Invited Trends Article:

Structural Role of Carotenoids in Photosynthetic Membranes

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Z. Naturforsch. **51 c,** 763–771 (1996); received January 23/February 20, 1996

Dedicated to Professor T. W. Goodwin on the occasion of his 80th birthday

Carotenoids, Higher Plants, Light-Harvesting Pigment-Protein Complex, Photoprotection, Purple Bacteria

Besides the light-harvesting and protecting role, carotenoids are also instrumental as structural components for the assembly of light-harvesting complexes in purple bacteria and green plants, as well as for the formation of photosystem II complex. Carotenoids stabilize those pigment-protein complexes, but have no effect on the formation of the reaction centers of purple bacteria and photosystem I of plants.

Carotenoids are a class of unsaturated C_{40}-hydrocarbons which are extensively distributed in Nature. They give red and yellow color to fruits, flowers, birds etc., although they are present in small amounts. Most of the carotenoids are located in photosynthetic organisms (plants, algae and bacteria) where they are usually masked by different types of chlorophyll (Chl). If the amount of Chl is small, carotenoids determine the color of some algae.

Molecules of bacteriochlorophyll (Bchl), Chl and carotenoids *in vivo* are bound noncovalently to (apo)proteins forming the pigment-protein complexes which, according to their functions, are divided into the light-harvesting complexes (LHC) and the reaction center (RC). The LHCs-complexes absorb photons and transform their energy into the energy of electronic excitation of Bchl (Chl) which is transferred to the RC where the primary charge separation occurs. Carotenoids do not participate in the photochemical reactions but perform the photoprotective and light-harvesting functions.

The most important function of carotenoids is protection of Chls by quenching their triplet state via transformation of energy into heat. That prevents the production of singlet oxygen, which is formed as a result of the interaction of Chl triplets with oxygen and causes the photooxidative destruction of membranes. The other function of carotenoids is to absorb the light energy within the 480–580 nm region, where Chl and Bchl have low absorption, and to transfer this energy with different efficiencies (20–100%) to Chl or Bchl. Both functions of carotenoids have been described by Siefermann-Harms (1985) and Cogdell and Frank (1987). In this review evidence for the third – structural – function of carotenoids in the organization of photosynthetic membranes is summarized. The idea of the structural role of carotenoids was at first proposed for bacterial light-harvesting complexes. A considerable amount of data has been recently accumulated which confirm the hypothesis that carotenoids are necessary for the assembly and stabilization of certain pigment-protein complexes of photobacteria and green plants.

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Carotenoids in Bacteria and Plants: Biosynthesis and Composition

As shown by Fig. 1 (A) carotenoids in plants and bacteria are synthesized from two molecules of geranylgeranyl diphosphate via hydrocarbon phytoene, this is followed by a series of desaturation reactions, then hydroxylation and O-methylation in purple bacteria or cyclization and intro-

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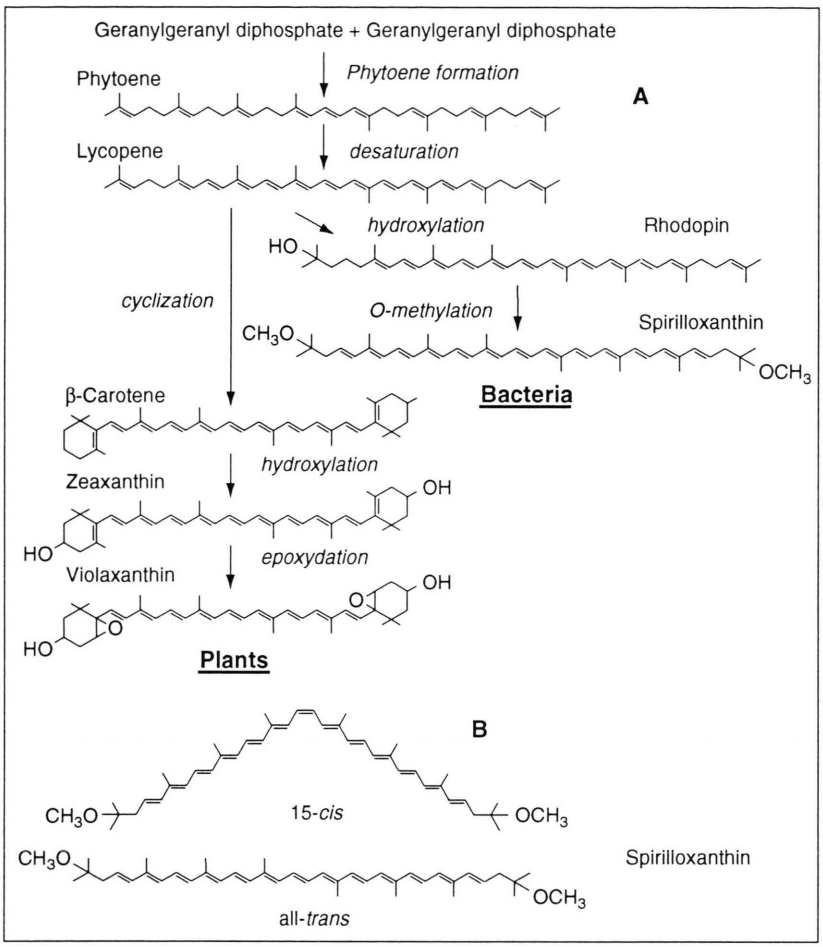

