attached onto a branched double bond.[11] This conjugation pattern is very similar to that of peridinin (**4**) (see Figure 2), which is a well-studied light-harvesting carotenoid found bound to the PCP protein.[12,13] RetInd is a simple molecule, relatively easy to synthesize, and the structure has been determined by X-ray crystallography.[11] As peridinin has a complicated structure, RetInd can be a simplified model to make access to their electronic properties.

In this study, we set out to answer the following question: How are the excited-state properties of RetInd perturbed by the electron withdrawing group? The answer was obtained by applying sub-μ-second time-resolved absorption spectroscopy using anthracene-sensitized triplet excitation.

Experimental Section

Sample Preparation. RetInd was synthesized and purified as described previously.[11] The purity of RetInd was confirmed by HPLC analysis (Column, LiChrosorb Si-60 (5 μm), 4 i.d. × 300 mm; eluent, 0.5% acetone in *n*-hexane) as well as by electronic absorption spectroscopy to be 100% and 99%, respectively, before and after the time-resolved absorption measurements.

Sub-μ-second Time-Resolved Absorption Spectroscopy. RetInd was dissolved in tetrahydrofuran (THF, Kishida Chemical, Japan) at a concentration of 7.5×10^{-5} M together with the triplet sensitizer, anthracene (Wako Chemical, Japan), at a concentration of 7.5×10^{-4} M. Triethylamine (TEA, Wako Chemical, Japan) was also added at a concentration of 5.25×10^{-3} M as a cation scavenger[14] when necessary. Deoxygenation was performed by bubbling with N_2 gas (G1 grade, 99.9999%, Tomoe Shokai, Japan) 30 min prior to the experiments unless otherwise noted. A white light continuum from an Ar−H_2 lamp (Nano Pulse Light NP 1-A, fwhm 75 ns, available wavelength 300−720 nm, operated at 10 Hz repetition, Sugawara, Japan) irradiated a quartz flow cell (optical path length, 2 mm) after passing through a glass filter (L39, HOYA). Therefore, the white continuum available in this setup is restricted between 400 and 720 nm. And then, the light transmitted through sample cell was passed through a single monochromator (Spectra-Pro275, Acton research corporation, U.S.A.) and detected by a CCD detector (LN/CCD-1340/400-EB1, Roper Scientific, Japan). The excitation pulse from a Nd:YAG laser (Surelite I-20, fwhm 10 ns, 355 nm, 1 mJ/pulse, operated at 10 Hz repetition, HOYA-Continuum, U.S.A.) irradiated the flow cell from the angle of ~30° with respect to the probe pulse. The delay time of the white continuum with reference to the excitation pulse was controlled electronically by using a digital delay pulse generator (DG535, Stanford Research, U.S.A.).

Chemical Oxidation with $FeCl_3$.[15,16] (a) RetInd was dissolved in 2,2,2-trifluoroethanol (TFE, Kishida Chemical, Japan) at a concentration of 1.25×10^{-2} M in a quartz cuvette ($1 \times 1 \times 4$ cm^3). Electronic absorption spectra were recorded spectrophotometrically (V-530, Jasco, Japan) while titrating in aliquots of 0.1% (w/v) $FeCl_3$ (Kishida Chemical, Japan).[15] (b) RetInd (6.6 μM) was dissolved in dichloromethane (Kishida Chemical, Japan) with different concentrations of $FeCl_3$. Electronic absorption spectra were recorded immediately after mixing with either 2.8 or 11.2 μM $FeCl_3$ in a quartz cuvette with a 10 mm optical path length.[16]

The large absorptions from $FeCl_3$ and neutral RetInd were fitted by using two and one Gaussian curves, respectively, and then, they were subtracted from raw spectra to reveal the absorptions due to the cation/dication.

All the measurements were performed at room temperature under darkness.

