Light-Induced Structural Change of β-Carotene in Thylakoid Membranes†

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ABSTRACT: The structure of β-carotene, in the thylakoid membranes of a thermophilic blue-green alga (*Synechococcus vulcanus* Copeland) and of spinach, has been studied by means of high-performance liquid chromatography (HPLC) analysis of isomers that were extracted from the membranes. The HPLC elution pattern showed that the extract contained about 80% all-trans isomer and 20% different kinds of cis isomers, namely, the 15-cis, 13-cis, 9-cis,13-cis, 9-cis,9′-cis, and 9-cis isomers; the result indicates that some of the β-carotene molecules take on cis or twisted structures around double bonds in the membranes. Most of the cis isomers originate from photosystem I. The amounts of the 15-cis and 13-cis isomers in the extract decreased when the thylakoids were exposed to light before extraction, indicating a light-induced structural change of β-carotene in the membranes; the change was found to be reversible. The same type of structural change in β-carotene was found for the intact photosynthetic membranes of both the blue-green alga and spinach; the extent of the structural change correlates with the intensity of the light cast on those organisms during their natural growth. The excitation of the chlorophyll molecules alone caused the change. Therefore, the structural change is ascribed to the formation of a triplet excited state of β-carotene. Possible implications of the structural change are discussed.

Carotenoids are bound to pigment–protein complexes in the photosynthetic membranes (Mathis & Schenck, 1982; Cogdell, 1985): In the case of a photosynthetic bacterium, *Rhodopseudomonas* (*Rps.*) *spheroides* for example, each of the reaction centers and the B875 and B800-850 light-harvesting complexes contain, respectively, one, two, and one molecule(s) of spheroidene as an intrinsic component. In the case of blue-green algae and of spinach, β-carotene has been considered to be bound exclusively to both the chlorophyll *a*–protein complexes of photosystem I (PS I) and those of photosystem II (PS II) and xanthophylls to be bound to the light-harvesting complexes. The number of β-carotene molecules bound to the PS I and PS II complexes is estimated to be 15–20 for the former and about 10 for the latter (Satoh, 1982; Omata et al., 1984; Satoh, personal communication).

The carotenoids have dual functions in the photosynthetic membranes (Mathis & Schenck, 1982; Cogdell, 1985): (1) They absorb light energy and transfer it to the (bacterio)-chlorophyll molecules in the reaction center; (2) they protect the organisms against photodynamic destruction, which is mediated by singlet oxygen (Krinsky, 1979). Singlet–singlet and triplet–triplet energy transfers play an important role in, respectively, the light-harvesting and photoprotective functions.

Resonance Raman spectroscopy has proved to be powerful in determining the configurations of the carotenoid in the membranes of photosynthetic bacteria (Lutz et al., 1976; Lutz et al., 1978; Agalidis et al., 1980; Merlin, 1985). In the case of *Rps. spheroides*, the carotenoid takes on a central cis configuration in the reaction center (Koyama et al., 1982, 1983) and it takes on the all-trans configuration in the light-harvesting complex. However, no cis configurations have been found by means of Raman spectroscopy for the PS I and PS II "reaction center" preparations. The PS I and PS II particles as well as the thylakoid membranes of a blue-green alga (*Synechococcus vulcanus* Copeland) showed essentially the same Raman spectrum of *all-trans*-β-carotene except for very small differences, when probed at 450–500 nm (Kito, Koike, & Koyama, unpublished results). It is probable that the Raman lines of the smaller amounts of cis isomers were obscured or blurred by the strong Raman lines of the all-trans isomer, which was under better resonant conditions. This assumption is based on the fact that the chromatophores of photosynthetic bacteria show the Raman spectrum of the all-trans carotenoid in the light-harvesting complex and that they gave no indication of the central cis carotenoid of the reaction center in the spectrum.

Small amounts of cis isomers may be detected when β-carotene extracted from the thylakoid membranes is analyzed by means of high-performance liquid chromatography (HPLC). The β-carotene molecules taking a cis or twisted configuration(s) around a double bond in the pigment–protein complexes should appear in the extract in the form of cis isomer(s), and the technique of HPLC analysis of β-carotene isomers has already been established (Tsukida et al., 1982). This indirect method enables the detection of the different configurations of β-carotene in the thylakoid membranes.

In the present investigation, we analyzed the isomers of β-carotene extracted from the thylakoid membranes, PS I, PS II, and the living organism for both a thermophilic blue-green alga (*S. vulcanus* Copeland) and spinach. The results showed that about 20% of the β-carotene in the thylakoid membranes is extracted in the form of cis isomers, which indicates that the β-carotene molecules take on not only the all-trans configuration but also cis or twisted configuration(s) around double bonds. Furthermore, we found that the central cis (or central twist) form(s) in the membranes undergoes a light-induced, reversible structural change into the all-trans (or a stretched) form.

