Pure & Appl. Chem., Vol. 63, No. 1, pp. 123–130, 1991.
Printed in Great Britain.

Carotenoids in photosynthesis: absorption, transfer and dissipation of light energy

Mamoru Mimuro[1] and Tetzuya Katoh[2]

[1]National Institute for Basic Biology, Myodaiji, Okazaki, Aichi 444 and [2]Department of Botany, Faculty of Science, Kyoto University, Kyoto 606 (Japan).

Abstract - The functioning of carotenoids in photosynthesis is discussed in relation to the reaction mechanism. The energy transfer process from the allenic carotenoid, fucoxanthin, to chl *a* was intensively investigated in the newly isolated fucoxanthin-chl *a*/*c* protein assembly (FCPA) from a brown alga *Dictyota dichotoma*. The transfer time was shorter than 3 ps at 15°C. The energy level responsible for transfer may not be the Qx band of chl *a*, contrary to the proposal for the transfer in the bacterial antenna system.

INTRODUCTION

Carotenoids are involved in several aspects of photosynthesis, notably light absorption and energy transfer to the reaction center (RC) complex and protection of the photosynthetic apparatus from damage by strong illumination. As these phenomena of function become clear, understanding of the reaction mechanisms becomes of major importance.

The distribution and localization of carotenoids in plant cells are now established (ref. 1). The chemical structures of almost all the carotenoids in the photosynthetic organelles are known. Their spectroscopic characteristics in various organic solvents have been analyzed in relation to their structure (ref. 2). However, these characteristics *in vivo* are different from those in the free form. Functional carotenoids are bound to apoproteins, giving rise to the pigment-protein complexes. Carotenoids in the complexes change their properties by interaction with polypeptides; this can be regarded as a "static tuning process" to give the functional form. This step is very critical because of its close relation to the dynamic reaction mechanism, but almost nothing is known about it. Our primary aim is the survey of this tuning process. The mechanism should be known at the molecular level. Thus, the molecular structure *in vivo* is very important for this approach. The main method of study is physical or physicochemical analysis.

In the functioning of carotenoids in photosynthesis, three processes and mechanisms are essential. 1. Light absorption; the main point is a red shift in the absorption maxima of the components. This seems to be an adaptive process to match the energy level for energy transfer, which is realized by interaction with a charged group of a polypeptide (ref. 3) or an electric field effect across the membranes (ref. 4). 2. Energy transfer; the main point is a transfer mechanism. There are two proposed mechanisms for this process, dipole-dipole interaction (ref. 5) or electron exchange interaction (ref. 6). The dynamic aspects of this process are still almost unknown. 3. Dissipation; the main point is the dynamic aspect of triplet-triplet (T-T) energy transfer in the RC. The T-T transfer is known to occur by an electron exchange mechanism (ref. 6) and this process is significantly related to the singlet-singlet (S-S) energy transfer which may be functional in the antenna system. The above three processes include various features relevant to the function of carotenoids in every kind of organism. Study of the reaction mechanisms of carotenoids in photosynthesis can, therefore, give rise to basic concepts about the functional reactions of carotenoids in general. The current status of the research on the absorption and dissipation processes will be summarized briefly and the energy transfer process then discussed in detail.

LIGHT ABSORPTION BY CAROTENOIDS IN THE COMPLEXES

The location of the absorption maximum of carotenoids depends on the number of conjugated double bonds; in the case of 9 double bonds (β-carotene), it is located around 450 nm in non-polar organic solvents. In the photosynthetic apparatus, the maxima are usually shifted to the red by ca. 30 nm ($\simeq$1500 cm^{-1}) (ref. 3). One possible explanation of this red shift is the presence of a point charge on the polypeptide (ref. 3). Another could be the electric field generated by the flow of electrons among

Abbreviations used: Bchl, bacteriochlorophyll; chl, chlorophyll; kDa, kilodalton; FCPA, fucoxanthin-chl *a*/*c* protein assembly; PS, photosystem; RC, reaction center; S, singlet; T, triplet.

the electron transfer components in the thylakoid membranes (ref. 4). The magnitude of the shift caused by the latter is only a few nm ($\simeq$50 cm^{-1}) (ref. 4), however, compared with the much larger red shift that is actually observed, so a different explanation becomes necessary. The point charge hypothesis is attractive for this, but there is no structural evidence of its existence in the actual antenna system.