Singular-Value Decomposition (SVD) Followed by Global Fitting Analysis. SVD followed by global fitting analysis was applied to a set of the time-resolved spectra shown in Figure 3b, from 500 to 715 nm (893 points) and from −3 to 90 μs (98 points) as reported previously.[17,18] This wavelength region was selected to minimize the effect of the large signal from T_1 anthracene. The decay profile of T_1 anthracene was extracted from the raw spectra by removal of the changes of the T_1 RetInd and then fitted with a single exponential curve for use as the excitation curve for the global fitting analysis.

Results and Discussion

Raw Time-Resolved Spectra. Figure 3 shows the time-resolved absorption spectra of RetInd in THF induced by anthracene-sensitized excitation (a) with and (b) without N_2 bubbling. As shown in Figure 3a, under N_2, a sharp $T_n \leftarrow T_1$ absorption of anthracene peaking at 430 nm rises and then decays. This is then followed by the rise of the broad $T_n \leftarrow T_1$ absorption of RetInd peaking around 580 nm. Figure 3b shows the decrease in the populations at 430 and 580 nm in the presence of oxygen and then confirms that both species are triplets. Homologues of β-carotene with the number of conjugated double bonds ranging from 7 to 19 show a linear relationship between their energies of $S_2 \leftarrow S_0$ and $T_n \leftarrow T_1$ transitions.[19] The transition energies of the $S_2 \leftarrow S_0$ and $T_n \leftarrow T_1$ of RetInd fit onto this trend diagram for the carotenoid homologues. This trend also indicates that the elongation of the effective conjugation length of RetInd due to the presence of an electron withdrawing group attached to the polyene backbone affects not only the singlet excited state but also the triplet excited state. In other words, RetInd behaves as a typical polyene with respect to its singlet and triplet excited states.

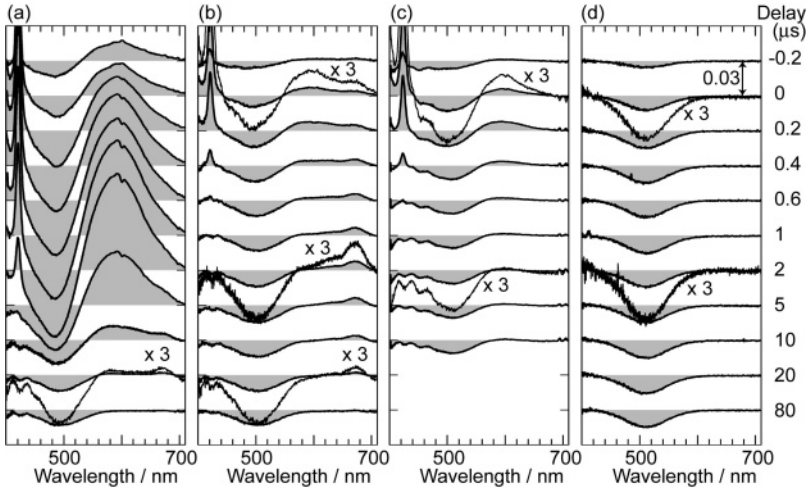

Figure 3. Selected time-resolved absorption spectra of RetInd in THF (a) with and (b), (c) without bubbling with N_2 by sensitized excitation as well as (d) by direct excitation (without sensitizer) without N_2 bubbling. The spectra in (c) were recorded in the presence of the cation scavenger, TEA, although the other conditions were the same as in (b) (see text). The labeled lines show a 3× expansion of original absorbance scale.

