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Factors controlling the efficiency of energy transfer from carotenoids to bacteriochlorophyll in purple photosynthetic bacteria

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Efficiencies of energy transfer from carotenoids to bacteriochlorophyll in purple photosynthetic bacteria have been studied with chromatophores, isolated pigment-protein complexes, and pigment-protein complexes reconstituted with a variety of carotenoids. Based on the efficiencies of energy transfer and the chemical structure of major carotenoids, photosynthetic bacteria used in this study are classified into two groups. (1) *Rhodobacter sphaeroides, Rhodobacter capsulatus,* and *Rhodocyclus gelatinosus* show relatively high efficiencies (> 70%) and contain spheroidene-series carotenoids which have nine or ten conjugated C=C bonds. (2) *Rhodopseudomonas palustris, Rhodospirillum rubrum,* and *Chromatium vinosum* show relatively low efficiencies (< 50%) and contain spirilloxanthin-series carotenoids which have 11 or 13 conjugated C=C bonds. Resonance Raman studies have shown that carotenoids in the former group of bacteria take planar polyene-chain structure, while carotenoids in the latter group take relatively distorted polyene-chain structure. Studies on carotenoids incorporated into carotenoid-less and carotenoid-deficient light-harvesting bacteriochlorophyll-protein complexes have led to a more decisive conclusion that the efficiency depends on not only carotenoids species but also apoproteins specific to bacterial species. As the number of conjugated C=C bonds in incorporated carotenoids increases, the efficiency of energy transfer decreases. This result gives strong support to the view that the energy transfer occurs from a forbidden excited state of carotenoids (2^1A_g). Carotenoids, when incorporated into the pigment-protein complex from *Ch. vinosum,* are more distorted and show lower efficiencies than when they are incorporated into the pigment-protein complex from *Rb. sphaeroides.* Thus, the energy-transfer efficiency is also associated with apoproteins which determine the molecular structure of the bound carotenoids and the interaction between carotenoids and bacteriochlorophyll.

Introduction

Carotenoids have two important roles in photosynthetic bacteria. First, carotenoids act as light-harvesting (LH) pigments by absorbing sunlight and transferring the excitation energy to bacteriochlorophyll (Bchl), which eventually reaches the reaction center (RC) [1–4]. By this mechanism, photosynthetic bacteria can utilize sunlight most effectively, because carotenoids absorb green light which is abundant in sunlight but is not absorbed directly by Bchl. Second, carotenoids quench triplet Bchl and singlet oxygen which are directly or indirectly related with the photodamage on cell membranes [3–5].

The efficiency of energy transfer from carotenoids to Bchl has been measured for cells, membranes and pigment-protein complexes from several purple photosynthetic bacteria. For example, *Rhodobacter sphaeroides* [6–8] shows relatively high efficiencies (70–100%), while *Chromatium vinosum* [9] and *Rhodospirillum rubrum* [1,6] show relatively low efficiencies (30–40%). Thus, the efficiencies of energy transfer are different from species to species.

Boucher et al. [10] have suggested that the efficiency of energy transfer depends on carotenoid species. They compared the energy transfer in RCs from *Rs. rubrum*

Abbreviations: Bchl, bacteriochlorophyll; Chl, chlorophyll; RC, reaction center; LH, light harvesting; DPA, diphenylamine; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; LDAO, lauryldimethylamine *N*-oxide; TGA, thioglycolic acid.

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G9 (carotenoid-less mutant) reconstituted with several species of carotenoids, and found that each carotenoid showed a specific efficiency. For example, spirilloxanthin showed a low efficiency, while spheroidene showed a high efficiency. It seems difficult, however, to apply their conclusion to carotenoids in the LH complexes, because the structures and functions of carotenoids are different between the RC and the LH complexes; e.g., carotenoids in the LH complexes take the all-*trans* form, while those in RCs take a mono-*cis* form [10,11].

On the other hand, Cogdell et al. [7] have suggested that carotenoid species are not responsible for the efficiency of energy transfer. They studied the energy transfer in the B800-850 complexes isolated from two green mutants of *Rb. sphaeroides* and a wild-type strain cultured under various conditions. From the result that the efficiencies of energy transfer were high in all these complexes in spite of different carotenoid compositions, Cogdell et al. concluded that the efficiency of energy transfer depended on the bacterial species but not on the carotenoids species. They pointed out the importance of the geometric arrangement between carotenoids and Bchl which should be determined by apoproteins in the pigment-protein complexes. However, the carotenoid species they studied were limited to spheroidene-series carotenoids having nine or ten conjugated C=C bonds. To reach a final conclusion, similar studies on various species of carotenoids, especially on spirilloxanthin-series carotenoids with 11 to 13 conjugated C=C bonds which are major components in the bacteria showing low efficiencies, are necessary.

More recently we have reported that another factor is associated with energy transfer [12]. Iwata et al. [13] studied the resonance Raman spectra of carotenoids in the LH systems of photosynthetic bacteria. Carotenoids in some species of bacteria (*Ch. vinosum, Rs. rubrum* and *Rp. palustris*) showed a relatively strong Raman band around 960 cm^{-1}. On the other hand, carotenoids in other species of bacteria (*Rb. sphaeroides, Rhodobacter capsulatus* and *Rhodocyclus gelatinosus*) showed a relatively weak Raman band around 960 cm^{-1}. The 960 cm^{-1} Raman band is assigned to the CH out-of-plane wagging mode [14,15], and can be used as a marker for discerning whether the polyene chain is distorted or not [12,13]. Thus, the polyene chains of carotenoids in the former group of bacteria are distorted. It is known that the efficiencies of energy transfer in these bacteria are low. In addition, spheroidene (one of the major carotenoids in *Rb. sphaeroides*) takes a distorted form and shows a low efficiency, when it is incorporated into the LH complex of *Ch. vinosum* [12]. Thus, distortion of the polyene chain of carotenoids seems to lower the efficiency of energy transfer.

The energy-transfer mechanism from carotenoids to Bchl or chlorophyll (Chl) has been discussed by several groups of authors [2–4,16–23]. Although theoretically the Dexter electron-exchange mechanism via a forbidden excited state (2^1A_g) is thought to be most probable, little experimental evidence in vivo has been reported. To understand the mechanism of energy transfer, it is necessary to know what kinds of factors affect energy transfer and how each factor controls the efficiency.