Materials and Methods

Sample Preparations. Thermophilic blue-green algae, *S. vulcanus* Copeland, were grown at 53–55 °C for 2–3 days in the medium of Hirano et al. (1980). Thylakoid membranes were prepared by the lysozyme treatment of the harvested cells

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and then by an osmotic shock to lyze the spheroplasts (Stewart & Bendall, 1979). The PS I and PS II particles were prepared by solubilizing the thylakoid membranes with digitonin, following the procedure of Satoh (1982). The purity of the particles was examined by SDS polyacrylamide gel electrophoresis (Chua, 1980).

Thylakoid membranes (chloroplasts) of spinach (commercial source) were prepared following the method of Satoh (1982). All the sample suspensions were stored at 77 K before use.

Irradiating Conditions. The thylakoid membranes of the blue-green alga were suspended in 50 mM HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid] and 10 mM $MgCl_2$ (pH 7.0), whereas those of spinach, in 0.4 M sucrose, 50 mM Tris, 10 mM NaCl, and 5 mM $MgCl_2$ (pH 7.2) at the concentrations of 7–10 μg of chlorophyll/mL. The suspensions were irradiated from a distance of 30–40 cm with the white light of a halogen lamp (13 000 lux passed through a water filter). For exciting only the chlorophyll molecules in the thylakoid membranes, a combination of filters, Kenko U330 and Toshiba O-56, was used. (The resultant wavelength of 50% transmission was around 570 nm.) The temperature of the suspension was kept at 0 °C for both the blue-green alga and spinach.

In the case of the intact blue-green algae, the cells were suspended in the culture medium (40–50 °C).

Extraction and HPLC Analysis of β-Carotene. For each analysis, 2 mL of acetone was added to the PS I particles, the PS II particles, the pellet of the thylakoid membranes, the pellet of algae, or the spinach leaf cut into pieces. Next, 1 mL of hexane and then 5 mL of water were added to the suspensions. When β-carotene entered the hexane layer, it was immediately subjected to HPLC. All the procedures were carried out in complete darkness. (β-Carotene could be extracted almost completely by this procedure; the debris after extraction for the PS I particles, the PS II particles, the thylakoid membranes, or spinach leaves was colorless and that for the cells of the alga showed the color of phycobilin (blue).)

HPLC analyses were performed on a Shimadzu LC2 liquid chromatograph, which was operated at 450 nm. A stainless-steel column (4 mm i.d. × 250 mm) packed with calcium hydroxide and the eluent of 0.3% acetone in *n*-hexane (flow rate 1 mL/min) were used. Chlorophylls and xanthophylls in the hexane extract were trapped at the top of the column, and β-carotene isomers alone eluted from the column under the conditions specified above. Each peak in the elution profile of the extract was assigned on the basis of its retention time (Tsukida et al., 1982) and its Raman spectrum (Koyama et al., 1983). The peak of the 9-cis,9′-cis isomer was newly identified by its ^1H NMR spectrum. The spectrum gave chemical shifts and coupling constants that are characteristic of the 9-cis configuration (Tsukida et al., 1981; Vecchi et al., 1981), and the lines of the spectrum were sharper than those of the 9-cis isomer, indicating the presence of the center of symmetry (the 9-cis,9′-cis isomer).

Injections of the extracts degraded the column and changed the retention time and the resolution of the peaks. However, the change was always kept small by replacing the degraded column with a new one.

The amount of each isomer was determined from the peak area. When the overlapping of peaks is present (in the case of the 13-cis, 9-cis,13-cis, 9-cis,9′-cis, or 9-cis isomer), we deconvoluted the elution profile into components by assuming asymmetric peaks. The validity of the procedure was confirmed by using columns with different retentions and resolutions.

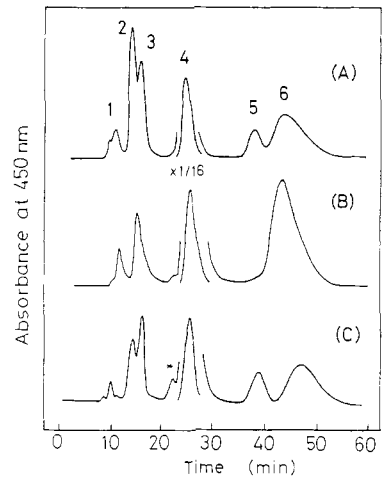

FIGURE 1: HPLC elution patterns of β-carotene that was extracted from the thylakoid membranes of a thermophilic blue-green alga, *S. vulcanus* Copeland. Each numbered peak is assigned to an isomer: (1) (15-cis); (2) 13-cis; (3) 9-cis,13-cis; (4) all-trans; (5) 9-cis,9′-cis; (6) 9-cis. The asterisk denotes the monoepoxide of *all-trans*-β-carotene. The same numbering of the peaks is used throughout the figures. The peak height of the all-trans isomer is reduced by a factor of 16. Peaks: (A) β-carotene extracted from the thylakoid (which had been kept in the dark) and then subjected to HPLC immediately after extraction. (B) The extracted β-carotene exposed to light for 30 min before application to HPLC. (C) β-carotene extracted from the thylakoids that had been irradiated with a halogen lamp for 60 min before extraction and subjection to HPLC.