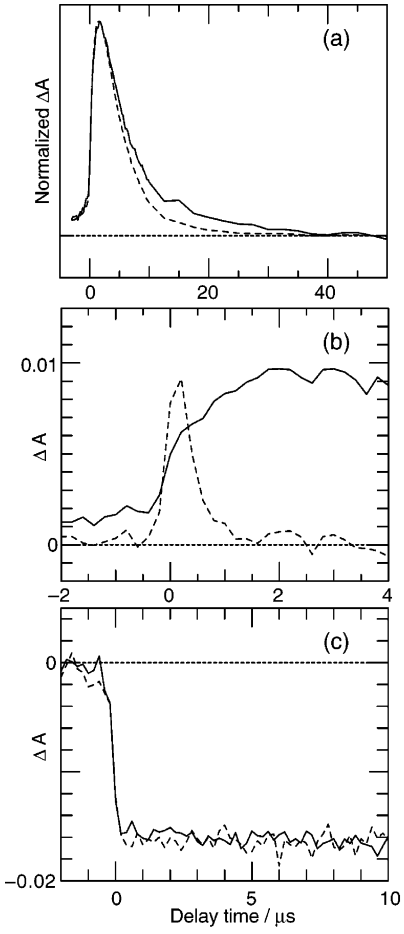

Figure 4. (a) Normalized time dependence of time-resolved absorbance changes corresponding to Figure 3a plotted at 580 nm (broken line) and 670 nm (solid line). (b) Comparison of the time dependence of time-resolved absorbance changes corresponding to Figure 3b plotted at the same wavelengths. (c) Comparison of the time dependences of time-resolved absorbance changes produced by direct excitation with (broken line, raw data not shown) and without (solid line, corresponding to Figure 3d) N_2 bubbling plotted at 510 nm.

The difference between the time profiles at 580 (broken line) and 670 nm (solid line) in Figure 4a indicates the presence of an additional species with an ~15 μs lifetime superimposed at 670 nm. In the absence of bubbling with N_2 (Figure 3b), the lifetime as well as the population of both the triplet species decreases because of deactivation by oxygen (vide supra), and under this condition, the spectral behavior of the 670 nm species is more clearly seen (see 3× expansion at 2 μs after excitation). The time-resolved spectra at 20 μs after excitation, with and without bubbling with N_2 (Figure 3a,b, respectively), are essentially the same, indicating that the generation and the lifetime of the 670 nm species are not affected by the presence of oxygen. The fact that the intensity of the 670 nm species is not affected by the intensity of the T_1 RetInd indicates that the 670 nm species is not generated from T_1 RetInd.

Figure 3c shows the same condition as Figure 3b except for the addition of cation scavenger, TEA. A comparison of the 0 μs spectra in Figure 3b and in Figure 3c shows that the 670 nm species disappears completely upon addition of the cation scavenger, TEA. The $T_n \leftarrow T_1$ absorptions due to both RetInd and anthracene remain unaffected in the presence of TEA. The reaction of the radical cation with oxygen is too slow to change its short lifetime.[4] The possibilities that the species is due to the cation of an impurity in the solvent or of anthracene are ruled out by the same sets of experiments with and without N_2 bubbling using only anthracene (data not shown) or only RetInd in THF (See Figure 3d). Under these conditions, the 670 nm species does not form. Therefore, the 670 nm species can be safely assigned as a kind of "cation" of RetInd.

Figure 4b shows that the T_1 RetInd (580 nm, broken line) decays in 1.5−2.0 μs after excitation, whereas the "cation" (670 nm, solid line) still shows a gradual increase over this time regime. As shown in Figure 3a and b, the population of the "cation" is not affected by that of T_1 RetInd. This kinetic mismatch and the fact that the extent of the "cation" formed is independent of T_1 RetInd indicate that the "cation" is not generated from T_1 RetInd. The comparison of the 2.0 μs spectra in Figure 3b and d clearly demonstrates that the "cation" is not generated by direct photoexcitation of RetInd in the absence of anthracene. Since the "cation" cannot be directly generated from T_1 RetInd, it is necessary to introduce an intermediate state, which has no absorption in the detected wavelength region and which is generated from T_1 anthracene (hereafter, we will call this state an "invisible state").