Fig. 1. Biosynthesis of carotenoids in bacteria and plants (A); *cis* and *trans* isomers of spirilloxanthin (B).

duction of oxygen in the case of plants (Britton, 1991). Carotenoids of purple bacteria are acyclic compounds which usually have a longer chromophore as compared with that of carotenes or xanthophylls in plants. The biosynthesis of spirilloxanthin is realized either from lycopene via rhodopin and rhodovibrin, or from neurosporene via spheroidene and hydroxyspheroidene. The carotenoid composition in the membranes of purple bacteria varies within a wide range depending on the growth conditions, age of culture etc. (Schwerzmann and Bachofen, 1989). Carotenoids from photosynthetic membranes of plants are divided into oxygen-free carotenes (α- or β-carotene) and xanthophylls containing one or several hydroxy or epoxy groups. The carotenoids usually found in the membranes of plants are 25–50% of β-carotene, 40–57% of lutein, 9–20% of violaxanthin and 5–13% of neoxanthin (Lichtenthaler, 1987).

The main approach to understand the role of carotenoids in the photosynthetic membranes is the comparison of the control and carotenoid-depleted membranes. There are several ways to remove carotenoids from the membranes or complexes: (i) photobleaching of carotenoids in purple bacteria under blue light; (ii) inhibition of the biosynthesis of carotenoids by herbicides or mutation; (iii) selective extraction of carotenoids from the membranes. We will discuss these data separately for each type of pigment-protein complexes of bacteria and green plants.

Light-Harvesting Complexes of Purple Bacteria

The purple bacteria contain two types of LHC: a core complex LH1 (B880) which surrounds the RC-complex, forming the B880-RC assembly, and a peripheral antenna complex LH2 (B800–850) associated with the assemblies (Fig. 2, A). Both LHCs sonsist of α- and β-polypeptides (4.5–8 kDa) in the stoichiometric ratio 1:1. Recent analysis of the LHC-complexes crystals reveals 9 subunits ($\alpha/\beta/Bchl_{2-4}/Car_{1-2}$) in the B800–850 complex and 16 subcomplexes ($\alpha/\beta/Bchl_2/Car$) in the B880 complex (McDermott *et al.*, 1995; Karrash *et al.*, 1995). Both complexes look as a ring with outer/inner diameter 116/68 Å for the LH1 of *R. rubrum* and 68/36 Å for LH2 of *Rps. acidophila*. The Bchl dimer is located adjacent to the periplasmic side in LH1 and LH2, and the Bchl monomers (or weakly interacting dimer) seem to be located near the cytoplasmic surface in LH2 (Fig. 2, A; Zuber and Brunisholz, 1991).

The analysis of LH2 crystal structure of *Rps. acidophila* has shown one carotenoid molecule for each α/β pair, i.e. 9 molecules of carotenoids are present in the crystal to compare with 14 molecules in the complex in the solution (McDermott *et al.*, 1995). Two different binding sites of carotenoids are present in LH2. The position of tightly bound carotenoid molecules was established in the crystal of *Rps. acidophila*. They interact with several hydrophobic residues of adjacent α- and β-polypeptides and with dimeric and monomeric Bchls. Since the helices of α- and β-polypeptides interact via the pigment molecules or buried water (McDermott *et al.*, 1995), it becomes evident that carotenoids are the important elements for the stabilization of the LH2 structure. A similar position of carotenoids was established for the LH1 too.