A carotenoid shift due to the electric field is observed in all the organisms that have so far been investigated (ref. 7, 8). Especially in the case of purple phototrophic bacteria, the shift is oberved only in LH2 (so-called B800-B850); LH1 (so-called B875 or B880) is not responsible for the shift. Both complexes consist of two polypeptides (α and β) with membrane-spanning α helix regions (ref. 9). The primary structures of the respective polypeptides of LH1 and LH2 are similar (ref. 9). Of course, some difference is observed in the sequence. The field effect is a long-distance effect, so there is no reason for the accumulation of an electric field on LH2. These results suggest that the small difference in the properties of the peptides is a critical factor. Substitution of even one amino acid could be responsible for the occurrence of the carotenoid shift.

The shift of the apparent absorption maximum is closely related to the relaxation of this absorption. It is known that the apparent absorption maximum corresponds to ^{1}Bu state; a second state, symmetry forbidden, is called ^{2}Ag, and the probability of transition to this state is substantially zero (ref. 10). The energy difference between these two states is reported to be about 3500 cm^{-1} in the case of up to 7 conjugated double bonds (ref. 11) and for spheroidene (10 conjugated double bonds) (ref. 12). If this energy difference is maintained in the carotenoids when they are bound to the photosynthetic organelles, the energy level of the ^{2}Ag state becomes close to that of the Qx band of chl or Bchl. The Qx band is believed to be the energy level responsible for energy transfer to chl or Bchl. Therefore, the red-shift of the maximum is critical for the energy transfer process, though the exact reason for the shift is not understood. The point charge (ref. 3) or polarizability of the peptide moiety (ref. 12) may be the main cause. This question may be resolved by X-ray crystallography of the antenna complex isolated from purple phototrophic bacteria (ref. 13).

PROTECTION OF THE PHOTOSYNTHETIC APPARATUS AGAINST LIGHT-INDUCED DAMAGE

There are several clear pieces of evidence for a protective effect of carotenoid against damage due to high photon density. Inhibition of carotenoid biosynthesis induces a lethal effect under natural environmental conditions (ref. 14), similar to that seen with carotenoid-less mutants. It is known that the molecular mechanism for the photo-protection is not simple. At least three reaction schemes have been suggested, namely quenching of triplet state, superoxide and singlet oxygen.

The first mechanism, triplet quenching, is observed in the RC. When photochemical charge separation is interrupted, a chl triplet is formed in about 1 μs (ref. 15). The energy of this chl triplet state is transferred to carotenoids by T-T transfer. The molecular arrangement of carotenoids in the bac-

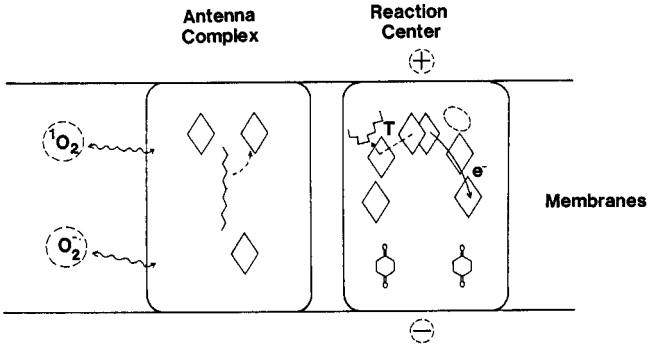

Fig. 1. Schematic representation of the functions of carotenoids in photosynthesis, i.e., triplet quenching in the RC complex, light absorption and energy transfer in the antenna complex. Two molecular species (singlet oxygen and superoxide anion radical) that are formed in the membrane diffuse and attack carotenoids. Each rhombus indicates a molecule of (B)chlorophyll or (B)pheophytin. A circle in the RC indicates the likely binding site of a second carotenoid molecule in the D1-D2 complex. The signs + or – outside the membrane indicate the electric field generated by the electron flow in the membrane.