As shown in the spectra at 80 μs after excitation in Figure 3, the bleaching of the ground state of neutral RetInd around 510 nm remains after all the detected transient species relax. The temporal profiles with and without N_2 bubbling at 510 nm (broken and solid lines in Figure 4c, respectively), upon direct

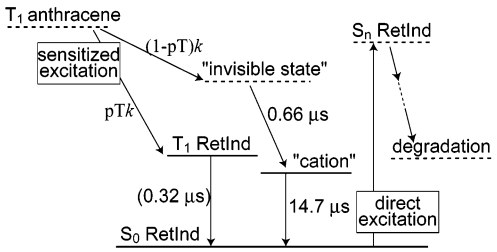

Figure 5. The three-component model used for the global fitting: Solid (broken) lines show the states directly (indirectly) used in the global fitting analysis.

excitation of RetInd, demonstrate that a component of this bleaching signal is generated immediately after excitation and is not affected by the presence of oxygen. This irreversible bleaching represents a degradation pathway which is generated by direct excitation of the RetInd, in other words, is generated via an optically allowed singlet excited state. Upon triplet-sensitized excitation at 355 nm, the direct excitation of RetInd also occurs. So, this degradation pathway is always present in Figure 3.

From the raw spectra, we can discern the following kinetic features: (1) The T_1 RetInd is generated by energy transfer from T_1 anthracene, (2) the "cation" state is generated via an "invisible state", which is generated by direct energy (or electron) transfer from T_1 anthracene, and (3) independently, there is a degradation pathway via the singlet excited state of RetInd, which probably has a short lifetime and is therefore beyond the temporal resolution of our instrument.

SVD Followed by a Global Fitting Analysis. To evaluate the kinetics, singular value decomposition (SVD) was applied to a set of spectra shown in Figure 3b. SVD is a mathematical method which allows a large data matrix to be expressed as a product of the matrices of basis spectra, singular values, and time courses. The number of major components is selected by consideration of the size of singular values, the physical significance of the shapes of basis spectra, and the time courses. In this analysis, we limited the wavelength region from 500 to 715 nm in order to extract the small changes due to the "cation" without interference from the large signal from the T_1 anthracene. The temporal profile of T_1 anthracene in the set of time-resolved spectra was extracted as described in the Experimental Section, and then, this was used as the response function of the excitation in the global fitting analysis. We could clearly select three components from the SVD analysis.

Then, we constructed a minimal model with these three detectable components, the triplet state, the "cation" state, and the bleaching (both degradation and "invisible state") of RetInd as shown in Figure 5 based on the features in the raw spectra summarized above

$$T_1 \text{ anthracene} + S_0 \text{ RetInd} \rightarrow S_0 \text{ anthracene} + T_1 \text{ RetInd} \quad (1)$$

$$T_1 \text{ anthracene} + S_0 \text{ RetInd} \rightarrow \text{"invisible state" RetInd} \quad (2)$$

$$\text{"invisible state" RetInd} \rightarrow \text{"cation" RetInd} \quad (3)$$

$$\text{"cation" RetInd} \rightarrow S_0 \text{ RetInd} \quad (4)$$

$$S_0 \text{ RetInd} + h\nu \text{ (355 nm)} \rightarrow S_n \text{ RetInd} \rightarrow \text{degradation} \quad (5)$$

The partition ratio of T_1 anthracene (pT) cannot be determined from these time-resolved absorption experiments. So, we fixed this value to be 0.5. As shown in Figure 4c, degradation happens

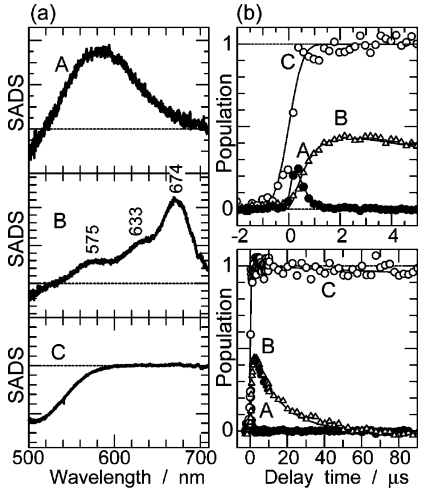

Figure 6. Results of singular value decomposition (SVD) followed by a global fitting using the three-component model shown in Figure 5: (a) The species-associated difference spectra (SADS) and time-dependent population change of the T_1 and "cation" states and the bleaching (degradation) component of RetInd, indicated by A (closed circles), B (open triangles), and C (open circles), respectively. The upper panel of (b) shows the initial stage of the temporal profile, while the lower panel shows the complete temporal profile.