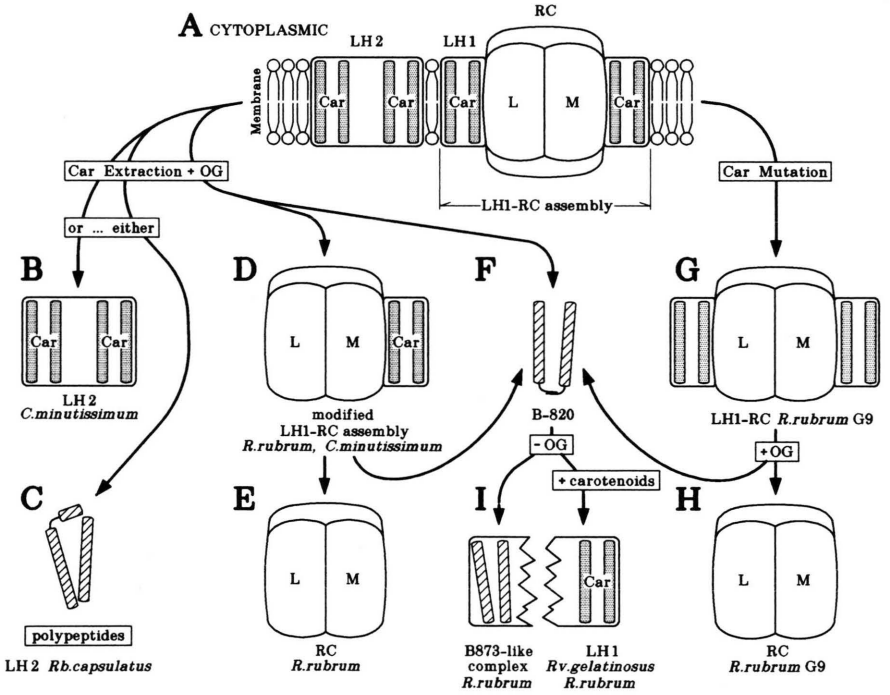

Fig. 2. Arrangement of the pigmehnt-protein complexes (RC, LH1, LH2) in the membrane (A) and the same complexes isolated from the membranes of different purple bacteria after extraction of carotenoids, using octyl-β-D-glucopyranoside (OG) (B–F), or isolated from the membranes, deleted of carotenoids by mutation, without OG (G) or with OG (F, H); reconstitution of LH complexes as a result of OG removal in the presence of carotenoids (I, right) or without carotenoids (I, left).
Car, carotenoids; RC – rection center complex; LH1 (or LH2) – light-harvesting complex 1 (or complex 2); L, M – polypeptides of the RC-complex; B820 complex or B873-like complex, LH complexes with absorption maxima at 820 or at 873 nm.

One end of the carotenoid is positioned very closely to the cytoplasmic surface and on the average the pigment molecules are oriented at about 45° to the membrane plane (Picorel *et al.*, 1988). The dimeric organization of carotenoids in the B880 complex was proposed (Zurdo *et al.*, 1991). The binding sites of carotenoids in the LHC are not conservative since complexes bind different carotenoids from phytoene to spirilloxanthin depending on growth conditions (Moskalenko *et al.*, 1991). Both LHCs can associate the same *(Rhodopseudomonas capsulatus, Rhodobacter sphaeroides)* or differrent *(Chromatium minutissimum)* types of carotenoids (Cogdell and Frank, 1987; Moskalenko *et al.*, 1991) which have the all-*trans* configuration (Fig. 1, B; Koyama, 1991).

The first assumption about the structural role of carotenoids in LH2 of *C. minutissimun* was based on photobleaching of pigments in the complex under illumination by blue (carotenoid region) and red light (Bchl region). The blue light effectively quenched the Bchl fluorescence, and the Bchl molecules in the complexes, especially in LH2, were more efficently destroyed as compared with the effect of the red light (Moskalenko, 1974). The photobleaching of Bchl molecules under blue light could not be explained by energy transfer from carotenoids as its efficiency was near 20–30%. The photooxidative degradation of the carotenoids under blue light in the presence of oxygen caused the destruction of the complexes.

The structural role of carotenoids was confirmed by the comparison of the properties of control complexes and those isolated from *C. minutissimum* cells grown with diphenylamine (Moskalenko *et al.*, 1983; 1991). Diphenylamine blocks the phytoene desaturase and causes about 95% inhibition of carotenoid biosynthesis. In the membranes of diphenylamine-grown cells the precursors (phytoene and phytofluene, 46%) and carotenoids (tetrahydrolycopene type, 33%; neurosporene type, 20%) have been identified. Both complexes (LH1 and LH2) were easily destroyed after treatment with 1% Triton X-100, while the control ones were stable during several days in the presence of the higher (up to 5%) detergent concentration. The complexes restored the structural stability only if the carotenoid content increased by more than 10% as compared with the control.