terial RC is clear; 1,2-dihydroneurosporene is found close to the accessory Bchl in the M-subunit of *Rhodopseudomonas viridis* (ref. 16). The T-T transfer time is much faster than that of Bchl triplet formation, thus the kinetics we observe in the RC refer to the process that yields Bchl triplet. The T-T transfer kinetics will give information on the S-S transfer from carotenoids to Bchl, but neither process is clear at this stage.

In the case of RC II of higher plants, two β-carotene molecules are associated with the D1-D2 polypeptide (K. Satoh et al., unpublished). Since the primary structures of the D1 and D2 polypeptides are homologous to those of the L and M subunits of the bacterial RC, respectively, the probable attachment site of one carotenoid is close to the accessory chl in the D2 subunit. The position of the second carotenoid might be in the D1-side, which is lost in the bacterial RC but is present in the RC of green plants. The configuration of the first β-carotene is reported to be all-*trans* (ref. 17), unlike that of the carotenoid in the bacterial RC. The D1-D2 polypeptide is known to be released by treatment with a high concentration of detergent (ref. 18), and this might cause loss of the native configuration. In the case of RC I of higher plants, formation of a carotenoid triplet is known, but the reaction mechanism is unclear due to lack of any structural information.

There are two other mechanisms, which involve carotenoid photobleaching for the protection against damage caused by superoxide and by singlet oxygen. The former is formed at the reducing side of PS I (ref. 19). Most of it is converted into hydrogen peroxide and oxygen by the enzyme, superoxide dismutase (ref. 19). However, part of the superoxide can diffuse within the thylakoid membranes, leading to reactions that bleach carotenoids. Singlet oxygen is formed via some sensitizers, e.g. pheophorbide (ref. 20) or protoporphyrin (ref. 21), which could be present in the membrane even if their contents are low. Singlet oxygen thus produced can be quenched by, or react with, carotenoids.

CLASSIFICATION OF CAROTENOID-PROTEINS IN THE ANTENNA SYSTEM

The presence of carotenoids in the pigment-protein complexes raises several essential points about their function *in vivo*. The energy transfer from carotenoids to (B)chl is observed in many kinds of photosynthetic organisms, from anaerobic bacteria to higher plants. However, it is not reasonable to apply exactly the same idea or mechanism to different organisms, because the molecular structures of the carotenoids or of polypeptides may be different. Before we begin to consider the transfer mechanism, we must reinvestigate the molecular organization of the antenna systems in various photosynthetic organisms.

In the case of phototrophic bacteria, especially the purple non-sulfur bacteria, linear polyenes (spirilloxanthin, spheroidene, spheroidenone, lycopene and rhodopin) are the major light-harvesting carotenoids, which are bound to a polypeptide which has a membrane-spanning α helix structure. The molar ratio of carotenoids to Bchl is 2 : 3 in LH2 (ref. 22). Similar features are also found in LHCII of higher plants. Lutein, a simple polyenic xanthophyll, is bound to a membrane-spanning α helix. The molar ratio of carotenoid to chl (chl *a* + chl *b*) is known to be less than 0.5 (ref. 1). Contrary to these examples, the fucoxanthin-chl or peridinin-chl complexes are very different. The structures of these carotenoids contain an allene group, and the polypeptides are water-soluble (peridinin-chl complex). The polypeptide may be globular, in which case the fully stretched polyene structure cannot be closely attached to the polypeptide. An allene-type structure may fit well to these globular polypeptides. The molar ratio of carotenoids to chl is in the range from almost one to more than 4. In this case, molecular interaction between carotenoids is possible. A similar molecular structure could be applicable to the fucoxanthin-chl complex.