instantaneously after excitation. The generation of the "invisible state" depends on the decay of T_1 anthracene, which also follows the time cause of the laser pulse (see the decay of 430 nm peak in Figure 3b). Therefore, these two bleaching signals could not be separated in this analysis.

After solving the rate equations for eqs 1−5, global fitting using this model was applied to the time courses of three components obtained from SVD analysis above. The resultant lifetimes are also indicated in Figure 5. Note that the T_1 lifetime of RetInd, from this analysis, includes the effect of quenching by oxygen.

Figure 6 shows the results of the global fitting: (a) the species-associated difference spectra (SADS) and (b) the time dependences of the populations of the T_1 RetInd (A, closed circles) and its "cation" (B, open triangles) states, as well as the bleaching (C, open circles). SADS is a difference spectrum between the excited state and the ground state. SADS of the excited state should therefore contain ground-state bleaching. SADS of T_1 RetInd clearly shows a typical triplet-state absorption of a carotenoid with ground-state bleaching (vide supra).[19] SADS of the "cation" shows distinct vibrational structures at 674, 633, and 575 nm. The energy gaps between these peaks are 1000 and 1600 cm^{-1}, respectively.

Chemical Oxidation Experiments. To confirm the assignment of the "cation", we oxidized RetInd with $FeCl_3$. Figure 7 shows (a) the electronic absorption spectra of RetInd in TFE in the presence of small and large additions of $FeCl_3$ (solid and broken lines, respectively) and (b) the electronic absorption spectra of RetInd (6.6 μM) in dichloromethane in the presence of 2.8 μM and 11.2 μM of $FeCl_3$ (solid and broken lines, respectively). Insets show the spectra after subtraction of the large background absorptions from S_0 of RetInd and of $FeCl_3$ (see Experimental Section in detail). When a small amount of $FeCl_3$ was added in TFE, a broad weak peak around 880 nm appeared. After the addition of an excess amount of $FeCl_3$, a shoulder around 640 nm appeared and then disappeared slowly over the next 5 min. In dichloromethane, both the broad peak (1013 nm) and the shoulder (675 nm) were observed immediately after mixing with 2.8 μM $FeCl_3$ (see Figure 7b). In contrast, only the shoulder (now as a single peak) was observed

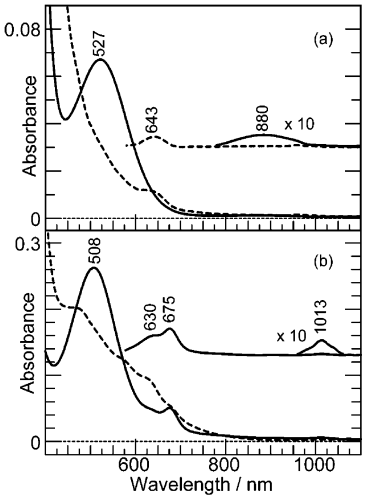

Figure 7. Chemical oxidation of RetInd using $FeCl_3$. (a) Electronic absorption spectra of RetInd in TFE in the presence of small and large amounts of $FeCl_3$ (solid and broken lines, respectively). The inset shows spectra after subtraction and expansion (see text). (b) Electronic absorption spectra of RetInd (6.6 μM) in dichloromethane in the presence of 2.8 and 11.2 μM of $FeCl_3$ (solid and broken lines, respectively). The inset shows the former spectrum (6.6 μM of RetInd with 2.8 μM of $FeCl_3$) after subtraction of the strong absorptions from ground-state RetInd and $FeCl_3$. 10× expansion around 1000 nm is also shown.