The isolation of subcomplexes $(\alpha/\beta/Bchl_2)_2$ from the control LHC was hindered by the presence of carotenoids and usually they dissociated into polypeptides and pigments under different treatments bypassing the subcomplex stage. The problem was resolved for LH1 by selective extraction of carotenoids from the dried membranes with petrol or benzene (they removed carotenoids only from the LHC and not from RC) followed by octyl-β-D-glucopyranoside treatment of LH1 of nonsulfur and sulfur bacteria (Miller *et al.*, 1987; Fig. 2, A and D–F). Thus, carotenoids prevent the dissociation of LH1 into the B820 subcomplexes but the B873-like structure may be easily reassociated without them (Fig. 2, F and I). The subcomplexes of *Rubrivax gelantinosus* and *C. minutissimum* reassociated into the B850-like complexes without carotenoids (Jirsakova and Reiss-Husson, 1994). Recently, the requirement of the carotenoids, bound to the complex in its native state, was established for the reconstitution of LH1 of *Rv. gelatinosus* (Fig. 2, F and I; Moskalenko *et al.*, 1995).

The selective extraction of carotenoids does not lead to the isolation of the subcomplexes from LH2. It causes a partial loss (20–40%) of carotenoids from LH2 *(C. minutissimum)* and a complete loss of carotenoids from LH2 *(Rps. capsulatus)*. In the first case the complex remained stable, in the second it dissociated into polypeptides and pigments during the isolation (Fig. 2, B and C; Moskalenko *et al.*, 1995). The structural role of carotenoids in LH2 of *Rps. capsulatus* was also concluded (Zurdo *et al.*, 1993). In contrast to LH1, the LH2 assembly *in vivo* depends on carotenoid availabiliy.

The carotenoidless mutants of purple bacteria have the intact RC and only one type of LHC with the absorption band centered at 850–880 nm (Fig. 2, G). The surviving complex is the B880 type or the B800–850 type which lost the binding site of the monomeric Bchl (800 nm). The compensatory mutations are generally required for surviving of the LHC. The LHC of *Rh. sphaeroides R26.1*, in contrast to the wild-type strain, contains a single amino acid exchange (Val_{24}→Phe) or the N-terminal methionine is missing (Zuber and Brunisholz, 1991). The conformation of the N-terminal regional of apoproteins is changed by a carotenoid in the LH1 of the wild straing of *R. rubrum* as compared with that of the carotenoidless mutant

G9 (Brunisholz *et al.*, 1986). Only the last one is known as a mutant without compensatory mutations in the N-region of α- or β-polypeptides. Therefore, its LH1 easily dissociates into the subcomplexes without any preliminary treatment (Fig. 2, F–H).

Thus, carotenoids keep under control the N-terminal region in both bacterial LHCs and participate in stabilization of α/β-helices interaction, in particular in LH2. Their elimination by the extraction of mutation causes either dissociation of LH2 into polypeptides and pigments or transformation of its structure into the LH1-like complex. This process does not influence significantly the organization of the LH1 complex, but it attenuates the interaction between the subcomplexes, and LH1 easily dissociates into B820 subcomplexes.

Light-Harvesting Complexes of Higher Plants

There are several Chl *a/b*-proteins (LHC) in the membranes of the higher plants binding 50–70% of the total Chl. The major LHC is the most abundant membrane protein in chloroplast membranes which binds 40–50% of Chl. It consists of 28, 27 and 25 KDa apoproteins. They penetrate the membranes three times (helices A, B and C) and each of them binds at least 12 Chl, 2 luteins and some other carotenoids of the xanthophyll group (Thornber *et al.*, 1991).

Xanthophylls but not carotenes play the structural role in the reassembly of stable LHC in the chloroplast membranes of higher plants (Plumley and Schmidt, 1987). In the course of reconstitution of LHC the apoproteins bind always the same amount of Chl *a*, Chl *b* and carotenoids, independently of the amount of that pigments in the medium that indicates the specific binding sites (Hobe *et al.*, 1994). Carotenoids are necessary for the correct assembly and stabilization of LHC apoproteins in the thylakoid membranes (Dahlin, 1988). This idea was proved by a LHC model, proposed on the basis of electron crystallography (Kühlbrandt *et al.*, 1994). The carotenoids present in this complex are in the all-*trans* configuration. The polyene chains of both luteins include a 50° angle with the membrane normal; the closest distance between them is nearly 11 Å with the head groups of the carotenoids roughly equidistant from both membrane surfaces. The lutein binding sites are located in the symmetrical extension of helices A and B. The polypeptide environment of both carotenoids is highly hydrophobic. The lutein molecules form an internal cross in the complex, stabilizing the conformation of LHC polypeptides (Fig. 3, A). This arrangement of two carotenoid molecules in the central part of LHC explains why they are essential for the reconstitution of the complex.