To clarify the problems described above, we incorporated several species of carotenoids having 9–13 conjugated C=C bonds into a carotenoid-less LH complex from *Rb. sphaeroides* and a carotenoid-deficient LH complex from *Ch. vinosum,* and examined the efficiency of energy transfer in these reconstituted LH complexes. From the results obtained, interrelationships among several factors such as carotenoid species, apoproteins, and polyene-chain structure will be disentangled. Mechanism of energy transfer will be discussed in the light of new information obtained in this study.

Materials and Methods

Purple photosynthetic bacteria were cultured phototrophically [24,25]. To suppress carotenoid synthesis in *Ch. vinosum*, a medium containing 12 mg/liter diphenylamine (DPA) was used [26,27]. Intracytoplasmic membranes were prepared as described previously [28].

The RC-B890 and B800-820, B800-850 complexes of *Ch. vinosum,* and the RC-B870 and B800-850 complexes of *Rp. palustris* were isolated as described previously [28,29]. The B800-850 complex of *Rb. sphaeroides* was isolated by the same detergent treatment as reported by Clayton and Clayton [30] and purified by chromatography on Sephacryl S-200. The RC-B870 and B800-850 complexes of *Rb. capsulatus* were prepared by the treatment with 1% sodium dodecyl sulfate (SDS) and 1% Triton X-100 on the membranes followed by SDS polyacrylamide gel electrophoresis (PAGE) (K. Shimada, private communication). The B870 and B800-850 complexes of *Rc. gelatinosus* were kindly supplied by Dr. K. Shimada [31]. The carotenoid-deficient B800-820, B800-850 complex was isolated from *Ch. vinosum* cultured in the presence of DPA. The carotenoid-less B870 complex from a blue-green mutant of *Rb. sphaeroides* was isolated as follows. The membrane was suspended in a 10 mM Tris-acetate buffer (pH 8.4) at a concentration having absorbance 50 at 870 nm (1 cm cell) and treated with 1% lauryldimethylamine *N*-oxide (LDAO). The mixture was layered over a 15%/30% (w/v) sucrose gradient and centrifuged at $200\,000 \times g$ for 2 h. The crude B870 complex was located at the 15%/30% sucrose interface. This crude B870 complex ($A_{870\,nm} = 50$) was treated with 1% SDS and 1% Triton X-100, and applied to gel filtration (Sephadex G-50) with a 50 mM Tris-thioglycolic acid (TGA) buffer (pH 8.4) containing 0.1% Triton X-100. Further purification was achieved by

PAGE with a 50 mM Tris-TGA buffer (pH 8.4) containing 0.01% EDTA, 0.05% SDS and 0.05% Triton X-100. The gel was 5 mm thick and contained 4% acrylamide, 0.1% *N,N'*-methylene-bis-acrylamide. 0.1% ammonium persulfate, 0.05% *N,N,N',N'*-tetramethylethylenediamine, and the same buffer. The electrophoresis was carried out at 40 V. The position and bandwidth of the Q_y band of Bchl in the B870 complex were monitored to confirm that there was no protein degradation during purification of the LH complex and reconstitution with carotenoids (see below). PAGE was performed in a cold room maintained at 4° C.

Neurosporene and methoxyneurosporene were extracted from *Rb. sphaeroides* strain Ga. Spheroidene was extracted from *Rb. sphaeroides* wild type. Rhodopin and spirilloxanthin were extracted from *Ch. vinosum*. these carotenoids were separated and purified by silica gel column chromatography. Neurosporene and methoxyneurosporene were eluted with a mixture of diethyl ether/petroleum ether (5 : 95), spheroidene was eluted with a mixture of acetone/petroleum ether (0.5 : 99.5), and rhodopin and spirilloxanthin were eluted with a mixture of acetone, chloroform, and petroleum either (3 : 20 : 77).

Reconstitution was performed with the carotenoidless and carotenoid-deficient complexes and carotenoids by essentially the same procedure as described previously [12]. The purified complexes were suspended in a Tris buffer (pH 8.0–8.5). Absorbance at 800 nm (1 cm light path) was adjusted to 30 for the complex from *Ch. vinosum*, and absorbance at 870 nm to 50 for the complex from *Rb. sphaeroides*. To these suspensions, purified carotenoids were added with a molar ratio of carotenoid/Bchl of 30 : 1 for the complex from *Ch. vinosum* and 50 : 1 for the complex from *Rb. sphaeroides*. These mixtures of the complexes and carotenoids were then sonicated for 5 min in the presence of 2.0% sodium deoxycholate. After dialyzing the mixtures against a Tris-acetate buffer (pH 8.0–8.5) overnight, excess carotenoid was separated from the complexes with SDS-PAGE. On the second PAGE, free carotenoid was removed completely. The reconstituted complex was dialyzed against a Tris buffer for 2–3 days to exclude excess detergent.

The efficiency of energy transfer from carotenoids to Bchl was obtained by evaluating the amount of light emitted from Bchl (via energy transfer) relative to the light absorbed by carotenoids. Fluorescence intensity from Bchl in the wavelength region longer than 800 nm was recorded using an actinic light source covering the wavelength region of 400–650 nm for excitation. The intensity of the excitation spectrum was so adjusted that the intensity around 590 nm corresponded to the absorption intensity of the Bchl Q_x band around 590 nm. The efficiency of energy transfer was directly calculated from the ratio of intensities at bands due to carotenoids between 400–550 nm in the absorption and excitation spectra, on the assumption that the energy transfer from the Q_x to Q_y levels of Bchl was 100%. An average of the values obtained from the 0–0 and 0–1 bands of carotenoids was used as the efficiency.