Table I: Amounts of Cis Isomers of β-Carotene Extracted from Thylakoid Membranes and from PS I and PS II Particles of the Thermophilic Blue-Green Algae[a,b]

isomer	thylakoids	PS I	PS II
(15-cis)	0.017	0.005	0.004
13-cis	0.073	0.042	0.010
9-cis,13-cis	0.051 (0.054–0.057)	0.069	0.010
9-cis,9′-cis	0.041 (0.037–0.039)	0.048	0.004
9-cis	0.092 (0.099–0.105)	0.130	0.007

[a] Amounts in this table are relative to that of the all-trans isomer. [b] Values in parentheses are the calculated amounts of isomers based on the amounts of each in PS I and PS II (see text).

Results and Discussion

Most of the experiments were done for the thermophilic blue-green alga. Therefore, the following description should be considered to concern this organism, when spinach is not specified.

Structure of β-Carotene in Thylakoid Membranes. Figure 1A shows the HPLC elution pattern of β-carotene extracted from the thylakoid membranes of blue-green algae that had been kept in the dark. All the numbered peaks originate from the cis–trans isomers of β-carotene; 2, 13-cis; 3, 9-cis,13-cis; 4, all-trans; 5, 9-cis,9′-cis; 6, 9-cis. Peak 1 is tentatively assigned to 15-cis; four different kinds of isomers are expected to give a peak in this region, and the assignment of the peak to the 15-cis isomer is not unequivocal [hereafter the isomer will be denoted by (15-cis)]. The configurations of these cis–trans isomers are depicted in Figure 2. Table I lists the amount of each cis isomer relative to that of the all-trans isomer, calculated by using the peak area and the molar extinction coefficient at 450 nm for each isomer (Tsukida et al., 1982; Koyama et al., 1983). The total amount of cis isomers in the extract reached approximately one-fourth of the amount of the all-trans isomer.

When a β-carotene molecule is bound to the apoprotein, the rotational angle around each double bond may deviate from that of an ordinary cis configuration, 0 ± 10° or from that

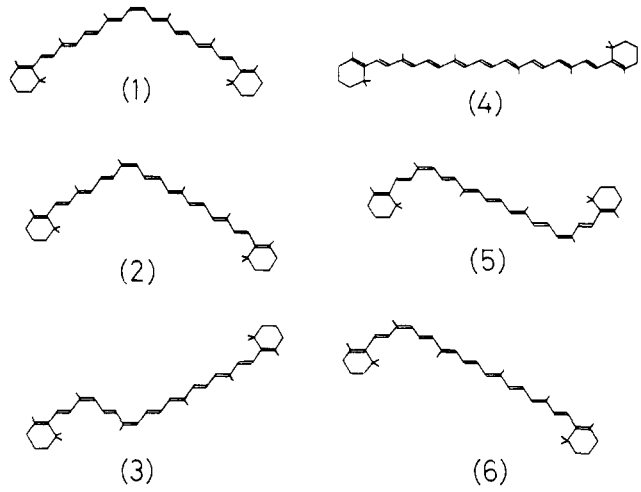

FIGURE 2: Configurations of the cis–trans isomers of β-carotene that appear in the elution patterns of thylakoid membranes: (1) 15-cis; (2) 13-cis; (3) 9-cis,13-cis; (4) all-trans; (5) 9-cis,9′-cis; (6) 9-cis.

of an ordinary trans configuration, 180 ± 10°. Therefore, we will use a notation "*n* twist" to call a configuration in which the rotational angle around the C_n=C_{n+1} bond is larger than that of the cis configuration and is in the range 0 ± 60°. (The rotational angles around all the other polyene double bonds are assumed to be in the range of the trans configuration.) We will also use the notation "stretched" to call a configuration in which the rotational angle of one double bond is in the range of 180 ± 60° and the rotational angles of other double bonds are in the range of the trans configuration.

The HPLC analyses showed, for example, that the relative amount of the 13-cis isomer in the thylakoid membranes in the dark is 0.073. The value does not necessarily indicate the amount of a cis or twisted structure around the 13–14 double bond. The relaxation and partition of a twisted double bond in the polyene chain of β-carotene into the trans and cis isomers around the particular double bond should be dependent on both the torsional angle and the nature of intermolecular interactions between β-carotene and the apoprotein (possibly chlorophyll also). However, the appearance of the 13-cis isomer in the extract strongly suggests the presence of the 13-cis or a twisted form around the 13–14 double bond in the pigment–protein complexes. Therefore, the cis isomers in the extract, in general, show the presence of cis or twisted structures around double bonds that are stabilized by intermolecular interactions.