after mixing with 11.2 μM $FeCl_3$. It has been shown previously that, during the oxidation of carotenoids with $FeCl_3$, several equilibria occur among the following possible species, Fe_3^+, Fe_2^+, Cl^-, the neutral carotenoid (Car), its radical cation ($Car^{\bullet+}$), and dication (Car^{2+}).[4] When excess amount of $FeCl_3$ is added,

$$FeCl_3 + Car \leftrightarrow Car^{\bullet+} + FeCl_2 + Cl^- \quad (6)$$

$$Car^{\bullet+} + FeCl_3 \leftrightarrow Car^{2+} + FeCl_2 + Cl^- \quad (7)$$

the equilibrium of eq 7 moves to the right. We can therefore assign the broad weak peak (either at 880 nm in TFE or at 1013 nm in dichloromethane) to the radical cation ($RetInd^{\bullet+}$) and the shoulder (at 640 nm in TFE or at 675 nm in dichloromethane) to the dication ($RetInd^{2+}$). The large shift of transition energies ($\sim$1500 cm^{-1}) of $RetInd^{\bullet+}$ in two solvents can be explained by assuming that $RetInd^{\bullet+}$ was stabilized by TFE.[15]

The shoulder or peak of $RetInd^{2+}$ generated in dichloromethane shows some structure. The inset in Figure 7b shows the $RetInd^{2+}$ spectrum after subtraction of the background. The energy difference between two peaks is $\sim$1000 cm^{-1}. This spectral shape is very similar to the SADS of the "cation" extracted from the time-resolved absorption spectra shown in Figure 6aB. On the basis of the similarities of the transition energies as well as spectral shapes of the shoulder/peak and SADS of the "cation", we can assign the "cation" detected by the time-resolved absorption experiments to the $RetInd^{2+}$. If this is the case, then the "invisible state" can be tentatively assigned to the $RetInd^{\bullet+}$, which has an absorption maximum at 880 or 1013 nm that could not be accessed in the present time-resolved studies. Further time-resolved absorption experiments detecting in the near-infrared region will be necessary to test this hypothesis.

Radical cations of carotenoids have two main absorption transitions, i.e., $D_2 \leftarrow D_0$ and $D_1 \leftarrow D_0$ transitions.[4] These two transitions from both the radical cation as well as the dication (the notation of $S_1' \leftarrow S_0'$ were used for dication in ref 16) have been reported for β-carotene analogues having ten conjugated double bonds together with an electron-withdrawing substituent (**5**, 7′-cyano-7′-ethoxycarbonyl-7′-apo-β-carotene, a "polar" carotenoid) and having the same number of conjugated double bonds with an electron-donating substituent (**6**, 7′,7′-dimethyl-7′-apo-β-carotene, "nonpolar" carotenoid). This latter study also reported these values for both canthaxanthin (**7**) and β-carotene (**3**).[16] The values obtained for the carotenoids **5**, **6**, **7**, and **3** were as follows: $D_2 \leftarrow D_0$, 890, 934, 887, and 970 nm; $S_1' \leftarrow S_0'$, 695, 758, 714, and 817 nm, respectively. The carotenoid **7** can be characterized as a "polar" carotenoid with two carbonyl groups at both ends. In comparison, the carotenoid **3** is a "nonpolar" carotenoid. There seems to be a shift to higher energy for the $D_2 \leftarrow D_0$ transition as well as the $S_1' \leftarrow S_0'$ transition in "polar" carotenoids (**5** and **7**) when compared to nonpolar carotenoids (**6** and **3**) with the same number of conjugated double bonds. Both the $D_2 \leftarrow D_0$ and $S_1' \leftarrow S_0'$ transition energies of RetInd are very similar to those of the carotenoid **5**. Since RetInd has the character of a "polar" carotenoid, the $RetInd^{\bullet+}$ peaks around 880 and 1013 nm can be assigned to the lower-energy transition, the $D_2 \leftarrow D_0$ transition.