Absorption spectra were measured on a Jasco UVIDEC 505 spectrophotometer at room temperature. For measuring the excitation spectra, the sample solution was illuminated with an actinic light, and the fluorescence from Bchl was monitored. The actinic light was obtained by a combination of a halogen lamp (12.5 V, 7 A), a monochromator (Ritsu Oyo Kougaku MC-50), and a light balancing filter (Hoya LB200). The fluorescence from Bchl was detected with a type S-1 photomultiplier (Hamamatsu Photonics 7102) electronically cooled to about −20° C and protected with a filter (Hoya R-72) which cut off the light in the region of wavelength shorter than 700 nm. The fluorescence intensity was corrected with the intensity of the actinic light, which was monitored with a photoquantum counter using an ethylene glycol solution of Rhodamine 640 (8 g/liter) [32,33]. Because of the small absorption coefficient of Rhodamine 640 above 600 nm, no reliable correction was possible in the region between 600 and 650 nm. (This must be the reason why, in some spectra to be shown later, fluorescence excitation curves are above absorption curves in the 600–650 nm region.) The slit width was 3 nm. Sample concentrations for the measurements of excitation spectra were adjusted to give absorbances less than 0.1 (1 cm path) at the absorption maxima of carotenoids.

Resonance Raman spectra of carotenoids were measured at room temperature using a rotating cell as reported previously [13]. The 457.9, 488.0, 501.7 and 514.5 nm lines of an Ar^+ laser were used for Raman excitation. The laser power was less than 50 mW at the sample point.

Results

Intrinsic carotenoids

The left side of Fig. 1 shows the absorption spectra and fluorescence excitation spectra of membranes from *Rb. sphaeroides* (A) and *Ch. vinosum* (B). From the intensities of the 0–0 and 0–1 bands in the absorption and excitation spectra, the efficiencies of energy transfer were estimated to be 94 ± 3% for *Rb. sphaeroides* and 28 ± 3% for *Ch. vinosum*. In the right side of Fig. 1, the resonance Raman spectra of carotenoids in the 1100–900 cm^{-1} region are shown. The band intensities in these spectra are so normalized that the intensities of the band around 1005 cm^{-1} (methyl rocking) are equal. Clearly, carotenoids in *Ch. vinosum* show a relatively strong band at 960 cm^{-1} (Fig. 1B), while the corresponding band of carotenoids in *Rb. sphaeroides* is very weak (Fig. 1A). The Raman band around 960 cm^{-1} of

carotenoids is assigned to a CH out-of-plane wagging mode [14,15]. A relatively strong intensity of this band indicates that the polyene chain is distorted, while a relativley weak intensity points to a planar structure [12,13]. Accordingly, carotenoids contained in *Rb. sphaeroides* have planar polyene chains, while those contained in *Ch. vinosum* have distorted polyene chains.

The efficiencies of energy transfer and the intensities of the 960 cm^{-1} Raman band measured for the membranes and the LH complexes from several photosynthetic bacteria are summarized in Table I. Both the membranes and the B800-850 complexes from *Rb. sphaeroides* wild type, *Rb. sphaeroides* strain Ga (green mutant), *Rb. capsulatus*, and *Rc. gelatinosus* showed high efficiencies (75–95%). The RC-B870 complex from *Rb. capsulatus* and the B870 complex from *Rc. gelatinosus* showed slightly lower efficiencies ($\approx$ 70%) than the membrane and the B800-850 complex of the respective bacteria. On the other hand, the membrane from *Rs. rubrum*, and the membrane, RC-B890 complex and B800-820, B800-850 complex from *Ch. vinosum* showed low efficiencies (20–30%). The membrane, the RC-B870 complex and the B800-850 complex from *Rp. palustris* showed medium efficiencies ($\approx$ 50%). The energy-transfer efficiencies of the B800-850 complex from *Rb. sphaeroides* and the membranes from *Rb. sphaeroides* wild type, *Rs. rubrum*, *Ch. vinosum*, and *Rp. palustris* were essentially the same as the results reported previously [6,7,9].

The relative intensities of the 960 cm^{-1} Raman band of carotenoids were weak for the membranes and the

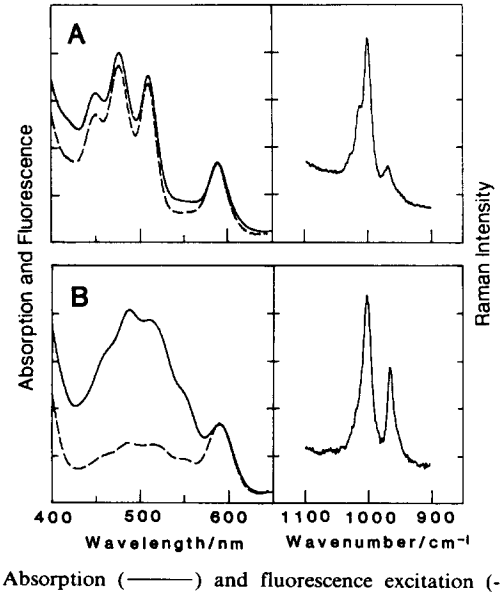

Fig. 1. Absorption (———) and fluorescence excitation (- - - - - -) spectra (left) and resonance Raman spectra (right) of chromatophores from *Rb. sphaeroides* (A) and *Ch. vinosum* (B). Absorption and fluorescence excitation spectra are normalized at the Q_x band of Bchl around 590 nm. Resonance Raman spectra were measured with 514.5 nm excitation in the 1100–900 cm^{-1} region. All the measurements were carried out at room temperature.

TABLE I

Energy-transfer efficiencies and intensities of the 960 cm^{-1} Raman band of carotenoids in native bacteria

Sample	Efficiency (%)[a]	960 cm^{-1} Raman band[b]	Carotenoid[c]
Rb. sphaeroides wild type			
Membrane	94	W	A
B800-850 complex	87	W	A
Rb. sphaeroides Ga			
Membrane	90	W	A
B800-850 complex	94	W	A
Rb. capsulatus			
Membrane	81	M	A
RC-B870 complex	74	M	A
B800-850 complex	82	W	A
Rc. gelatinosus			
Membrane	75	W	A
B870 complex	72	M	A
B800-850 complex	84	W	A
Rs. rubrum			
Membrane	32	M	B
Ch. vinosum			
Membrane	28	S	B
RC-B890 complex	22	M	B
B800-820, B800-850 complex	32	S	B
Rp. palustris			
Membrane	49	M	B
RC-B870 complex	51	M	B
B800-850 complex	50	M	B

[a] Experimental error is ± 3.

[b] S, strong; M, medium; and W, weak.

[c] A, spheroidene-series carotenoids which have nine or ten conjugated C=C bonds (neurosporene, methoxyneurosporene, chloroxanthin, spheroidene, etc.). B, spirilloxanthin-series carotenoids which have 11–13 conjugated C=C bonds (lycopene, rhodopin, spirilloxanthin, etc.).