The above features clearly suggest that the energy transfer process and thus the transfer mechanism should be considered separately in allenic carotenoids. Therefore, we have extensively investigated this type of complex; procedures for their isolation, and the energy transfer process in which they participate.

FUCOXANTHIN-CHL *a/c* PROTEIN ASSEMBLY (FCPA)

Fucoxanthin is found in many orders of algae: Phaeophyta, Bacillariophyta, Chrysophyta, Raphidophyta, Haptophyta and Dinophyta (ref. 1). The light absorbed by fucoxanthin is as effective for driving photosynthesis as the light absorbed by chl *a* itself (ref. 23). Isolation of their pigment-protein complexes has been attempted with partial success, by using many kinds of detergents (ref. 1). In the case of the diatom *Pheodactylum tricornutum* (ref. 24), the isolated complex consists of a 16.5 kDa polypeptide as a unit. The pigment content per unit peptide was reported to be 1.0 : 0.09 : 0.28 : 2.22 for chl *a* : chl c_1 : chl c_2 : fucoxanthin. The absorption maxima were located at 670 nm (chl *a*) and around 500 nm (fucoxanthin). Compared with the absorption spectrum, the fluorescence excitation spectrum for chl *a* emission was different in the contribution of the fucoxanthin region, due to the blue-shift of the

fucoxanthin in the isolated complexes. This situation becomes more clear when the spectrum of intact cells is compared, showing that the most important criterion for the intactness of the isolated complex is the absence of the blue-shift of carotenoid absorption (Fig. 2).

In 1989, we reported a new isolation method for fucoxanthin-chl complexes from a brown alga *Dictyota dichotoma* (ref. 25). The most critical point is the detergent. We used a sugar ester, decanoyl-sucrose, which is a very mild detergent that permits selective solubilization of the complexes. The isolated complex shows a high molecular mass on a sucrose density gradient and is brownish-orange in color, turning green on standing at room temperature. After the color changes, the apparent molecular mass on the sucrose density gradient is much smaller. These results show two essential points about the complex. One is that the pigment-protein complex forms an assembled form, and the second, that the blue-shift of the carotenoid is accompanied by dissociation of the assembled form. This explains why the complexes previously isolated did not retain the spectroscopic properties of the native complexes; most of the isolated complexes had already been dissociated. The assembly is essential for the functioning of the complexes. Based on these observations, we propose to call this functional form the fucoxanthin-chl *a*/*c* protein assembly (FCPA).

Our recent study on the FCPA isolated from other species shows that, even in the dissociated form, the blue-shift was not observed. This suggests that the band shift of the absorption maxima and the dissociation are independent (T. Katoh, unpublished).

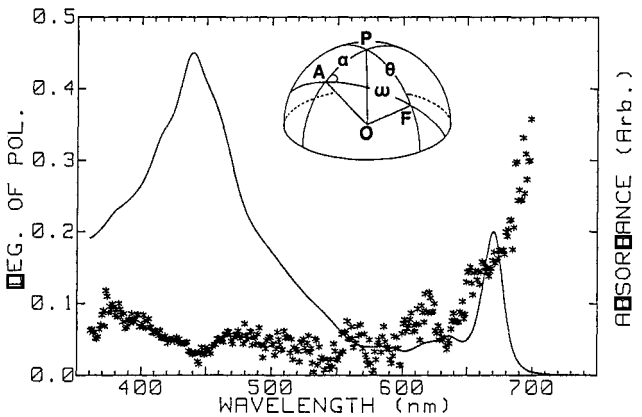

Fig. 2. Absorption spectrum (full line) and excitation polarization spectrum (asterisks) of FCPA at 15°C. Fluorescence was monitored at 715 nm. In the coordinate system, A indicates transition moment of the donor molecule (fucoxanthin) and F, that of the acceptor molecule (chl *a*).