Reaction Centers of Purple Bacteria

The isolated RC-complex consists of three (H, M and L) subunits, four Bchl and two bacteriopheophytin molecules, and one carotenoid molecule. The RC core is made up of the L and M subunits. Each subunit has five membrane spanning helices. Bchls, bacteriopheophytins and quinones form two structurally equivalent brances. One of them more closely associated with the L subunit in the active branch, the other with the M subunit in the nonactive branch (Deisenhofer and Michel, 1989). The organization of the RC pigments, including the carotenoids, was determined for *Rps. viridis* and *Rh. sphaeroides*. X-ray analysis of the crystals of RC-complexes isolated from the species allowed to determine the precise location of carotenoids for the purple bacteria. RC of *Rh. sphaeroides* contains one molecule of spheroidene in the *cis*-conformation which locates in the nonactive branch of the RC between the B and C helices of the M subunit near to monomeric Bchl (Yeates *et al.*, 1988). The latter lies between carotenoid and the dimeric Bchl (primary electron donor).

In discrepancy with the bacterial LHC, the structure of the carotenoidless RC-complex is not essentially changed therefore the RC-complex is always present in that mutants. Indeed, the loss of carotenoids decreases the stability of the mutants, since the special pair donates excess energy only to molecular oxygen. Carotenoids with the terminal polar functional groups can be incorporated back into the carotenoidless RC with restoratin of their native characteristics and protective function (Cogdell and Frank, 1987). The carotenoidless RC-complex of *Rh. sphaeroides R26* binds pure spheroidene (neither β-carotene, nor spirilloxanthin) in molar ratio 1:1 with respect to P870 (Agalidis *et al.*, 1980). The carotenoid binding to RC

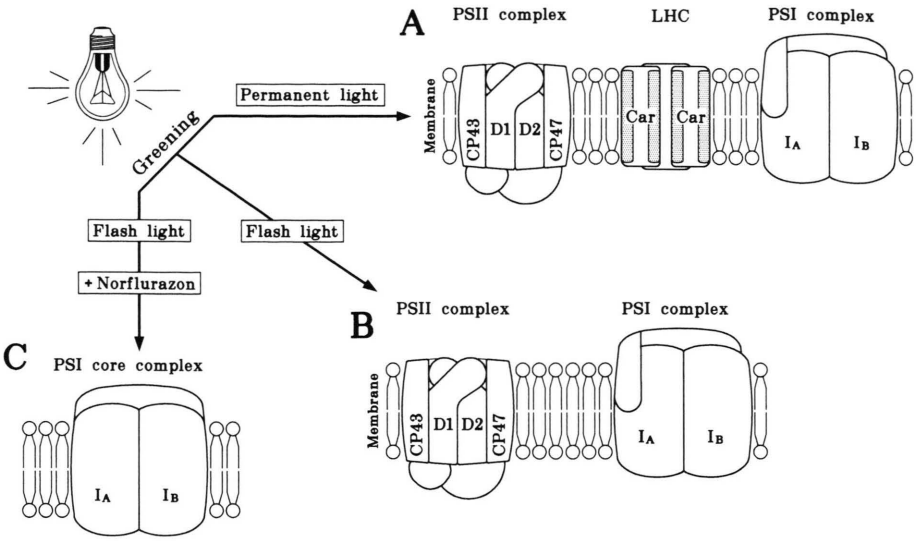

Fig. 3. Arrangement of the pigment-protein complexes (PS II, PS I, LHC) in the membrane of chloroplasts of higher plants grown under illumination with permanent (A) or flash light without (B) or with herbicide norflurazon which inhibits the bioynthesis of carotenoids (C).
Car – carotenoid; PS I (or PS II), photosystem I (or photosystem II); LHC, light-harvesting complex; D1, D2, CP43, CP47 – polypeptides of PS II core complex; I_A, I_B – polypeptides of PS I core complex.

most likely occurs at the same site as in the wild-type RC, since speroidene displays light-induced absorbance changes. Thus, carotenoids are not essential components for the structure of the bacterial RCs which can assemble and exist without this type of pigment.

Reaction Center Complex of Photosystem II

The isolated RC-complex of photosystem II (PS II) is composed of D1 and D2 proteins, the cytochrome b559 (α- and β-subunits) and 4.8 kDa protein (Nanba and Satoh, 1987). It is the main part of the PS II core complex which also includes Chl-proteins CP47 and CP43 (Fig. 3, A; Thornber *et al.*, 1991). Despite the close homology between L and M subunits of bacterial RC with the D1 and D2 proteins of PS II RC, there are significant differences in their chromophore content. The RC-complex of PS II consists of 5–6 Chl molecules and 1–2 β-carotene(s) depending on the isolation procedure (Telfer *et al.*, 1991). The β-carotene molecules have *all-trans* conformation and closely interact with each other (Newell *et al.*, 1991). On the basis of the different photooxidation rates of two β-carotene molecules it was concluded that one molecule was bound to the D1 protein and the other to the D2-protein (Telfer *et al.*, 1991). One β-carotene was removed without a loss of Chl content and significant changes in properties of PS II RC-complex. The remaining β-carotene molecule can be removed by intensive washing of the PS II RC-complex but only a very small amount of PS II RC-complex maintained their structure (De Las Rivas *et al.*, 1993).