B800-850 complexes from *Rb. sphaeroides* wild type, *Rb. sphaeroides* strain Ga, and *Rc. gelatinosus*. The intensities of that band were medium for the membrane from *Rb. capsulatus*, the RC-B870 complex from *Rb. capsulatus*, and the B870 complex from *Rc. gelatinosus*. The 960 cm^{-1} band was strong for the membrane and the B800-820, B800-850 complex from *Ch. vinosum*, and medium for the membrane from *Rs. rubrum*, the RC-B870 complex from *Ch. vinosum*, and the membrane, the RC-B870 and the B800-850 complexes from *Rp. palustris*.

In Table I, types of the dominant carotenoids in these bacteria are also shown. The carotenoid composition depends on the pathway of carotenoid synthesis [34]. *Rs. rubrum*, *Ch. vinosum* and *Rp. palustris* synthesize carotenoids by the normal pathway of spirilloxanthin synthesis, and accumulate spirilloxanthin (number of the conjugated C=C bond, $n_{C=C} = 13$), rhodopin

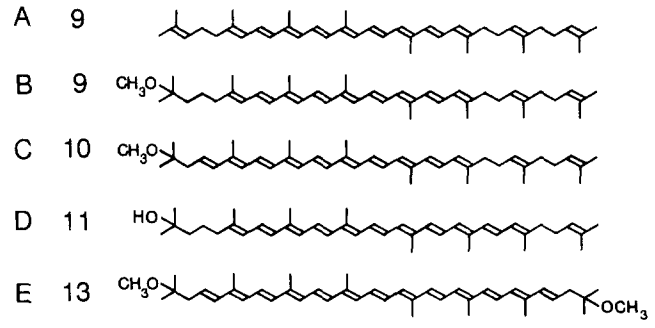

Fig. 2. Chemical structures of carotenoids used for reconstitution experiments. A, neurosporene; B, methoxyneurosporene; C, spheroidene; D, rhodopin; and E, spirilloxanthin. The number of conjugated C=C bonds is also shown.

($n_{C=C} = 11$), lycopene ($n_{C=C} = 11$), etc. (spirilloxanthin-series carotenoids). On the other hand, *Rb. sphaeroides, Rb. capsulatus* and *Rc. gelatinosus* synthesize carotenoids by an alternative pathway of spirilloxanthin synthesis via spheroidene. However, in these bacteria the synthesis does not proceed to the final product, and intermediates in the pathway, such as spheroidene ($n_{C=C} = 10$), OH-spheroidene ($n_{C=C} = 10$), neurosporene ($n_{C=C} = 9$), methoxyneurosporene ($n_{C=C} = 9$), chloroxanthin ($n_{C=C} = 9$), etc. (spheroidene-series carotenoids) are accumulated.

Incorporated carotenoids

The following species of carotenoids were used in reconstitution experiments; neurosporene ($n_{C=C} = 9$), methoxyneurosporene ($n_{C=C} = 9$), spheroidene ($n_{C=C} = 10$), rhodopin ($n_{C=C} = 11$), and spirilloxanthin ($n_{C=C} = 13$). The chemical structures of these carotenoids are shown in Fig. 2.

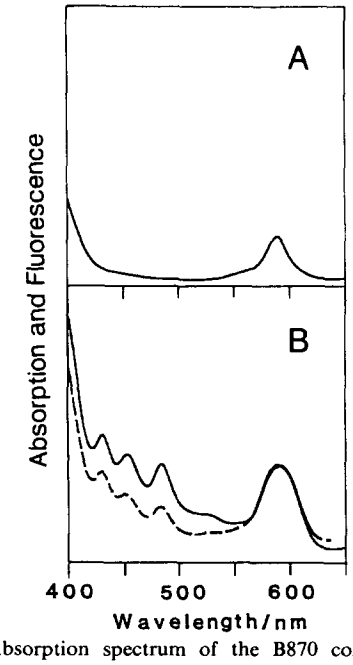

Fig. 3. (A) Absorption spectrum of the B870 complex from the carotenoid-less mutant of *Rb. sphaeroides*; (B) absorption (———) and fluorescence excitation (- - - - - -) spectra of the B800-820, B800-850 complex from the carotenoid-deficient *Ch. vinosum* cultured in the presence of diphenylamine (12 mg/liter).

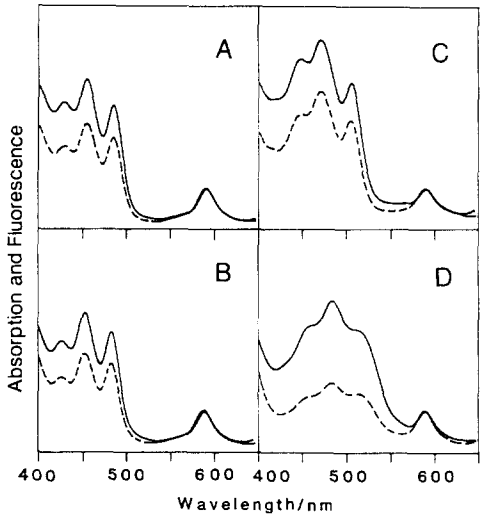

Fig. 4. Absorption (———) and fluorescence excitation (- - - - - -) spectra of the B870 complex from the carotenoid-less mutant of *Rb. sphaeroides* reconstituted with neurosporene (A), methoxyneurosporene (B), spheroidene (C) and rhodopin (D). All the spectra are normalized at the Q_x band of Bchl around 590 nm.

Two kinds of LH complexes were used for reconstitution with carotenoids. One was the B870 complex from a blue-green mutant of *Rb. sphaeroides*, which was devoid of carotenoids (see the absorption spectrum in Fig. 3A). This strain is a carotenoid-less mutant similar to the well-known blue-green mutant, strain R26. Davidson and Cogdell [35] reported incorporation of carotenoids into the LH complex from a blue-green mutant of *Rb. sphaeroides*. However, the strain they used was R26.1 and the complex used for reconstitution was a B800-850 type complex lacking B800 Bchl [36]. Thus, the carotenoid-less complex used in the present study is different from the complex used by Davidson and Cogdell.