In order to elucidate from which pigment–protein complex (PS I or PS II) the cis or twisted configurations originate, we recorded the elution patterns of their extracts. Figure 3A,B show the elution patterns for PS I and PS II. (The peak marked with an asterisk is ascribable to the monoepoxide of β-carotene, the characterization of which will be described elsewhere.) Table I lists the amount of cis isomers. It is to be noted that the amounts of the (15-cis) and 13-cis isomers were decreased by exposing the samples to diffused room light during the preparation procedures. The amounts of cis isomers relative to that of the all-trans isomer are much larger for PS I than for PS II. The ratio of PS I to PS II particles in the thylakoid membranes is reported to be 2–3 (Kawamura et al., 1979). Therefore, the cis isomers in the extract of the thylakoid membranes are regarded as originating mainly from the PS I complex.

In the case of spinach also, the cis isomers originate mainly from the PS I complex; the amounts of cis isomers that were extracted from the PS II particles were considerably smaller

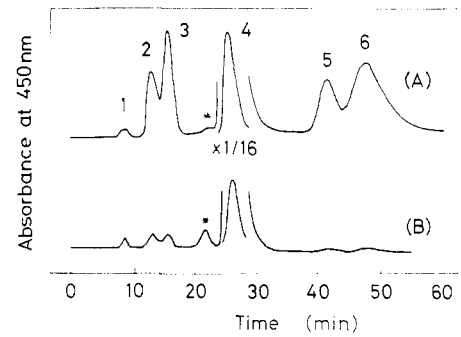

FIGURE 3: HPLC elution patterns of β-carotene that was extracted from (A) PS I and (B) PS II of the blue-green alga.

than those from the chloroplasts (data not shown). The results suggest that the amounts of cis or twisted configurations in PS II should generally be smaller than those in PS I.

Evidence against Isomerization during Extraction or HPLC Analysis. When exposed to light or heat, some isomers of β-carotene undergo cis–trans isomerization in organic solvents. Therefore, one can suspect that the cis isomers that were found in the hexane extract might originate from isomerization of *all-trans*-β-carotene during the process of extraction and/or HPLC analysis. The following observations show that this is not the case.

First, if the isomers originate from gradual thermal isomerization of the all-trans isomer, the elution pattern should change with time after extraction. However, storage of the extract in the dark at room temperature as long as 3 h slightly decreased the amount of the 9-cis,13-cis isomer, but the amounts of other isomers remained unchanged. Second, the extract from the thylakoid membranes contains a fairly large amount of the 9-cis,13-cis and 9-cis,9′-cis isomers, although it is difficult to produce those isomers from the all-trans isomer by means of thermal isomerization in organic solvents. The isomers could be produced only by melting the crystals of the all-trans isomer at 190–200 °C. Third, using hexane in the extraction procedure instead of acetone did not change the elution pattern. Fourth, the patterns of the change in the relative quantity of isomers that are caused by irradiation are quite different for the hexane extract (Figure 1B) and the intact thylakoid membranes (Figure 1C; see below). Figure 1B shows the elution pattern when the *hexane extract* from the thylakoid membranes (which had been kept in the dark) was irradiated; the amounts of the 9-cis,13-cis and 9-cis,9′-cis isomers decreased to almost zero, and the amount of the 9-cis isomer increased. Figure 1C shows the elution pattern when the *thylakoid membranes* were irradiated before extraction; the amounts of the (15-cis) and 13-cis isomers decreased (compare with A) and those of other cis isomers remained unchanged (see below for detailed discussion). Fifth, the elution patterns of the extracts from the PS I and PS II complexes, the thylakoid membranes, and the intact cells of blue-green algae as well as those from the thylakoid membranes and the leaves of spinach are unique and different from one another. Sixth, the amount of the 9-cis,13-cis, 9-cis,9′-cis, and 9-cis isomers (which are independent of the previous irradiating conditions) calculated for the thylakoid membranes of blue-green algae roughly agreed with those actually found for the thylakoid membranes. The calculation was based on (a) the amounts of isomers in the extracts from the PS I and PS II complexes, (b) the number of β-carotene molecules bound to the PS I (15–20) and PS II (10) complexes, and (c) the ratio of PS I and PS II complexes in the thylakoid membranes (2:1). (See Table I. The calculated values are listed in parentheses.)