It was established that barley seedlings grown with norflurazon, with the amount of carotenoids not exceeding 3%, show no PS II activity (Öquist *et al.*, 1980; Lehoczki *et al.*, 1982). The native electrophoresis of the complexes from the tylakoids of the treated seelings has revealed that disappearance of PS II activity is the result of the absence of PS II pigment-protein complex (Karapetyan *et al.*, 1991). The seedlings grown with norflurazon under flash light contained no LHC and PS II core complex (Fig. 3, B and C), although they have the apoproteins and Chl *a*, and the bound Chl molecules appear located in photosystem I (PS I). Carotenoids are necessary also for the formation of primary thylakoids (Bolychevtseva *et al.*, 1995).

Another proof of the structural role of carotenoids during the biosynthesis of PS II-complex

was obtained with mutant cells of *Scenedesmus obliquus* (Humbeck *et al.*, 1989). The dark-grown cells contain only Chl *a*, precursors of carotenoids (lycopene, neurosporene, ζ-carotene, β-zeacarotene) and show only the PS I aktivity. The complex of PS II and the corresponding activity developed immediately upon the transfer of the culture to light and coincided with the formation of β-carotene and lycopene. Other xanthophylls were synthesized only after a 30 min lag. Inhibition of the transformation of precursors into carotenoids by nicotine prevents the light-induced development of the PS II activity and the PS II core complex; it was suggested that lutein is the necessary prerequisite for the assembly of PS II complex. These results prove that carotenoids are essential components for the assembly of active PS II units. The exact mode of action of carotenoids in this process remains unknown.

Photosystem I Complex (Chl *a*-P700 Protein)

Although the structure of the RC of cyanobacterial PS I-complex, containing about 90 Chl *a* molecules per P700, was established using X-ray analysis (Krauss *et al.*, 1993), the exact location of β-carotenes and the stoichiometry of carotenoids and Chls in the PS I core complex is not clear. The PS I particles from higher plants contain mostly β-carotene which show high optical activity that reflects the high carotenoid ordering on the PS I complex (Schafernicht and Junge, 1981). Carotenoids in cyanobacterial PS I trimers enriched with the long-wavelength Chls are higher oriented as compared with PS I monomers (Shubin *et al.*, 1993). Extraction of carotenoids from the PS I particle has no effect on the activity of P700 (Searle and Wessels, 1978).

The thylakoids isolated from norflurazon-treated barley seedlings have been enriched with the RC-complex of PS I: Chl/P700 was 60 in treated samples as compared with 150 in the control at the same amount of Chl *a*, and P700 remained active (Fig. 3, C), i.e. the formation of PS I does not require the carotenoids(Lehoczki *et al.*, 1982; Karapetyan *et al.*, 1992). It was shown for the mutant of *Scenedesmus obliquus*, containing only carotenoid precursors, that the RC of PS I existed in the dark-grown cells, and the assembly of the core complex of PS I and its function were independent from the presence of carotenoids (Römer *et al.*, 1990). On the other hand, it was suggested that carotenoids in PS I complex may play the strucutral role and they are necessary for binding the core complex and the peripheral antenna. Probably, carotenoids may also stabilize the RC-complex, since Chl molecules in the absence of carotenoids are very sensitive to the effect of light or detergents.

Concluding Remarks

The data discussed prove the structural role of carotenoids in photosynthesis. As a structural component for some pigment-protein complexes, carotenoids are very important at start of the development of photosynthetic membranes. Carotenoids stabilize the structure of bacterial and plant LHCs, and serve as an important component for the assembly of the PS II core comples and LCH from plants. However, they have no influence on the structure and assembly of bacterial or PS I RC-complex from plants. In future new and more detailed rules will be established as to whether carotenoids participate in the formation of pigment-protein complexes in different types of photosynthetic organisms and stabilize the structure of that complexes.

Acknowledgements

We gratefully acknowledge The Scientific and Environmental Affairs Division of NATO (grant HTECH.LG.931646) and The Russian Foundation of Basic Research (grants No. 95-04-12998 and 96-04-48809) for financial support.

Agalidis I., Lutz M. and Reiss-Husson F. (1980), Binding of carotenoids on reaction centers from *Rhodopseudomonas sphaeroides R-26.* Biochim. Biophys. Acta **589,** 264–274.

Bolychevtseva Y. V., Rakhimberdieva M. G., Karapetyan N. V., Popov V. I., Moskalenko A. A. and Kuznetsova N. Y. (1995), The development of carotenoid-deficient membranes in plastids of barley seedlings treated with norflurazon. J. Photochem. Photobiol. B: Biol. **27,** 153–160.