DISTRIBUTION OF FCPA IN FUCOXANTHIN-CONTAINING ALGAE

The FCPA are found in many classes of algae that contain fucoxanthin (T. Katoh, unpublished). Thus it can be regarded as a common functional form of fucoxanthin in the antenna system. The molecular masses of the unit peptides seem to be similar in all cases (17 to 19 kDa). When the sequences of these unit peptides are known, evolutionary trends among the peptides can be deduced.

BIOCHEMICAL PROPERTIES OF FCPA ISOLATED FROM *D. DICHOTOMA*

Several features of the FCPA isolated from a brown alga *D. dichotoma* will be described, as an example of FCPA. Of course minor but critical differences in the spectroscopic characteristics and assembly forms are observed in different species.

The FCPA of *D. dichotoma* shows an apparent molecular mass of 590 kDa including the pigments. Its shape consists of a bundle of unit peptides with the dimensions 10.2 nm in height and 11.2 nm in diameter (ref. 26). In their center, a small hole was observed, around which seven or eight subunits were assembled. When a detergent such as 0.02 % of Triton X-100 was added, this assembly was dissociated into smaller subunits with a molecular mass of 76 kDa. At the moment, we do not have any structural data on the FCPA.

The molecular mass of the FCPA unit peptide was 17.3 kDa; its amino acid composition has been reported (ref. 25). The content of hydrophobic amino acids is high, as is the case with other membrane proteins. A notable feature was a small amount of histidine (3 per unit peptide), which could be a ligand for chl. The pigment composition per dimer was 13 : 3 : 10 : 1 for chl *a* : chl *c* : fucoxanthin : violaxanthin. Thus, it is reasonable to assume that amino acids other than histidine must also be a ligand of chl in the FCPA.

The FCPA consists of seven identical subunits (ref. 26). This stoichiometry is unusual, compared with the unit structure of other antenna complexes. For example, the LHCII in higher plants (ref. 27) and C-phycocyanin in cyanobacteria (ref. 28) show C_3 symmetry and LH2 in the bacterial antenna system shows C_6 symmetry (ref. 9) At this experimental stage, it is not known if there is a common assembly form of FCPA; it could be species dependent. A comparative study of this point is very critical.

BIOPHYSICAL PROPERTIES OF FCPA ISOLATED FROM *D. DICHOTOMA*

The FCPA is brownish-orange in color, indicating the absence of a blue-shift of fucoxanthin. The absorption maxima at 15°C were observed around 670 nm (chl *a*), 633 nm (chl *c*), 510 nm (fucoxanthin) and the Soret bands of chls (Fig. 2). Note that the absorption of fucoxanthin is extended to 540 nm, indicating a large red-shift of the absorption spectrum. The fluorescence excitation spectrum of chl *a* is very similar to the absorption spectrum, indicating energetically tight coupling among the pigments. The quantum yield of fucoxanthin was almost equal to that of chl *a*. Triton-induced dissociation gave a blue-shift of the fucoxanthin, as shown by the difference spectrum (ref. 29). After dissociation, energy transfer from fucoxanthin to chl *a* hardly occurred. One important feature of energy transfer in the FCPA is that chl *c* is not a mediator to chl *a* (ref. 29). When fucoxanthin was excited, fluorescence from chl *c* was never observed; the energy level of chl *c* is in between those of fucoxanthin and chl *a*, so energy transfer to chl *a* is independent.

The energy transfer process will be more clearly resolved by time-resolved spectroscopy. We have attempted to detect fluorescence from fucoxanthin in the FCPA, but we could not detect any fluorescence in the wavelength region from 550 to 600 nm (Mimuro *et al*, unpublished). Even in the case of isolated fucoxanthin, fluorescence was not detectable, contrary to the case of β-carotene (ref. 30). The fluorescence properties of allenic carotenoids may be different from those of the linear polyenes. Transient absorption spectroscopy indicates that the transfer time from fucoxanthin to chl in the cells of *P. tricornutum* is in the range of 200 femtoseconds (fs) (ref. 31).

