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Selective isomerization of retinal upon two-photon excitation

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Abstract

The products of photo-isomerization when excited directly up to the two-photon-allowed $^1A_g^-$ excited state of all-*trans* and five *cis* isomers of retinal were determined. The composition of the isomers at the photo-stationary state was drastically changed as compared to one-photon excitation. The production of the 13-*cis* isomer was selectively increased, and the production of the *dicis* isomers was observed in the case of a direct excitation upon the $3^1A_g^-$ state, although they were hard to be produced by one-photon excitation in *n*-hexane solution. These results clearly evidenced that there exist isomerization pathways via two-photon-allowed excited states.

1. Introduction

Photochemical reactions of π-electron-conjugated molecules in vitro are more complicated than those in vivo. A mixture of various geometrical isomers is usually produced under a photo-stationary state in vitro. On the contrary, the selective *trans* → *cis* or *cis* → *trans* isomerization of retinal, for example, takes place in bacteriorhodopsin (all-*trans* → 13-*cis*) or in rhodopsin (11-*cis* → all-*trans*), respectively [1]. Understanding and mimicking the way in which nature controls these complicated photochemical reactions would make it possible to control the reactions optically and choose a single pathway among them. This may give us an opportunity to establish one of the key technologies to speed up the information transfer. Miyazawa et al. [2,3] recently reported a highly selective photoreaction for one of the most simple π-electron-conjugated molecules, *cis*-stilbene, in organic solvent by the use of a non-resonant two-photon excitation method. One-photon excitation of *cis*-stilbene produced *trans*-stilbene together with dihydrophenanthrene (a cyclized product), while non-resonant two-photon excitation of it resulted in the production of only *trans*-stilbene. No intermolecular cyclization occurred in the latter case. This result suggested that there are two pathways for the initial photoreaction of π-electron-conjugated molecules that have two different singlet excited states. One is from the ^1A_g state, and the other is from the ^1B_u state.

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Photo-isomerization of a biologically important π-electron-conjugated molecule, retinal, by one-photon and/or by triplet-sensitized excitation has been studied extensively to understand the reason for the natural selection of a particular isomer as well as the highly selective *cis–trans* isomerization in biological systems [4,5]. A comparison between the one-photon and triplet-sensitized excitation revealed that the photo-isomerization of retinal isomers in *n*-hexane mainly occurred via the lowest excited triplet state [4]. On the other hand, femtosecond time-resolved UV–VIS absorption spectroscopy of all-*trans* retinal in *n*-hexane by one-photon excitation showed the isomerization pathway via the 2^1A_g excited state (the lowest excited state), which is the precursor of the perpendicularly twisted singlet excited state [6].

In this study, we demonstrate the photo-isomerization of retinal isomers by direct excitation up to a two-photon-allowed excited state and show direct evidence for the isomerization pathway via the $3^1A_g^-$ state of retinal isomers by direct excitation to the $3^1A_g^-$ state by the use of a non-resonant two-photon excitation method.

2. Experimental

All-*trans* retinal was synthesized starting from commercially available retinyl acetate. Retinyl acetate was purchased from BASF (Switzerland) and used after purification with silica–gel column chromatography (20% diethyl ether/*n*-hexane). It was reduced with $LiAlH_4$ and then oxidized using activated MnO_2 to produce retinal. The retinal thus obtained was subjected to silica–gel column chromatography (15% diethyl ether/*n*-hexane), and the all-*trans* component was collected. All-*trans* retinal was further purified by recrystallization from an *n*-hexane solution of this particular component.

Mono-*cis* isomers of retinal were prepared from an isomeric mixture produced by irradiating all-*trans* retinal. All-*trans* retinal in acetonitrile was irradiated with a 250 W tungsten–halogen lamp for 1 h at room temperature under a nitrogen atmosphere. Each isomer was separated from the mixture of isomers by silica–gel column chromatography (15% diethyl ether/*n*-hexane) and further purified using silica gel (LiChrosolb Si-60) HPLC ($4\phi \times 300$ mm column and 7% diethylether/*n*-hexane). *n*-Hexane, diethyl ether, and acetonitrile (special grade) were purchased from Wako Chemical Industry and used as received. The 11,13-*dicis* isomer was separated and purified from the isomeric mixture obtained by the irradiation of all-*trans* retinal in *n*-hexane with nanosecond laser pulses of 490 nm (see below).