Vibrational Structure of the Dication Spectrum. As we mentioned above, SADS of the dication shows vibrational modes of 1000 and 1600 cm^{-1}. The peak interval corresponds to the vibrational mode in the Franck−Condon state, which is the excited state conserving the nuclear conformation of the ground state. The electronic structures of ground-state carotenoid cations have been studied precisely by NMR spectroscopy.[20−22] In the case of the dication, the electron density is localized in both ends, and the central parts of the conjugated double bonds still show the characteristics of the alternative hydrocarbon chain.[20−22] The vibrational structure is not clear in the ground-state absorption spectrum of neutral RetInd. This can be explained by applying inhomogeneous broadening caused by fluctuations of the β-ionone ring.[23,24] The dication spectrum with clear structure indicates that the conformation of β-ionone is stabilized or that the fluctuations are canceled out by other effects.

Possible Mechanism of Generation of the Dication in Time-Resolved Absorption Spectra. The "cation" was assigned to the dication of RetInd. If the "invisible state" is the $RetInd^{\bullet+}$, anthracene should have accepted an electron to generate an anion radical of anthracene ($anthracene^{\bullet-}$). $Anthracene^{\bullet-}$ has a broad absorption around 330 nm;[25,26] this was outside our detection limits and therefore could not be measured. Since it has been shown that the "invisible state" is a precursor of the dication, it is simplest to assume that this state is a monocation radical. So, a plausible model to explain the kinetics of these species can be proposed as follows:

$$T_1\ anthracene + S_0\ RetInd \rightarrow (anthracene^{\bullet-}) + (RetInd^{\bullet+}) \quad (2')$$

$$(RetInd^{\bullet+}) \rightarrow RetInd^{2+} + e^- \quad (3')$$

$$RetInd^{2+} + 2e^- \rightarrow S_0\ RetInd \quad (4')$$

Equation 2′ represents a diffusion-limited second-order reaction and, consequently, depends on concentrations of the donor and the acceptor. Therefore, this could be tested by concentration dependence experiments. These were not possible in the present study due to S/N problems.

Conclusions

We have identified, for the first time, a cationic state of a polar carotenoid analogue (RetInd) peaking at 670 nm in non-

halogenated solvent, THF, by the use of sub-μ-second time-resolved absorption spectroscopy with triplet-sensitized excitation. The absorption spectrum and population profile of the "cation" have been extracted from the set of time-resolved absorption spectra by the use of the SVD followed by a global fitting analysis. The absorption spectrum of the "cation" shows vibrational structure. It is generated via an "invisible state" produced by energy transfer from T_1 anthracene and then decays in 15 μs. Its population was not affected by the presence of oxygen, but was seriously affected by the presence of a cation scavenger. Oxidation of RetInd by addition of $FeCl_3$ in 2,2,2-trifluoroethanol (dichloromethane) produced a broad absorption band around 880 (1013) nm which then decayed into a state giving a shoulder around 640 (675) nm. The former can be assigned to the radical cation of RetInd and the latter to the dication. Therefore, on the basis of both spectral and energetic similarities, the "cation" detected in time-resolved absorption spectroscopy can be assigned to the dication of RetInd.

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References and Notes

(1) Frank, H. A.; Cogdell, R. J. In *Carotenoids in Photosynthesis*; Young, A., Britton, G., Eds.; Chapman & Hall: London, 1993; p 253.

(2) Hanley, J.; Deligiannakis, Y.; Pascal, A.; Faller, P.; Rutherford, A. W. *Biochemistry* **1999**, *38*, 8189.

(3) Frank, H. A.; Brudvig, G. W. *Biochemistry* **2004**, *43*, 8607.

(4) Kispert, L. D.; Konovalova, T.; Gao, Y. *Arch. Biochem. Biophys.* **2004**, *430*, 49.

(5) Polivka, T.; Sundstroem, V. *Chem. Rev.* **2004**, *104*, 2021.