MECHANISM OF ENERGY TRANSFER FROM CAROTENOIDS TO CHLOROPHYLL

The Förster model (ref. 5), that is, inductive resonance, has been thought to be inadequate to explain the energy transfer from carotenoids; the main reason is that excited carotenoid is a short-lived species and is not fluorescent. The second point is that the energy level of the acceptor pigments (chl or Bchl) must be lower than that of the apparent absorption maximum of the donor; an energy level lower than that corresponding to the apparent absorption maximum should be present for the efficient energy transfer. These points must be included when the electron exchange mechanism (ref. 6) is applied to this transfer process. One important requisite is the close location of the donor-acceptor pair, because electron exchange can be achieved only by contact of the electron clouds of the two compounds. Taking these situations into account, the currently most promising hypothesis for the energy transfer is an electron exchange mechanism through the symmetry forbidden energy level (^{2}Ag) to the (B)chl Qx band. This hypothesis must be checked in more detail.

The presence of the ^{2}Ag state has been detected by time-resolved Raman spectroscopy (ref. 32) in all-*trans* β-carotene. Its presence was confirmed in chromatophores isolated from *Rhodobacter sphaeroides* (ref. 33). Thus, this could be the energy level responsible for the transfer. The significance of the Qx level is shown in the bacterial antenna system. When carotenoid-chl complexes were reconstituted, the transfer efficiency was highest with carotenoids for which the energy level of the ^{2}Ag state matches the Qx level of antenna Bchl. In this case, the linear polyene structure is somewhat distorted by interaction with the polypeptide, indicating "static tuning". These results clearly indicate that the above mentioned hypothesis for the transfer is correct. The actual process can be monitored by kinetic analysis.

The above process may be applicable to carotenoids with a linear polyene structure, e.g. lutein in LHCII, and β-carotene in the RC I complex. However, there is no real basis for its application to the allenic carotenoids. Therefore the energy transfer process in the FCPA must be investigated.

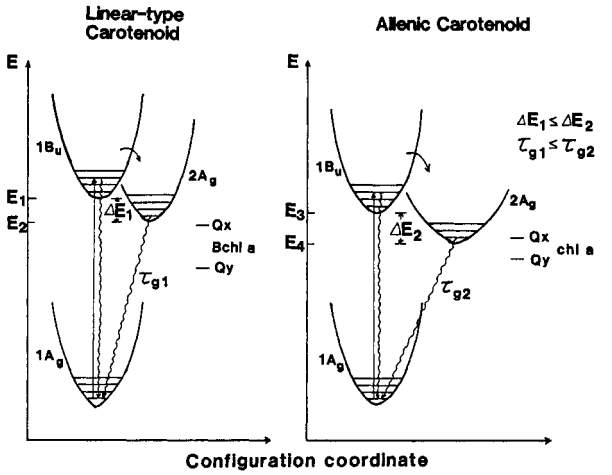

Fig. 3. A working hypothesis for the energy transfer from an allenic carotenoid to chl *a* in comparison with the transfer from a linear-type carotenoid to Bchl. ΔE, energy gap between the ^{1}Bu and ^{2}Ag states; τ_g, ground-state recovery time; Qx and Qy, the energy levels of the Qx and Qy transitions of (B)chl.

ENERGY LEVEL RESPONSIBLE FOR THE TRANSFER

We analyzed the energy level responsible for the transfer, by polarized fluorescence spectroscopy. Figure 2 shows the excitation polarization spectrum of FCPA at 15°C. When the chl *a* fluorescence was monitored at 715 nm, it was possible to deduce the transfer sequence from the degree of polarization. Every time energy is transferred, the degree of polarization of the donor molecules becomes close to zero due to a depolarization effect. When we compared the energy level of fucoxanthin (in the wavelength region from 475 to 550 nm) with that of the chl *a* Qx transition (580 to 600 nm), they were observed to be almost the same. This indicates that the transition moments of the two are nearly parallel, a very unusual property.