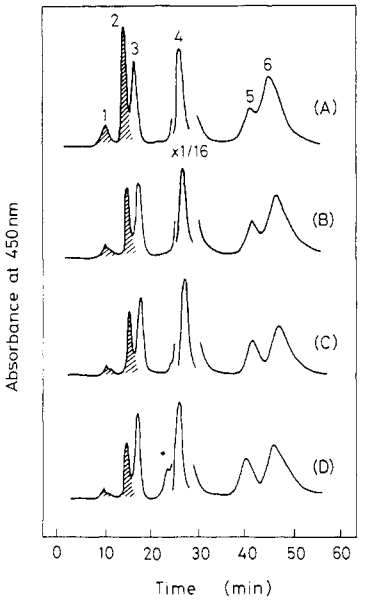

FIGURE 4: HPLC elution patterns of β-carotene that was extracted from the thylakoid membranes of the blue-green alga (A) before and (B–D) after irradiation. The membranes were irradiated with a halogen lamp (without filters) before extraction for (B) 40 min, (C) 135 min, and (D) 255 min. The peaks of the (15-cis) and the 13-cis isomers are hatched.

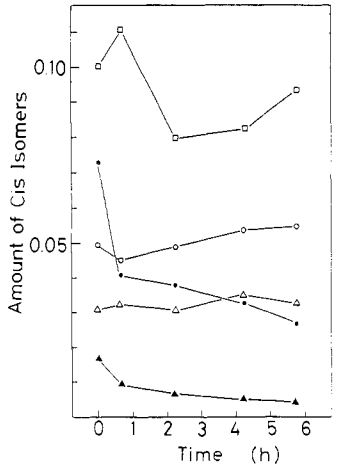

FIGURE 5: Changes in the relative amounts of the cis isomers of β-carotene in the thylakoid membranes of the blue-green alga upon irradiation: ▲, (15-cis); ●, 13-cis; ○, 9-cis,13-cis; △, 9-cis,9′-cis; □, 9-cis.

The above evidence strongly supports the idea that the amount of each isomer in the extract should reflect the structure(s) of β-carotene in the thylakoid membranes. Furthermore, the sixth evidence described above suggests that most of the β-carotene molecules are bound to either the PS I or PS II particles.

Change in the Structure of β-Carotene in the Thylakoid Membranes Caused by Irradiation. As mentioned above, the amounts of the (15-cis) and 13-cis isomers decrease when the thylakoid membranes are irradiated before extraction. Figure 4 shows the change in the composition of the isomers in the extract, when the suspension of the thylakoid membranes was irradiated by a halogen lamp for 40 (B), 135 (C), and 255 min (D). The peaks of the (15-cis) and 13-cis isomers (hatched peaks) lowered with reference to the major all-trans peak. The peaks of the 9-cis,13-cis, 9-cis,9′-cis, and 9-cis isomers remained almost unchanged.

More quantitatively, we calculated the amount of each isomer relative to the all-trans isomer and plotted it against

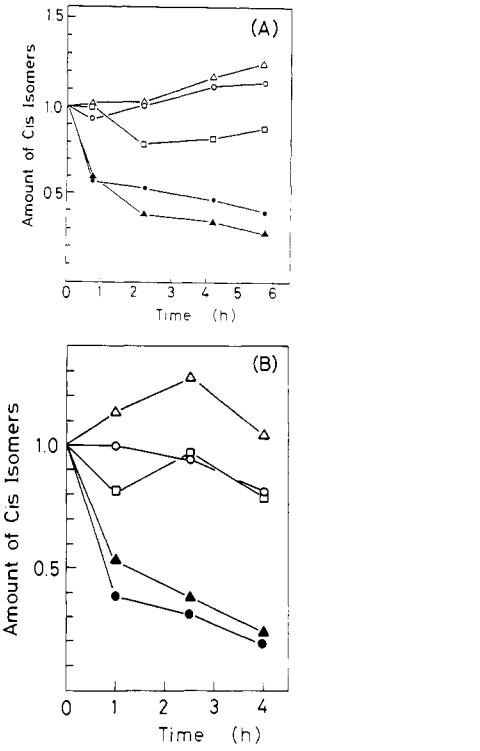

FIGURE 6: Changes in the relative amounts of the isomers of β-carotene in the thylakoid membranes of the blue-green alga upon irradiation. The amount of each isomer before irradiation was normalized to 1.0. Irradiation was made with a halogen-lamp (A) without filters and (B) with filters (Kenko U-330 and Toshiba O-56) that pass the light with a wavelength above 570 nm: ▲, (15-cis); ●, 13-cis; ○, 9-cis,13-cis; △, 9-cis,9′-cis; □, 9-cis.

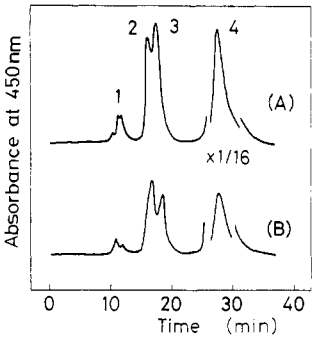

FIGURE 7: HPLC elution patterns of β-carotene that was (A) extracted from the thylakoid membranes of the blue-green alga after irradiation and (B) extracted from the particular membranes stored in the dark at 77 K as long as 20 days.

the time of irradiation (Figure 5). Within 6 h the amount of the 13-cis isomer decreased from ~0.07 to ~0.03 and that of the (15-cis) isomer from ~0.02 to ~0.005. The change in the amount of each isomer is more clearly seen when the initial amount of each isomer is normalized to 1.0 (Figure 6A).