Britton G. (1991), The biosythesis of carotenoids: a progress report. Pure Appl. Chem. **63,** 101–108.

Brunisholz R. A., Zuber H., Valentine J., Lindsay J. G., Wooley K. J. and Cogdell R. J. (1986), The membrane location of the B890-complex from *Rhodospirillum rubrum* and effect of carotenoid on the conformation of its two apoproteins exposed at cytoplasmic surface. Biochim. Biophys. Acta **849,** 295–303.

Cogdell R. J. and Frank H. A. (1987), How carotenoids function in photosynthetic membranes. Biochim. Biophys. Acta **895,** 63–79.

Dahlin C. (1988), Correlation between pigment composition and apoproteins of the light-harvesting complex II (LHC II) in wheat (*Triticum aestivum*). Physiol. Plant. **74,** 342–348.

Deisenhofer J. and Michel H. (1989), The photosynthetic reaction center from purple bacterium *Rhodopseudomonas viridis.* The EMBO J. **8,** 2149–2169.

De Las Rivas J., Telfer A. and Barber J. (1991), Two coupled β-carotene molecules protect P680 from photodamage in isolated photosystem II reaction centers. Biochim. Biophys. Acta **1142,** 155–164.

Hobe S., Prytulla S., Kühlbrandt W. and Paulsen H. (1994), Trimerization and crystallization of reconstituted light-harvesting chlorophyll *a/b* complex. The EMBO J. **13,** 3423–3429.

Humbeck K., Römer S. and Senger H. (1989), Evidence for an essential role of carotenoids in the assembly of an active photosystem II. Planta **179,** 242–250.

Jirsakova W. and Reiss-Husson F. (1994), A specific carotenoid is required for reconstitution of the *Rubrivax gelatinosus* B875 light-harvesting complex from its subunit form B820. FEBS Lett. **353,** 151–154.

Karapetyan N. V., Bolychevtseva Y. V. and Rakhimberdieva M. G. (1991), The necessity of carotenoids for the assembly of active photosystem II reaction centers. In: Light in Biology and Medicine (R. H. Duglas, J. Moan and G. Rönto, eds.). Plenum Press, New York, vol. **2,** 45–54.

Karapetyan N. V.., Bolychevtseva Y. V., Rakhimberdieva M. G., Moskalenko A. A., Kuznetsova N. Y. and Popov V. I. (1992), Biogenesis of chloroplasts and pigment-protein complexes in barley seedlings with blocked biosynthesis of carotenoids. In: Regulation of Chloroplast Biosynthesis (J. H. Argyroudi-Akoyunoglou, ed.). Plenum Press, NATO ASI series A, vol. **226,** pp. 349–354.

Karrash S., Bullough P. A. and Ghosh R. (1995), The 8.5 Å projection map of the light-induced complex I from *Rhodospirillum rubrum* reveals a ring composed of 16 subunits. The EMBO J. **14,** 631–638.

Koyama Y. (1991), Structure and function of carotenoids in photosynthetic systems. J. Photochem. Photobiol. B: Biol. **9,** 265–280.

Krauss N., Hinrichs W., Witt I., Fromme P., Pritzkow W., Dauter Z., Betzel C., Wilson K. S., Witt H. T. and Sänger W. (1993), Three-dimensional structure of system I of photosynthesis at 6 Å resolution. Nature **136,** 326–331.

Kühlbrandt W., De Neng Wang and Fujiyoushi Y. (1994), Atomic model of plant light-harvesting complex by electron crystallography. Nature **376,** 614–621.

Lehoczki E., Rakhimberdieva M. G. and Karapetyan N. V. (1982), Effect of blocking of carotenoid synthesis by norflurazon on photosystem II formation in barley leaves. Plant Physiol. (Russ.) **29,** 682–686.

Lichtenthaler H. K. (1987), Chlorophylls and carotenoids: pigments of photosynthetic membranes. In: Methods in Enzymology **148,** 350–382.

McDermott G., Prince S. M., Freer A. A., Hawthornthwaite-Lawless A. M., Papiz M. Z., Cogdell R. J. and Isaacs N. W. (1995), Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. Nature **374,** 517–521.

Miller J. F., Hinchigeri S. B., Parkes-Loach P. S., Callakan P. M., Sprinkle J. R., Riccobono J. R. and Loach P. L. (1987), Isolation and characterization of a subunit form of the light-harvesting complex of *Rhodospirillum rubrum.* Biochemistry **26,** 5055–5062.

Moskalenko A. A. (1974), Investigation of pigment-protein complexes of *Chromatium minutissimum.* Ph. D. Thesis (Russ.) Minsk.