In the absorption spectrum of chl *a* (cf. Fig. 2), three progressive absorption bands were found at 670, 625 and 585 nm, and have been assigned to the Qy(0-0), Qy(0-1) and Qx(0-0) transitions. However, the fluorescence polarization spectrum (ref. 34) clearly indicates that the Qx transition is located around 635 nm, where the degree of polarization is almost zero. The real value of the Qx transition should be negative, as deduced from the coordinates of the molecular axis. When we assume that energy transfer occurs from fucoxanthin to the Qx transition of chl *a*, the mutual orientation of the transition moments between donor and acceptor is estimated to be larger than 70 degrees. This is regarded as an unfavourable condition. Thus it is reasonable to assume that the probable transfer pathway is from fucoxanthin to a higher vibrational level of the Qy transition of chl *a*.

A similar spectrum of fluorescence polarization was also observed for the peridinin-chl *a* protein isolated from a marine dinoflagellate, *Amphidinium carterae* (Plymouth 450) (ref. 34). In this case, the degree of polarization of peridinin is clearly higher than that of the Qx transition of chl *a*. Therefore, transfer through the Qx transition of chl *a* does not seem to occur.

The above results clearly indicate that energy transfer from fucoxanthin to chl *a* is mediated not by the Qx transition, but through another transition [Qy or a band of different assignment (cf. ref. 35)] whose absorption maximum is located at wavelengths longer than 600 nm. This is in contrast to the case of the bacterial antenna system in which the Qx transition may play an essential role in the transfer.

We propose a working hypothesis for energy transfer from allenic carotenoids (Fig. 3). There are two major differences compared to the situation with simple linear polyene carotenoids. One is a lower energy level of the ^{1}Bu state, the other a larger energy gap between the ^{1}Bu and ^{2}Ag states. The potential surface of ^{2}Ag may be different from that of the ^{1}Ag or ^{1}Bu states. A longer ground-state recovery time for fucoxanthin [30 ps (ref. 31)] than for the linear carotenoids [10 ps (ref. 36)] may support the above hypothesis. Work is now in progress to detect the ^{2}Ag state in allenic carotenoids.

FUTURE PROSPECTS

Developments over the past three years in the study of the function of carotenoids are enhanced by crystallographic data for the bacterial RC (ref. 37) and light-harvesting complex (ref. 13). Spectrosocopy with time resolution of several hundreds of fs will provide direct information on the transfer kinetics. Theoretical investigation either of dipole-dipole (or quadropole) interaction or of electron exchange interaction would be critical for the understanding of the observed phenomena.