For photo-isomerization experiments, the concentration of each isomer in *n*-hexane solution was adjusted to be 2×10^{-4} M. The solutions were irradiated with laser pulses (5 ns pulse duration; 0.8 mJ at 245 nm for one-photon excitation and 8.5 mJ at 490 nm for two-photon excitation). The wavelength dependence for the photo-isomerization of two-photon excitation was determined under a fixed condition of exposure time (30 min) and laser power (70 mW). Laser light between 440 and 820 nm was used for this purpose. All procedures were performed at room temperature and under dim red light. Isomerization without laser irradiation was negligible. The isomeric composition after each irradiation period of time was determined by HPLC, as described above.

3. Results and discussion

A π-electron-conjugated molecule has several singlet excited states belonging to particular symmetry groups. PPP–MRD–CI calculations of model polyenes by Tavan and Schulten [7] predicted that the energy of each singlet excited state for the polyene with six conjugated C=C double bonds was in the order of $3^1A_g^- > {}^1B_u^+ > {}^1B_u^- > 2^1A_g^-$. Recently, a two-photon-allowed singlet excited state, $3^1A_g^-$, was newly identified for some carotenoids by the measurements of resonance-Raman excitation profiles [8] and electro-modulation spectroscopy [9]. There is little information concerning the energy of the optical-forbidden singlet excited states of retinal; hence, it is supposed that the order of each singlet excited state in *n*-hexane is $3^1A_g^-,\ {}^1A_g^+ > {}^1B_u^+ > {}^1B_u^- > 2^1A_g^- > n\pi^*$. Note that we here adopt the notation of the electronic states assuming the C_{2h} symmetry of the polyene backbone.

The specific wavelength of the laser light to be used for the two-photon excitation experiment was selected according to the following protocol. In a previous transient absorption study of the direct excitation upon the $2^1A_g^-$ state using femtosecond laser pulses [10], 800 nm excitation was applied to two-photon excitation. Fig. 1 shows the absorption spectra of isomeric retinal in an *n*-hexane solution at room temperature. None of the isomers shows a one-photon-allowed transition above 440 nm. At first, we tried 800 nm excitation of nanosecond laser pulses with no success. No isomerization was observed after 2 h of exposure. This result may be due to the fact that the absorption cross section of the two-photon-allowed electronic transition ($^1A_g^- \rightarrow 2^1A_g^-$) was small as well as the fact that the nonlinear optical effect induced by the femtosecond laser excitation was 3–4 orders of magnitude larger than that induced by the nanosecond laser excitation. In order to identify the suitable wavelengths for the two-photon excitation using nanosecond laser pulses, we closely inspected the 440–820 nm wavelength region, which is hardly affected by one-photon excitation. Fig. 2 shows the results of the all-*trans* isomer by two-photon excitation. Isomerization was not detected at all above 540 nm excitation; however, 440–540 nm excitations did induce isomerization. There was a peak around 480 nm that showed the selective production of the 13-*cis* isomer. A half wavelength of that may correspond to a transition to the third two-photon-allowed excited state ($3^1A_g^-$) because of its energy. Therefore, in order to fulfill the requirements to satisfy the effective two-photon excitation as well as to avoid the interference of photoisomerization via the $^1B_u^+$ state that is induced by one-photon excitation, we selected a 490 nm excitation for subsequent studies. The yield of the *cis* species was proportional to the 1.9th power of the laser power when using pulses of 490 nm. This result shows that the isomerization by this excitation wavelength is a two-photon reaction.