(6) Polivka, T.; Pullerits, T.; Frank, H. A.; Cogdell, R. J.; Sundstroem, V. *J. Phys. Chem. B* **2004**, *108*, 15398.

(7) Kodis, G.; Herrero, C.; Palacios, R.; Marino-Ochoa, E.; Gould, S.; de la Garza, L.; Van Grondelle, R.; Gust, D.; Moore, T. A.; Moore, A. L.; Kennis, J. T. M. *J. Phys. Chem. B* **2004**, *108*, 414.

(8) Marder, S. R.; Torruellas, W. E.; Blanchard-Desce, M.; Ricci, V.; Stegeman, G. I.; Gilmour, S.; Bredas, J.-L.; Li, J.; Bublitz, G. U.; Boxer, S. G. *Science* **1997**, *276*, 1233.

(9) Hashimoto, H.; Hattori, K.; Yamada, T.; Kobayashi, T. *Int. J. Mod. Phys.* **2001**, *15*, 3773.

(10) Hashimoto, H.; Nakashima, T.; Hattori, K.; Yamada, T.; Mizoguchi, T.; Koyama, Y.; Kobayashi, T. *Pure Appl. Chem.* **1999**, *71*, 2225.

(11) Hashimoto, H.; Hattori, K.; Okada, Y.; Yoda, T.; Matsushima, R. *Jpn. J. Appl. Phys.* **1998**, *37*, 4609.

(12) Zigmantas, D.; Polivka, T.; Hiller, R. G.; Yartsev, A.; Sundstroem, V. *J. Phys. Chem. A* **2001**, *105*, 10296.

(13) Zigmantas, D.; Hiller, R. G.; Yartsev, A.; Sundstroem, V.; Polivka, T. *J. Phys. Chem. B* **2003**, *107*, 5339.

(14) Bobrowski, K.; Das, P. K. *J. Phys. Chem.* **1985**, *89*, 5733.

(15) Krawczyk, S. *Chem. Phys.* **1998**, *230*, 297.

(16) Jeevarajan, J. A.; Wei, C. C.; Jeevarajan, A. S.; Kispert, L. D. *J. Phys. Chem.* **1996**, *100*, 5637.

(17) Fujii, R.; Furuichi, K.; Zhang, J.-P.; Nagae, H.; Hashimoto, H.; Koyama, Y. *J. Phys. Chem. A* **2002**, *106*, 2410.

(18) Zhang, J.-P.; Inaba, T.; Watanabe, Y.; Koyama, Y. *Chem. Phys. Lett.* **2000**, *331*, 154.

(19) Koyama, Y.; Hideki, H. In *Carotenoids in Photosynthesis*; Young, A. J., Britton, G., Eds.; Chapman & Hall: London, 1993; p 327.

(20) Lutnaes, B. F.; Kildahl-Andersen, G.; Krane, J.; Liaaen-Jensen, S. *J. Am. Chem. Soc.* **2004**, *126*, 8981.

(21) Lutnaes, B. F.; Bruas, L.; Kildahl-Andersen, G.; Krane, J.; Liaaen-Jensen, S. *Org. Biomol. Chem.* **2003**, *1*, 4064.

(22) Lutnaes, B. F.; Bruas, L.; Krane, J.; Liaaen-Jensen, S. *Tetrahedron Lett.* **2002**, *43*, 5149.

(23) Krawczyk, S.; Olszowka, D. *Chem. Phys.* **2001**, *265*, 335.

(24) Yanagi, K.; Gardiner, A. T.; Cogdell, R. J.; Hashimoto, H. *Phys. Rev. B* **2004**, *69*, 205103/1.

(25) Martin, M. M.; Plaza, P.; Changenet-Barret, P.; Siemiarczuk, A. *J. Phys. Chem. A* **2002**, *106*, 2351.

(26) Shida, T. In *Electronic absorption spectra of radical ions*; Elsevier: Amsterdam, 1988; Physical Sciences Data; Vol. 34.