The other complex was the B800-820, B800-850 complex from carotenoid-deficient cells of *Ch. vinosum* cultured in the presence of DPA. The B800-850 and B800-820 complexes of *Ch. vinosum* could not be separated by SDS-PAGE. Since these two complexes have almost the same carotenoid and protein compositions [37], we used a mixture of these complexes without further separation. Fig. 3B shows the absorption and fluorescence excitation spectra of the B800-820, B800-850 complex from DPA-cultured *Ch. vinosum* before reconstitution. The amount of carotenoids in this carotenoid-deficient complex is less than 1/10 of that in the complex from normally cultured cells, although it depends on the growth phase [12]. Chloroxanthin ($n_{C=C} = 9$) is the dominant remaining carotenoid in this carotenoid-defi-

cient complex, which shows an energy-transfer efficiency of 50% [12].

In Fig. 4, the absorption and fluorescence excitation spectra of the B870 complex of *Rb. sphaeroides* reconstituted with four different carotenoids are shown. Both the absorption and fluorescence excitation spectra are normalized in intensity at the Q_x band of Bchl around 590 nm. The amount of carotenoid incorporated into the B870 complexes was 80–100% of that in the native B870 complex. Spirilloxanthin could not be incorporated into the B870 complex from *Rb. sphaeroides*. The energy-transfer efficiency (72%) and the position of the 0–0 peak (505 nm) of spheroidene incorporated into the B870 complex from the *Rb. sphaeroides* blue-green mutant (Fig. 4C) are essentially the same as the values of the native B870 complex intrinsically containing spheroidene (70% and 504 nm [8]). This means that the reconstitution procedure used in this study was appropriate.

The absorption and fluorescence excitation spectra of the B800-820, B800-850 complex from DPA-cultured *Ch. vinosum* reconstituted with five carotenoids are shown in Fig. 5. Spirilloxanthin could be incorporated into this complex, in contrast to the case of the complex from *Rb. sphaeroides*. The amount of carotenoid in the reconstituted complexes was 70–90% of that in the native B800-820, B800-850 complex. To correctly evaluate the efficiency of only the incorporated carotenoid, contribution of the remaining carotenoid was eliminated by subtracting the absorption (or excitation) spectrum of the complex before reconstitution from the absorption (or excitation) spectra shown in Fig. 5, after normalizing all the band intensities in the manner described above. The resultant spectra are shown in Fig. 6. It should be noted that the bands around 590 nm are not seen because of the spectral subtraction. The B800-820, B800-850 complex from normally cultured *Ch. vinosum* mainly contains rhodopin [13], and the efficiency of energy transfer is 32% (Table I). The efficiency in the B800-820, B800-850 complex reconstituted with rhodopin is 29% (Fig. 6D). This value is close to that for the native complex, indicating again that the reconstitution was successfully carried out.

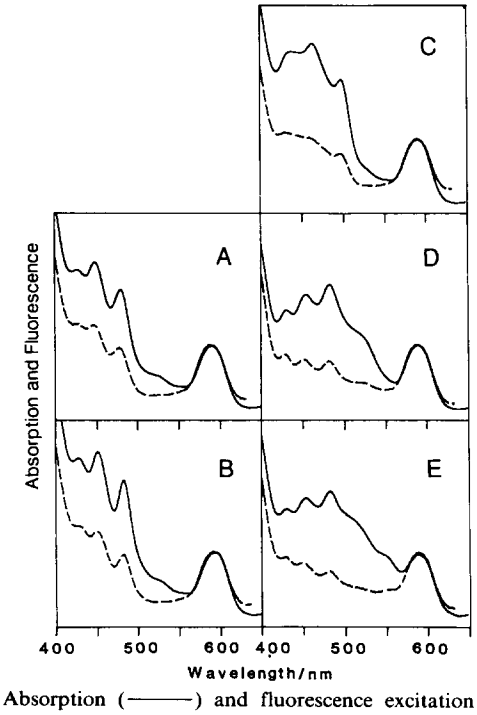

Fig. 5. Absorption (———) and fluorescence excitation (- - - - - -) spectra of the B800-820, B800-850 complex from carotenoid-deficient *Ch. vinosum* reconstituted with neurosporene (A), methoxyneurosporene (B), spheroidene (C), rhodopin (D) and spirilloxanthin (E). All the spectra are normalized at the Q_x band of Bchl around 590 nm.

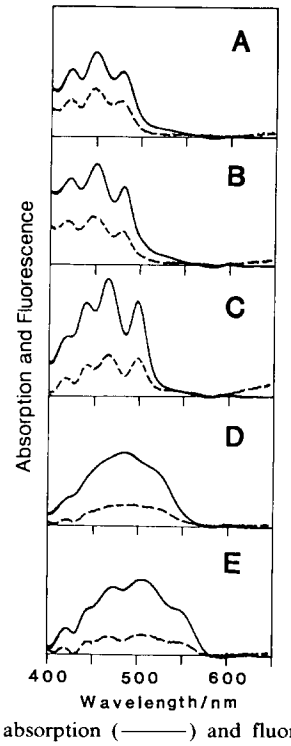

Fig. 6. Subtracted absorption (———) and fluorescence excitation (- - - - - -) spectra of the B800-820, B800-850 complex of *Ch. vinosum* reconstituted with neurosporene (A), methoxyneurosporene (B), spheroidene (C), rhodopin (D) and spirilloxanthin (E). The absorption (or excitation) spectrum of the carotenoid-deficient complex (Fig. 3B) was subtracted from the absorption (or excitation) spectra of the reconstituted complexes (Fig. 5) after normalization at the Bchl Q_x band.

The efficiencies of energy transfer from carotenoids to Bchl in the reconstituted complexes are summarized in Table II. In the B870 complex from the blue-green mutant of *Rb. sphaeroides*, neurosporene ($n_{C=C} = 9$) and methoxyneurosporene ($n_{C=C} = 10$) show an equal efficiency of 70%, and spheroidene ($n_{C=C} = 10$) shows an efficiency of 72%, while rhodopin ($n_{C=C} = 11$) shows a lower efficiency of 43%. In the B800-820, B800-850 complex from DPA-cultured *Ch. vinosum*, the efficiency of neurosporene and methoxyneurosporene are 56 and

TABLE II

Energy-transfer efficiencies from carotenoids to bacteriochlorophyll in the reconstituted light-harvesting complexes

Values are expressed as %; n.b., not bound.