The change was found to be reversible, although the change in the opposite direction is very slow and sometimes difficult to produce. Figure 7A shows the elution pattern for the thylakoid membranes that were irradiated, and Figure 7B shows the elution pattern for the particular thylakoid membranes which were *then* stored as long as 20 days in the dark at 77 K. The relative amounts of the (15-cis) and 13-cis isomers increased when kept in the dark at a low temperature. The result indicates that the decrease of these isomers is not due to photobleaching but to a structural change of β-carotene in the thylakoid membranes.

The structural change upon irradiation is expected to cause an increase in the amount of the all-trans isomer, because no

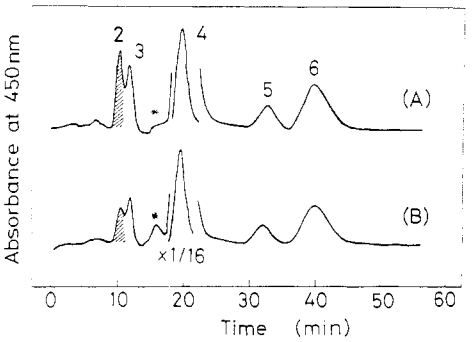

FIGURE 8: HPLC elution patterns of β-carotene extracted from the spinach thylakoid membranes (chloroplasts) (A) before and (B) after irradiation with a halogen lamp (without filters).

drastic increase of other cis isomers was found to compensate for the decrease of the (15-cis) and 13-cis isomers. Since the amounts of those central mono cis isomers are less than 1 order of magnitude smaller than that of the all-trans isomer, the increase in the amount of the all-trans isomer upon irradiation is very small. Therefore, we can safely show the changes in the amounts of cis isomers referring to that of the all-trans isomer as was done above, instead of referring to the total amount of the isomers including cis and all-trans.

We investigated whether the same type of light-induced structural change of β-carotene occurs in the case of the thylakoid membranes of spinach. Figure 8 shows the elution patterns for the extracts from the membranes (A) before irradiation and (B) after 40 min of irradiation with the halogen lamp. The same type of decrease in the amount of the (15-cis) and the 13-cis isomers was observed, suggesting that the structural change of β-carotene is generally found for thylakoid membranes. In the case of spinach, the amount of the cis isomers in the extract relative to that of the all-trans isomer is less when compared to the case of the blue-green algae, which probably reflects the fact that the amount of PS I relative to that of PS II in spinach is smaller (~1) (Hachnel, 1976) than that in blue-green algae (2–3).

A very similar light-induced structural change was observed also at room temperature (23 °C) for the thylakoid membranes of both the blue-green alga and spinach, indicating that the change is independent of temperatures in this range.

Light-Induced Structural Change of β-Carotene in Living Organisms. In order to investigate whether the structural change occurs also in the intact blue-green algae, we suspended the cells in the culture medium, irradiated them with the halogen lamp, and analyzed the β-carotene isomers that were extracted from them. The amounts of the (15-cis) and 13-cis isomers decreased upon irradiation as in the case of the isolated thylakoid membranes (data not shown). In this experiment, both white and red light (see below) were used; the change occurred similarly in both cases. These results indicate that the structural change of β-carotene upon irradiation is not confined to isolated thylakoid membranes but also occurs in intact cells.

The above result raised the question whether the change may occur in the cells under growing conditions, because the intensity of light cast on each cell of the alga will decrease as the cells multiply. The cells are exposed to a fairly strong light shortly after each inoculation, because the initial concentration of the cells is low. When the growth of the cells proceeds, the penetration of light decreases because the dense population of the cells causes most of the cells to stay in the dark. Figure 9 shows the elution patterns for the isomers of β-carotene that were extracted from (A) cells used for inoculation and cells

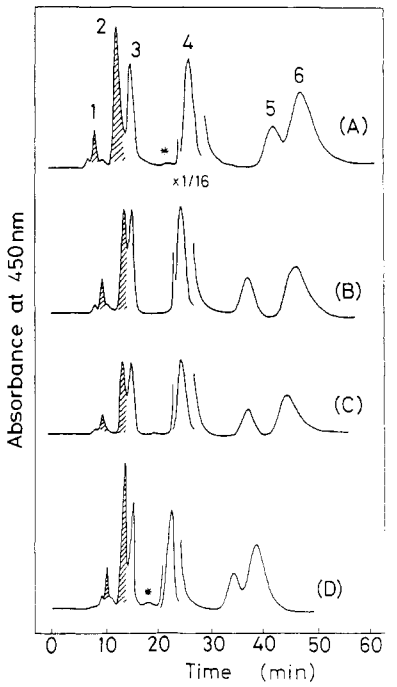

FIGURE 9: Change in the elution patterns of β-carotene that was extracted from the blue-green algae, caused by cell growth; (A) cells used for inoculation; (B–D) cells grown 1, 3, and 7 days after inoculation, respectively.