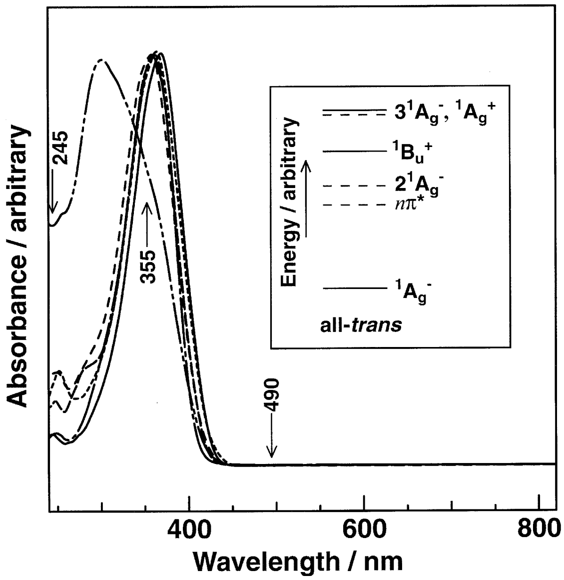

Fig. 1. Absorption spectra of all-*trans* (solid), 13-*cis* (dot), 11-*cis* (short dash), 9-*cis* (dash), 7-*cis* (dot dash), and 11,13-*dicis* (2-dot dash) retinal in *n*-hexane. Arrows show the excitation wavelength for one- and two-photon excitation measurements. Inset shows the order of singlet energy levels of all-*trans* retinal in *n*-hexane. Solid lines are one-photon allowed excited-states and dashed lines are one-photon forbidden.

Two-photon excitation of nanosecond laser pulses showed quite different photo-isomerization characteristics from those of the one-photon excitation and/or the triplet-sensitized excitation [4]. We tried to examine three excitation wavelengths, i.e., 245, 355, and 490 nm. They correspond, respectively, to the *cis*-peak ($^1A_g^+$ state), the region nearby the one-photon-allowed transition to the $^1B_u^+$ state, and a half energy of 245 nm (no absorption coefficient exists at 490 nm). A ground ($^1A_g^-$) to $^1A_g^+$ one-photon transition is supposed to be partially allowed even for the all-*trans* isomer due to the twisting of the polyene backbone. Table 1 shows the photo-stationary state composition of isomers excited at 245, 355, and 490 nm. Excitation of 355 nm resulted in 70% all-*trans* and 27% 13-*cis*, while 245 nm excitation resulted in 71% all-*trans* and 24% 13-*cis*. The compositions of these isomers were almost the same for both excitations. On the basis of the comparison of the photo-stationary state mixture of isomers produced by direct excitation up to the $^1B_u^+$ state (355 nm excitation) and that by triplet-sensitized excitation, Mukai et al. [4] showed that isomerization by one-photon excitation took place via a triplet excited state that was formed by an intersystem crossing from the $^1B_u^+$ state. Therefore, the above result suggests that the isomerization induced by the

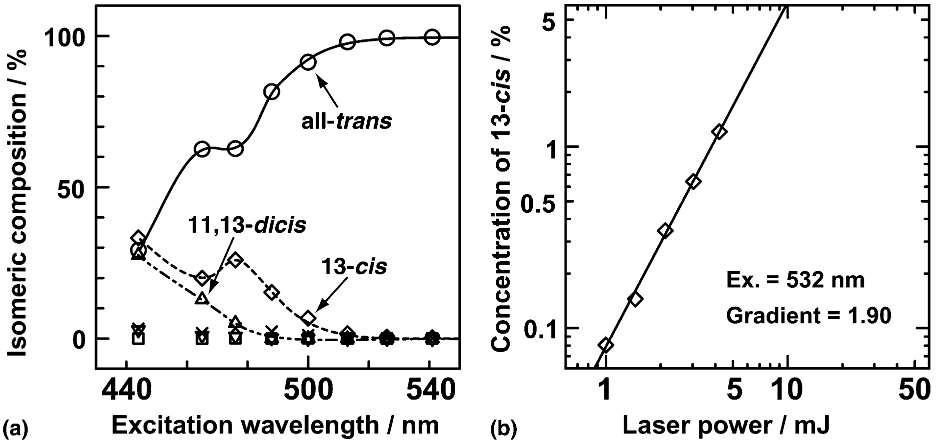

Fig. 2. (a) The excitation-wavelength dependence on the isomeric composition for 30 min irradiation starting from all-*trans* retinal. Each mark shows all-*trans* (○), 13-*cis* (◇), 11-*cis* (▽), 9-*cis* (×), 7-*cis* (□), and 11,13-*dicis* (△). (b) The square power dependence of the isomerization from all-*trans* to 13-*cis* retinal upon exposure of 532 nm nanosecond laser pulses.