Apoprotein	Carotenoid [a]				
	Neu(9)	MeO-neu(9)	Sph(10)	Rho(11)	Spi(13)
Rb. sphaeroides B870 complex	70 [b]	70	72	43	n.b.
Ch. vinosum B800-820, B800-850 complex	56	44	37	29	27

[a] Neu, neurosporene; MeO-neu, methoxyneurosporene; Sph, spheroidene; Rho, rhodopin; and Spi, Spirilloxanthin. The number of conjugated C=C bonds is given in parentheses.
[b] Experimental error is ±3%.

44%, respectively. It has been reported that the efficiency of chloroxanthin ($n_{C=C} = 9$) intrinsically contained in this carotenoid-deficient LH complex is about 50% [12]. The efficiencies of spheroidene, rhodopin and spirilloxanthin ($n_{C=C} = 13$) in this complex are 37, 29 and 27%, respectively.

The resonance Raman spectra (1100–900 cm^{-1}) of the carotenoids bound to the reconstituted LH complexes are shown in Fig. 7. All the spectra are normalized at the intensity of the methyl rocking band at about 1005 cm^{-1}. It is clearly seen in Fig. 7 that the relative intensities of the 960 cm^{-1} band are more dependent on apoproteins than the carotenoid species. The carotenoids bound to the B870 complex from the *Rb. sphaeroides* blue-green mutant show relatively broad bands with weak or medium intensities, while those bound to the B800-820, B800-850 complex from DPA-cultured *Ch. vinosum* show sharp, strong bands. Therefore, it is reasonable to consider that the polyene-chain

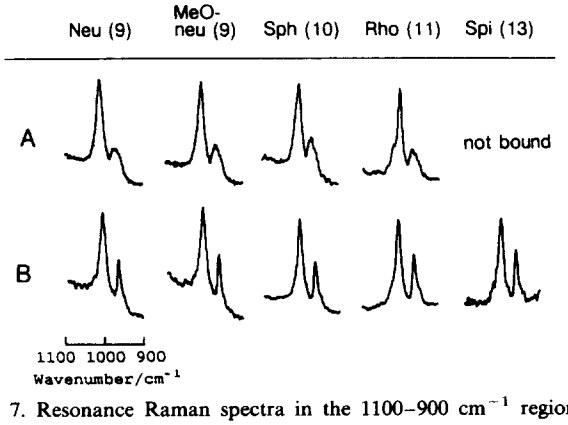

Fig. 7. Resonance Raman spectra in the 1100–900 cm^{-1} region of carotenoids incorporated into the B870 complex from the carotenoid-less mutant of *Rb. sphaeroides* (A) and the B800-820, B800-850 complex from the carotenoid-deficient *Ch. vinosum* (B). From left to right, the spectra of neurosporene (Neu), methoxyneurosporene (MeO-neu), spheroidene (Sph), rhodopin (Rho) and spirilloxanthin (Spi) are shown. Spirilloxanthin was not bound to the B870 complex from *Rb. sphaeroides*. The number of conjugated C=C double bonds are shown in parentheses. Intensities are normalized at the methyl rocking band (approx. 1005 cm^{-1}).

structures of carotenoids are determined by their apoproteins, and are distorted slightly in the B870 complex of *Rb. sphaeroides* and more distorted in the B800-820, B800-850 complex of *Ch. vinosum*.

Absorption maxima of carotenoids in the LH complexes

Carotenoids exhibit different absorption maxima in different LH complexes. Table III shows the absorption maxima of spheroidene in three kinds of LH complexes, together with the efficiencies of energy transfer. The B800-850 complex of *Rb. sphaeroides* was prepared from a wild-type strain. Since spheroidene dominates more than 90% of carotenoids in this complex [7], it greatly determines the absorption maxima and the efficiency. The other values were obtained from spheroidene incorporated into the B870 complex from the carotenoid-less mutant of *Rb. sphaeroides* and the B800-820, B800-850 complex of DPA-cultured *Ch. vinosum*. the 0–0 absorption peaks were found at wavelengths longer than those observed in *n*-hexane by 14, 21 and 26 nm, respectively, in the B800-820, B800-850 complex

TABLE III

Absorption maxima and energy-transfer efficiencies of spheroidene in the light-harvesting complexes

Complex	Absorption maxima [a] (nm)			Efficiency (%)
Ch. vinosum B800-820, B800-850 complex [b]	442 (16)	466 (14)	498 (14)	37
Rb. sphaeroides B870 complex [c]	448 (22)	471 (19)	505 (21)	72
Rb. sphaeroides B800-850 complex	450 (24)	477 (25)	510 (26)	87

[a] The difference from absorption maxima in *n*-hexane is given in parenthesis.
[b] The complex reconstituted with the carotenoid-deficient complex from DPA-cultured *Ch. vinosum*.
[c] The complex reconstituted with the B870 complex from the *Rb. sphaeroides* blue-green mutant.

from *Ch. vinosum,* the B870 complex from *Rb. sphaeroides,* and the B800-850 complex from *Rb. sphaeroides.* It is noted that the energy-transfer efficiency increased from 37 to 87% in parallel with the extent of the red shifts of absorption maxima.

The red shifts of the absorption spectra of carotenoids in the LH complexes were interpreted in terms of permanent local fields due to charged amino-acid residues [38]. However, the primary structures of LH proteins [39–43] indicate that most of those proteins do not have charged amino-acid residues in the hydrophobic region where carotenoids are probably located. Although a few proteins have an arginine residue in this region [43,44], it is doubtful that this residue is ionized in the hydrophobic environment. Thus, permanent local fields are not likely to be responsible for the red shifts. Very recently, Andersson et al. [45] have suggested that the red shifts are due to dispersive interactions of carotenoids with surrounding aromatic amino-acid residues. In addition to such an effect from the protein moiety, interactions between carotenoids and Bchl might also be a cause of the red shifts. Moore et al. [46] synthesized a covalently linked carotenoid-porphyrin ester with an interchromophore distance of ≈ 4 Å, and observed the energy transfer (efficiency $\approx 25\%$) from carotenoid to porphyrin as well as red shifts of carotenoid absorption bands. In fact, close proximity of carotenoids to Bchl in the LH complexes has been suggested from the existence of triplet–triplet energy transfer from Bchl to carotenoids [47].