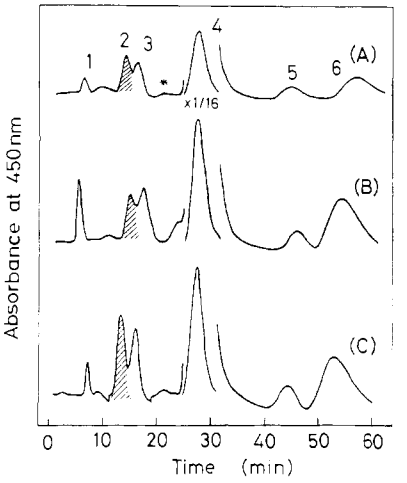

FIGURE 10: HPLC elution patterns of β-carotene extracted from spinach leaves: (A) a leaf harvested before sunrise; (B) a leaf harvested in the daytime; (C) a leaf harvested in the daytime but covered with aluminum foil for 40 h.

grown (B) 1 day, (C) 3 days, and (D) 7 days after inoculation. The elution patterns show clearly that the amount of the (15-cis) and 13-cis isomers responded to the light intensity cast on the cells; those amounts decreased after 1 day, but they recovered to the initial level of inoculum after 7 days.

In order to investigate whether the same type of structural change takes place in the case of a living higher plant, we extracted β-carotene from spinach leaves that were harvested before sunrise and in the daytime and compared the elution patterns for the extracts. Figure 10 shows the elution patterns for the leaves (A) before sunrise and (B) in the daytime. The amount of the 13-cis isomer in the extracts decreased in the sunlight. Figure 10C shows the elution pattern for a leaf that was harvested in the daytime but had been covered with aluminum foil for 40 h. The amount of the 13-cis isomer roughly corresponds to that of the leaf harvested before sunrise. Therefore, the difference between (A) and (B) should originate

from the difference not in the harvesting time but in the light intensity. Thus, we conclude that the same type of reversible structural change of β-carotene takes place also in the case of spinach growing outdoors. (No systematic change in the peak of (15-cis) was found in this set of experiments.)

Origin of the Structural Change of β-Carotene. When a β-carotene molecule is bound to the apoprotein and its structure is stabilized, the most probable cause of the light-induced structural change is the excitation of the pigment molecule up to a higher electronic state, which can be either singlet or triplet. The β-carotene molecule can be excited to a singlet state by direct light absorption. But, it relaxes to the ground state within less than picoseconds (Dallinger et al., 1981). On the other hand, the triplet state of β-carotene can be produced only by triplet–triplet energy transfer from a sensitizer because of this fast relaxation of the singlet excited state. The triplet–triplet energy transfer from chlorophyll to carotenoid in the thylakoid membranes has been well established (Mathis and Schenck, 1982; Cogdell, 1985 and references therein). The triplet state of chlorophyll can be produced easily by the absorption of strong light through either intersystem crossing or charge recombination (Mathis et al., 1979; Frank et al., 1979; Kramer & Mathis, 1980; Satoh & Mathis, 1981). Therefore, when the chlorophyll molecules alone are excited, the triplet state of β-carotene will be produced (Mathis et al., 1979; Kramer & Mathis, 1980; Satoh & Mathis, 1981).

In order to investigate whether the generation of the triplet state is essential to the structural change of β-carotene, we attempted to excite the chlorophyll molecules alone by irradiating the thylakoid membranes of the blue-green alga with a red light ($\lambda > 570$ nm). Figure 6B shows the time course of the change in the amount of each isomer (the initial amount was normalized to 1.0). The time course for each isomer is very similar to that shown in Figure 6A, which was obtained by a white light. The result indicates that the structural change of β-carotene is due to the formation of the triplet state, if we ascribe it to the formation of an excited state.

Mathis and Schenck (1982) have shown the formation of a carotenoid cation radical when the evolution of oxygen is blocked in PS II. However, as described above, the cis isomers that change their structures mainly originate from PS I, and changing the redox state by adding ascorbate or ferricyanide to the suspension did not affect the structural change. Yamashita et al. (1969) described the photobleaching of carotenoids related to the electron transport in chloroplasts. However, the structural change described in this paper is reversible and should not be related to the bleaching of β-carotene. Therefore, it is unlikely that an electron-transfer reaction involving β-carotene is relevant to the present structural change. Thus, we ascribe the structural change to the formation of the triplet state. However, the final conclusion should be postponed until the relation between the structural change and the electron-transfer reaction through PS I and PS II is established.