Table 1
The average value of isomeric composition starting from each retinal isomer at photo-stationary state excited at 245, 355, and 490 nm with nanosecond laser pulse

Excitation wavelength (nm)	Isomeric composition (%)					
	all-*trans*	13-*cis*	11-*cis*	9-*cis*	7-*cis*	11,13-*dicis*
245	71	24	2	2	0	0
355	70	27	0	2	0	0
490	24	42	9	6	0	18

245 nm excitation should also take place via a triplet excited state that was formed through internal conversion from the $^1A_g^+$ to the $^1B_u^+$ states followed by the intersystem crossing.

Since there is no absorption band of each retinal isomer at 490 nm, this particular wavelength should allow us to excite the two-photon-allowed singlet excited state, i.e., the $3^1A_g^-$ state. Even when any retinal isomers were irradiated, the composition of photo-stationary state isomers upon 245 and 355 nm excitation was almost the same value to each other; the all-*trans* isomer was the main component, and the 13-*cis* isomer was less than half of the all-*trans* isomer (see above). On the contrary, in the case of two-photon excitation at 490 nm, the 13-*cis* isomer was produced as the main component, and the 11,13-*dicis* isomer was also produced, although this particular isomer was hardly detected for 245 and 355 nm excitations. This result is direct evidence of the existence of a unique isomerization pathway via the two-photon-allowed singlet excited state. It is interesting to note that the relaxation from the $3^1A_g^-$ to the $^1B_u^+$ states does not play a major role in the isomerization induced by two-photon excitation. The amount of 13-*cis* isomer under the photo-stationary state produced by two-photon (490 nm) excitation was obviously larger than that produced by one-photon (245 and 355 nm) excitation. On the other hand, preliminary experiments using femtosecond laser pulses for two-photon excitation up to the $2^1A_g^-$ state revealed that the all-*trans* and 13-*cis* isomers had an almost equivalent composition at the photo-stationary state. If we assume that isomerization takes place along the adiabatic potential energy curves of the $3^1A_g^-$, $2^1A_g^-$, and $^1B_u^+$ states, we can explain the difference of the isomeric composition at the photo-stationary state when excited up to each excited state. The 13-*cis* isomer was the major product when excited