Determination of relative contributions of the above-mentioned causes to the red shifts observed in the absorption spectra of the LH complexes seems to be an interesting problem to be studied in the future. This would clarify implications in the parallelism found between the red shifts and the efficiency of energy transfer.

Discussion

Factors controlling the efficiency of energy transfer from carotenoids to Bchl

The present study shows that the efficiency of energy transfer from carotenoids to Bchl is related to at least three factors; carotenoid species (chemical structure of carotenoids), the nature of apoproteins of LH complexes, and the polyene-chain structure (planarity of the CH=CH moiety).

From the results presented in Table I, photosynthetic bacteria used in this study can be divided into the following two groups with respect to the energy-transfer efficiency and carotenoid composition: (1) *Rb. sphaeroides, Rb. capsulatus,* and *Rc. gelatinosus.* These bacteria show high efficiencies of energy transfer ($\geqq 70\%$) and contain spheroidene-series carotenoids (neurosporene, methoxyneurosporene, chloroxanthin, spheroidene, etc.) which have nine or ten conjugated C=C bonds. (2) *Rs. rubrum, Ch. vinosum,* and *Rp. palustris.* These bacteria show low efficiencies of energy transfer ($\leqq 50\%$), and contain spirilloxanthin-series carotenoids (lycopene, rhodopin, spirilloxanthin, etc.) which have 11 or 13 conjugated C=C bonds.

This classification seems to indicate that the carotenoid species is a significant factor determining the efficiency, and that increase in the polyene-chain length ($n_{C=C}$) lowers the efficiency. However, it is difficult at this stage to discuss the effect of the carotenoid species separately from that of apoproteins, because each bacterium contains specific apoproteins which may affect the efficiency. To investigate the effects of carotenoid species and apoproteins independently, we have examined energy transfer from extrinsic carotenoids incorporated into the LH complexes.

From the results of reconstitution experiments shown in Table II, it is clear that the efficiency of energy transfer is affected by both carotenoids and apoproteins. In the case of the LH complex from *Rb. sphaeroides,* the efficiency decreases from 70–72% for the three kinds of carotenoids with nine or ten C=C bonds to 43% for rhodopin with 11 C=C bonds. In the case of the LH complex from *Ch. vinosum,* neurosporene ($n_{C=C} = 9$) and methoxyneurosporene ($n_{C=C} = 9$) show efficiencies of 56 and 44%, respectively. Chloroxanthin ($n_{C=C} = 9$), which is accumulated in the carotenoid-deficient LH complex, shows an efficiency of 50% [12]. Thus, in the LH complex from *Ch. vinosum,* carotenoids having nine C=C bonds show efficiencies around 50%. The carotenoids having 10, 11 and 13 C=C bonds in this LH complex show efficiencies of 37, 29 and 27%, respectively. Although it is difficult to evaluate the effect of end groups in carotenoids on the efficiency, the data show a definite trend that the efficiency decreases as $n_{C=C}$ increases from 9 to 13. Therefore, we conclude that the efficiency is closely related to the number of conjugated C=C bonds; i.e., the efficiency decreases with increasing polyene-chain length when their apoprotein remains the same. In addition, each species of carotenoid shows a higher efficiency when it is incorporated into the LH complex from *Rb. sphaeroides* than when incorporated into the LH complex from *Ch. vinosum.* Thus, it is clear that the efficiency of energy transfer also depends on the nature of apoproteins.

In the LH complexes, apoproteins control many factors relating to energy transfer, e.g., the geometric arrangement between carotenoids and Bchl, the polyene-chain structure of carotenoids, etc. In this study, the polyene-chain structure of carotenoids has been examined by resonance Raman spectroscopy.

As shown in Table I, in the LH complexes showing efficiencies higher than 80%, carotenoids have planar polyene chains (weak 960 cm^{-1} Raman bands), while in those showing efficiencies lower than 30%, they have

distorted polyene chains (strong or medium 960 cm^{-1} Raman bands). Although it is difficult to express quantitatively the distortion of polyene chain, it may be possible to conclude that the distortion generally causes a decrease in the efficiency of energy transfer. The results for incorporated carotenoids strongly supports this relationship. As shown in Fig. 7, carotenoids always showed stronger Raman bands at 960 cm^{-1} (distorted polyene chains) when they were incorporated into the LH complex from *Ch. vinosum* than into the LH complex from *Rb. sphaeroides*. The efficiency of energy transfer is lower in the former case (Table II). Thus, the apoprotein of the LH complex determines the polyene-chain structure of the bound carotenoid independently of the carotenoid species, and a carotenoid with a more distorted polyene chain gives a lower efficiency. Therefore, the polyene-chain structure is one of the factors which affect the efficiency of energy transfer. The distortion of polyene chain changes the relative arrangement between carotenoid and Bchl. Also, it may affect the lifetimes and energy levels of excited electronic states of the carotenoid, which are very important factors for the energy transfer as will be discussed later.

The mechanism of energy transfer from carotenoids to Bchl

As for the physical mechanism of energy transfer from carotenoids to Bchl or Chl, two different mechanisms, namely, the Förster dipole–dipole interaction mechanism [48] and the Dexter electron-exchange mechanism [49] have been proposed. The Förster mechanism allows energy transfer from donor to acceptor over a relatively long distance, but requires a high emission quantum yield of the donor molecule. In contrast, the Dexter mechanism is limited to the transfer within a short range where electron clouds of the donor and acceptor overlap each other. Since the emission quantum yields of carotenoids are generally very low ($< 10^{-4}$) [20,23,50] and the distance between carotenoids and Bchl is probably short [46,47], the Dexter mechanism has been claimed to be more plausible for the energy transfer from carotenoids to Bchl [2,4,17,19–23].