Possible Implications of the Structural Change. The β-carotene molecules in the thylakoid membranes are characterized as follows: (1) 15–20 molecules of β-carotene are bound to the PS I complex, while 10 molecules of β-carotene are bound to the PS II complex. (2) The β-carotene molecules extracted from the thylakoid membranes take on the all-trans (~80%); 15-, 13-, and 9-mono-cis and 9,13- and 9,9′-di-cis configurations (total of the cis isomers ~20%). (3) The amounts of the 9-cis,13-cis, 9-cis,9′-cis, and 9-cis isomers (relative to that of the all-trans isomer) in the extract of the thylakoid membranes could be calculated by using the compositions of those isomers in the PS I and PS II particles, the number of β-carotene molecules bound to the PS I and PS II particles, and the ratio of the PS I to PS II particles in the thylakoid membranes. The fact suggests that most of the β-carotene molecules are bound either to the PS I or to the PS II complex. (4) Irradiation of the thylakoid membranes caused a change in the composition of the remaining isomers in the extracts, i.e. the (15-cis), 13-cis, and all-trans isomers; the amounts of the former two decreased, and that of the last increased. The simplest interpretation of this change is to ascribe it to a structural change of bound β-carotene from a central cis (or a central-twisted) form into the all-trans (or a stretched) form. (5) The above structural change is ascribed to the formation of the triplet state of β-carotene.

The carotenoid molecules in the membranes of photosynthetic bacteria can be characterized on the basis of results in the literatures as follows: (1) In the case of *Rps. spheroides*, one, two, and one molecule(s) of spheriodene is (are) bound to the reaction center and the B875 and B800-850 light-harvesting complexes, respectively. (2) The carotenoid molecule takes on a central cis configuration in the reaction center (Boucher et al., 1977; Koyama et al., 1982, 1983) and the all-trans configuration in the light-harvesting complexes (Iwata et al., 1985). (3) Reconstitution of the wild-type reaction center (Boucher et al., 1977; Agalidis et al., 1980) using a carotenoid-less mutant [*Rhodospirillum* (*Rs.*) *rubrum* G9 or *Rps. spheroides* R26) and the intrinsic all-trans carotenoid (spirilloxanthin or spheroidene] revealed that the central cis configuration is stabilized by intermolecular interaction with the apoprotein and possibly with bacteriochlorophyll also. The central cis configuration of the carotenoid was formed upon binding, in spite of its instability in solution especially in the presence of bacteriochlorophyll. (4) A *cis*-spirilloxanthin that is bound to the reaction center of *Rs. rubrum* undergoes "cis to trans" isomerization under the conditions that lead to the formation of the triplet state p^R of the primary electron donar (Boucher & Gingras, 1984). It is interesting that cis to trans isomerization of the carotenoid, which is ascribed to triplet formation upon irradiation, is found common in the photosynthetic membranes of an alga, a plant, and a photosynthetic bacterium.

Some insight into the biological implications of the structural change of in vivo β-carotene, which is formed in the present investigation, can be obtained from the results of investigations on the triplet state of in vitro β-carotene. In an organic solvent, the 15-cis and 13-cis isomers of β-carotene readily transform into the all-trans isomer when they are excited to the triplet state by triplet–triplet energy transfer from a sensitizer, i.e. anthracene (Teraoka et al., 1985), chlorophyll (Jensen et al., 1982), or bactriochlorophyll (Hashimoto and Koyama, unpublished results); the quantum yield of the isomerization is very high. Chlorophyll-sensitized formation of the carotenoid triplet state in the in vivo systems, which has been widely accepted (Mathis and Schenck, 1982; Cogdell, 1985), is most probably responsible for the structural change in the thylakoid membranes. However, the quantum yield of the isomerization in the present in vivo system is extremely low, which is additional strong evidence that the β-carotene molecules are bound to the pigment–protein complexes and that their configurations are stabilized through binding. Presumably, most of the central cis-β-carotene molecules are forced to return to the original configuration in the process of relaxation from the triplet state through intermolecular interactions. Some portion of the molecules may undergo isomerization into the all-trans configuration; the structural change back to central

cis carotenoid molecules must be facilitated by incubation of the thylakoid membranes in the dark at low temperature.

Further investigation is necessary to establish the involvement of the triplet state in the structural change of β-carotene that has been found in the present investigation.

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Registry No. 15-*cis*-β-Carotene, 19361-58-1; 13-*cis*-β-carotene, 6811-73-0; 9-*cis*,13-*cis*-β-carotene, 81703-03-9; *all-trans*-β-carotene, 7235-40-7; 9-*cis*,9′-*cis*-β-carotene, 81703-02-8; 9-*cis*-β-carotene, 13312-52-2.

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