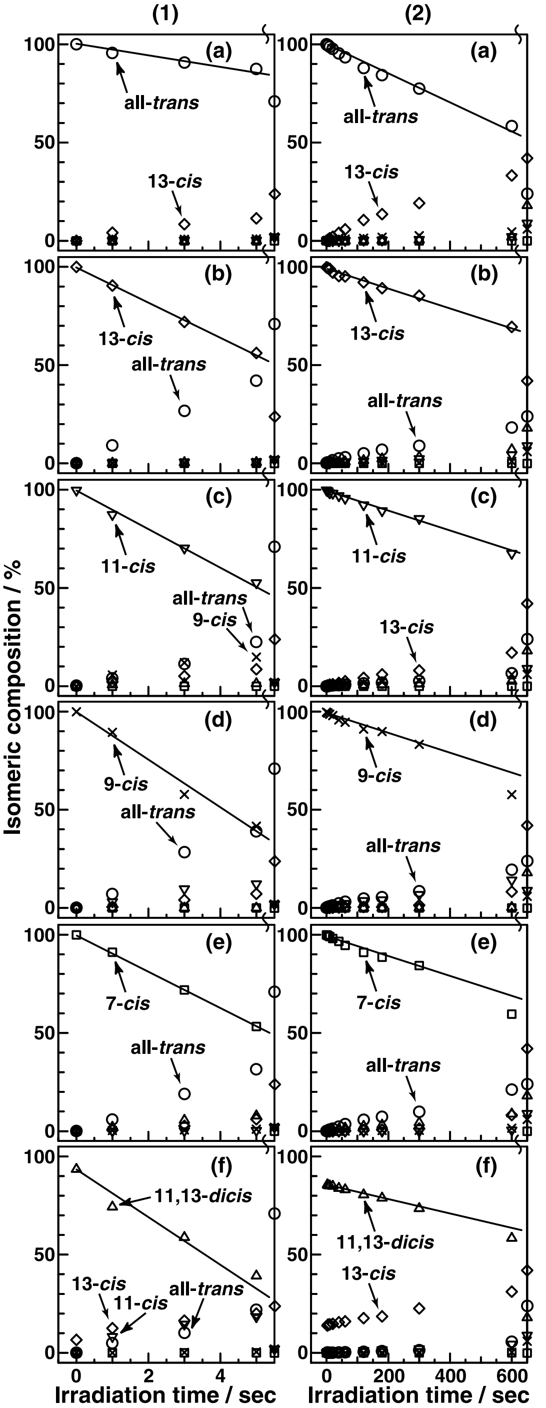

Fig. 3. Time dependence of isomeric composition excited at (1) 245 and (2) 490 nm nanosecond laser pulses starting from (a) all-*trans* (○), (b) 13-*cis* (◇), (c) 11-*cis* (▽), (d) 9-*cis* (×), (e) 7-*cis* (□), and (f) 11,13-*dicis* (△) retinal in *n*-hexane.

directly up to the $3^1A_g^-$ state. This may be due to the fact that, on the adiabatic potential energy curve of C13=C14 torsion, the 13-*cis* configuration has a more stable minimum than the all-*trans* configuration.

Fig. 3 shows the time course of the isomerization excited at 245 and 490 nm starting from the all-*trans*, 13-*cis*, 11-*cis*, 9-*cis*, 7-*cis*, and 11,13-*dicis* isomers of retinal in *n*-hexane solutions. The relative speed of isomerization for one-photon excitation was in the order of 9-*cis* ≈ 11,13-*dicis* > 11-*cis* > 7-*cis* > 13-*cis* ≫ all-*trans*, while that for two-photon excitation was in the order of all-*trans* > 7-*cis* ≈ 9-*cis* ≈ 11-*cis* ≈ 13-*cis* > 11,13-*dicis*. If we compare the relative rate of isomerization at the initial stage of one-photon and two-photon excitations, the all-*trans* isomer was the slowest by one-photon excitation but the fastest by two-photon excitation. The 11,13-*dicis* isomer showed the opposite trend of the all-*trans* isomer. Based on the inference that is drawn from the above discussion concerning the different isomeric composition at photo-stationary states, the all-*trans* isomer would have the most stable potential minimum in the one-photon-allowed excited state, while the 11,13-*dicis* isomer would have it in the two-photon-allowed excited state. This interpretation does not contradict the fact that the 11, 13-*dicis* isomer was not detected at all by one-photon excitation although it was substantially produced by two-photon excitation.

4. Conclusion

In the previous photochemistry of retinal and carotenoids, isomerization by one-photon or triplet-sensitized excitation was well documented, while that through the two-photon-allowed $3^1A_g^-$ state was ignored in most cases. However, the present study clearly demonstrated that this should not always be the case. Enhancement of the production of the 13-*cis* isomer was evidenced by way of the $3^1A_g^-$ state, and the presence of a unique pathway that produces the 11,13-*dicis* isomer was found by two-photon excitation. Since the energies and orders of the excited states of polyene molecules change systematically depending on their conjugated chain lengths, we expect to find a highly selective isomerization pathway and thus control the yield of isomerization products by applying our method of non-resonant two-photon excitation to various biological polyene molecules. However, in order to determine the role of $3^1A_g^-$ excited state in vision and/or proton-pump of biological systems, further investigation using retinal proteins as well as theoretical studies in terms of multi-photon isomerization processes are necessary.

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