REFERENCES

1. D. Siefermann-Harms, *Biochim. Biophys. Acta, 811*, 325-355 (1985).
2. H. Pfander, *Key to Carotenoids*, 2nd Edn., Birkhäuser, Basel, 1987.
3. T. Kakitani, B. Honig and A.R. Crofts, *Biophys. J., 39*, 57-63 (1982).
4. S. Schmidt, R. Reich and H.T. Witt, *Proc. II International Congr. Photosynthesis Res.*, pp. 1087-1090, Jung, The Hague, 1972.
5. T. Förster, *Ann. Phys. Leipzig*, 2, 55-75 (1948).
6. D.L. Dexter, *J. Chem. Phys., 21*, 836-850 (1953).
7. D.C. Fork and J. Amesz, *Photochem. Photobiol., 6*, 913-918 (1967).
8. H. Inoue, K. Sugawara and M. Nishimura, *Proc. VII Internatl. Seaweed Symp.*, (K. Nishizawa, ed.) pp. 515-520, Univ. Tokyo Press, Tokyo, 1972
9. H. Zuber, *Photochem. Photobiol., 42*, 821-844 (1985).
10. R.J. Thrash, H. L-B. Fang and G.E. Leori, *Photochem. Photobiol., 29*, 1049-1050 (1979).
11. R. Snyder, E. Arvidson, C. Foote, L. Harrigan and R.L. Christensen, *J. Am. Chem. Soc., 107*, 4117-4122 (1985).
12. T. Gillbro, P. Andersson and R.J. Cogdell, *Current Research in Photosynthesis*, Vol. II. pp. 11-16, Kluwer, Dordrecht, 1990.
13. M.Z. Papiz, A.M. Hawthornthwaite, R.J.Cogdell, K.J. Woolley, P.A. Wightman, L.A. Ferguson and J.G. Lindsay, *J. Mol. Biol., 209*, 833-835 (1989)
14. K-J. Kunert and P. Böger, *Z. Naturforsch., 34c*, 1047-1051 (1979).
15. H.T. Witt, *Quart. Rev. Biophys.*, 365-477 (1971).
16. J. Deisenhofer and H. Michel, *EMBO J., 8*, 2149-2170 (1989).
17. M. Fujiwara, H. Hayashi, H. Tasumi, M. Kanaji, Y. Koyama and K. Satoh, *Chem. Lett.*, 2005-2009 (1987).
18. O. Nanba and K. Satoh, *Proc. Natl. Acad. Sci. USA., 84*, 109-112 (1987).
19. M. Takahashi and K. Asada, *Arch. Biochem. Biophys., 267*, 714-722 (1988).
20. S. Kimura and Y. Takahashi, *Photomed. Photobiol., 3*, 73-74 (1981).
21. A.A. Krasnovsky, Jr., *Photochem. Photobiol., 29*, 29-36 (1979).
22. M.B. Evans, R.J. Cogdell and G. Britton, *Biochim. Biophys. Acta, 935*, 292-298 (1989).
23. T. Tanada, *Am. J. Bot., 38*, 276-283 (1951).
24. T.G. Owens and E.R. Wold, *Plant Physiol., 80*, 732-738 (1986).
25. T. Katoh, M. Mimuro and S. Takaichi, *Biochim. Biophys. Acta, 976*, 233-240 (1989).
26. T. Katoh and T. Ehara, *Plant Cell Physiol., 31*, 439-447 (1990).
27. W. Kühlbrandt, *Nature London., 307*, 478-440 (1984).
28. T. Shirmer, W. Bode, R. Huber, W. Sidler and H. Zuber, *J. Mol. Biol., 184*, 257-277 (1985).
29. M. Mimuro, T. Katoh and H. Kawai, *Biochim. Biophys. Acta, 1015*, 450-456, (1990).
30. J. Watanabe, S. Kinoshita and T. Kushida, *Chem. Phys. Lett., 126*, 197-200 (1986).

31. J.K. Trautman, A.P. Shreve, T.G. Owens and A.C. Albrecht, *Current Research in Photosynthesis*, Vol. II. pp. 289-292, Kluwer, Dordrecht, 1990.

32. H. Hashimoto and Y. Koyama, *Chem. Phys. Lett., 163*, 251-256 (1989).

33. M. Kuki, H. Hashimoto and Y. Koyama, *Chem. Phys. Lett., 165*, 417-422 (1990).

34. P. Koka and P.-S. Song, *Biochim. Biophys. Acta, 495*, 220-231 (1977).

35. M. Fragata, R. Norden and T. Khrugsev, *Photochem. Photobiol., 47*, 133-143 (1988).

36. M.R. Wasielewsky and L.D. Kispert, *Chem. Phys. Lett., 128*, 238-243 (1986).

37. J. Deisenhofer, O. Epp, K. Miki, R. Huber and H. Michel, *Nature London, 318*, 618-624 (1985).