In general, the energy-transfer efficiency (η) is expressed as:

$$\eta = K_{ET}/(K_D + K_{ET}) \quad (1)$$

where K_{ET} is the rate constant of energy transfer and K_D is the decay rate constant of the donor excited state. $1/K_D$ equals the intrinsic lifetime of the donor excited state. According to the Dexter mechanism, the rate constant of energy transfer is expressed as:

$$K_{ET} = (2\pi/\hbar)Z^2 \int F_D(\nu)\varepsilon(\nu)\, d\nu \quad (2)$$

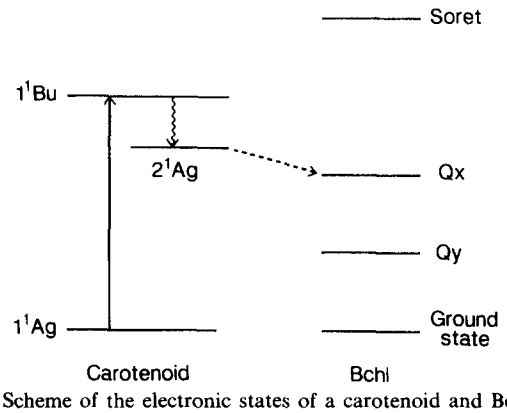

Fig. 8. Scheme of the electronic states of a carotenoid and Bchl, and the flow of the excited energy.

where $F_D(\nu)$ is the normalized spectral distribution of the donor emission, $\varepsilon(\nu)$ is the normalized spectral distribution of the acceptor absorption, and Z is a factor determined by the relative arrangement between donor and acceptor [17,49]. From the Eqns. 1 and 2, the energy-transfer efficiency is affected by the lifetime of the donor excited state ($1/K_D$) and the overlap between energy levels of the donor and acceptor ($\int F_D(\nu)\varepsilon(\nu)\, d\nu$); i.e., a longer lifetime and a larger overlap result in a higher efficiency.

The electronic states of a carotenoid and Bchl are schematically shown in Fig. 8. A carotenoid molecule is excited from the ground state to the allowed 1^1B_u excited electronic state by absorbing light. Then, this state rapidly relaxes to the forbidden 2^1A_g excited electronic state by internal conversion. Since the lifetime of the 1^1B_u state is thought to be extremely short (< 1 ps) [23,50] and the energy level of the 2^1A_g state of β-carotene is close to that of the Bchl Q_x state [51], the energy transfer to Bchl is thought to occur from the 2^1A_g state [2,4,16,17,19–23] (Fig. 8). However, Snyder et al. [18] have predicted from results for shorter polyenes that the energy level of the 2^1A_g state of β-carotene is lower than the Q_x state, and argued in favor of the possibility of energy transfer from the 1^1B_u state. Further, some photosynthetic bacteria contain carotenoids longer than β-carotene, which have energy levels of the 2^1A_g state lower than that of the Q_x state, and have some overlap of the 1^1B_u state with the Q_x state (see below). Thus, more experimental support is needed before it can be concluded that the energy transfer proceeds via the 2^1A_g state. Dependence of the energy-transfer efficiency on the polyene chain length demonstrated in the present study seems to provide important information for the discussion of the energy-transfer mechanism.

The lifetime of the 2^1A_g state of carotenoids is expected to be shorter for a longer carotenoid, because increase in $n_{C=C}$ lowers the quantum yield of emission from the 2^1A_g state [18]. In fact, it has been reported that the lifetime of the 2^1A_g state of β-8′-apocarotenal,

β-carotene and canthaxanthin (longer in this order) are 25.4, 8.4 and 5.2 ps, respectively [20]. Values of 17230 cm^{-1} [51] and 16600 cm^{-1} [52] have been reported for the energy level of the 2^1A_g state of β-carotene. Although these values are not in exact agreement, they are quite close to the Bchl Q_x state (approx. 16900 cm^{-1}). As $n_{C=C}$ increases from 9 to 13, it is expected that the energy level of the 2^1A_g state becomes lower than that of the Q_x state, and that the separation between the two energy levels increases. Therefore, the overlap between the energy levels of the 2^1A_g and Q_x states is also expected to become smaller with increasing $n_{C=C}$. On the other hand, the absorption spectra (due to the transition from the ground state to the 1^1B_u state) indicate that increase in $n_{C=C}$ from 9 to 13 lowers the energy level of the 1^1B_u state from about 20000 to 18000 cm^{-1}. As a result, the level of the 1^1B_u state would approach the Q_x level of Bchl, and the overlap between the two levels would increase.

The above considerations lead to a conclusion that, if the excited energy is transferred from the 2^1A_g state, increase in $n_{C=C}$ would decrease the efficiency, while if the 1^1B_u state is the energy-transferring state, increase in $n_{C=C}$ would increase the efficiency. Our result, i.e., decrease in the efficiency with increasing $n_{C=C}$, is consistent with the former case. Thus, the experimental results obtained in this study give strong, if not definitive, support to the energy-transfer mechanism via the 2^1A_g state.

Finally, in order to make clearer the views described above, we show diagrammatically in Fig. 9 the interrelationships among various factors controlling the efficiency of energy transfer. The length of polyene chain affects the lifetime and energy level of the 2^1A_g state and, as a result, controls the energy transfer through K_D and $F_D(\nu)$ in Eqns. 1 and 2. Apoproteins determine the distance and orientation between carotenoids and Bchl, and control the efficiency through parameter Z in Eqn. 2. Furthermore, the polyene-chain structure of carotenoids is determined by apoproteins, and affects the relative arrangment between carotenoids and Bchl (effect on Z) and possibly the lifetime and energy level of the 2^1A_g excited state (effects on K_D and $F_D(\nu)$).

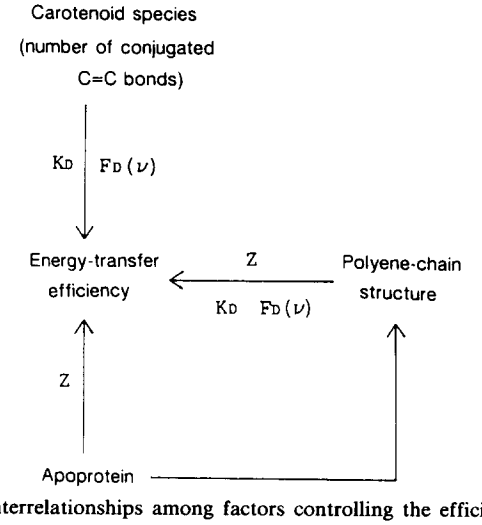

Fig. 9. Interrelationships among factors controlling the efficiency of energy transfer from carotenoids to Bchl. K_D, the decay constant of the donor excited state; $F_D(\nu)$, the normalized spectral distribution of the donor emission; and Z, a factor determined by the relative arrangement between donor and acceptor (see text).

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