Moskalenko A. A., Kuznetsova N. Y. and Erokhin Y. E. (1987), Isolation, spectral and photochemical properties of three types of the pigment-protein complexes of *Chromatium minutissimum* with inhibited synthesis of carotenoids. Dokl. AN SSSR (Russ.) **269,** 1248–1251.

Moskalenko A. A., Britton G., Connor A., Young A. and Toropygina O. A. (1991), The carotenoid content in the chromatophores and pigment-protein complexes isolated from cells of *Chromatium minutissimum* grown with diphenylamine. Biol. Membr. (Russ.) **8,** 249–260.

Moskalenko A. A., Toropygina O. A. and Kuznetsova N. Y. (1995), The isolation of the B812 subcomplex of the B880 core complex and the B800–850 complex from membranes of *Chromatium minutissimum* with exctracted carotenoids: the structural role of carotenoids. J. Photochem. Photobiol. B: Biol. **27,** 199–208.

Nanba O. and Satoh K. (1987), Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome *b*559. Proc. Natl. Acad. Sci. USA **84,** 109–112.

Newell W. R., van Amerongen H., Barber J. and van Grondelle R. (1991), Spectroscopic characterization of the reaction center of photosystem II uisng polarised light: Evidence for β-carotene exitons in PS II reaction centers. Biochim. Biophys. Acta **1057,** 223–228.

Öquist G., Samuelsson G. and Bishop N. I. (1980), On the role of β-carotene in the reaction center chlorophyll *a* antennae of photosystem I. Physiol. Plant. **50,** 63–70.

Picorel R., Holt R. E., Cotton T. M. and Seibert M. (1988), Surface-enhanced resonance Raman scattering spectroscopy of bacterial photosynthetic membranes. J. Biol. Chem. **263,** 4374–4380.

Plumley F. G. and Schmidt G. W. (1987), Reconstitution of chlorophyll *a/b* light-harvesting complexes: xanthophyll-dependent assembly and energy transfer. Proc. Natl. Acad. Aci. USA **84,** 146–150.

Römer S., Humbeck K. and Senger H. (1990), Relationship between biosynthesis of carotenoids and increasing of complexity of photosystem I in mutant C-6D of *Scenedesmus obliquus.* Planta **182,** 216–222.

Schafernicht H. and Junge W. (1981), Analysis of the complex band spectrum of P700 based on photoselection studies with photosystem I particles. Photochem. Photobiol. **34,** 223–232.

Schwerzman R. U. and Bachofen R. (1989), Carotenoids profiles in pigment-protein complexes of *Rhodospirillum rubrum.* Plant Cell Physiol. **30,** 497–504.

Searle G. F. W. and Wessels J. S. C. (1973), Role of β-carotene in the reaction centers of photosystem I and II of spinach chlorplasts prepared in non-polar solvents. Biochim. Biophys. Acta **504,** 84–99.

Siefermann-Harms D. (1985), Carotenoids in photosynthesis. 1. Location in photosynthetic membranes and light-harvesting complexes. Biochim. Biophys. Acta **811,** 325–355.

Shubin V. V., Tsuprun V. L., Bezsmertnaya I. N. and Karapetyan N. V. (1993), Trimeric forms of the photosystem I reaction center complex pre-exist in the membranes of the cyanobacterium *Spirulina platensis.* FEBS Lett. **334,** 79–82.

Telfer A., De Las Rivas J. and Barber J. (1991), β-carotene within the isolated photosystem II reaction center: photooxidation and irreversible bleaching of this chromophore by oxidised P680. Biochim. Biophys. Acta **1060,** 106–114.

Thornber J. P., Morishige D. T., Anandan S. and Peter G. (1991), Chlorophyll-carotenoid proteins of higher plant thylakoids. In: The Chlorophylls (H. Scheer, ed.). CRC Press Boca Raton, Boston and London, pp. 549–585.

Zuber H. and Brunisholz R. A. (1991), Structure and function of antenna polypeptides and chlorophyll-protein complexes: principles and variability. In: The Chlorophylls (H. Scheer, ed.). CRC Press Boca Raton, Boston and London, pp. 627–703.

Zurdo J., Fernandez-Cabrera C. and Ramirez J. M. (1993), A structural role of carotenoids in the light-harvesting complex. Biochem. J. **290,** 531–537.

Zurdo J., Losano R. M., Fernandez-Cabrera C. and Ramirez J. M. (1991), Dimeric carotenoid interaction in the light-harvesting antenna of purple photosynthetic bacteria. Biochem. J. **274,** 881–884.

Yeates T. O., Komiya H., Chirino A., Rees D. C., Allen J. P. and Feher G. (1988), Structure of the reaction center from *Rhodobacter sphaeroides R-26* and 2.4.1: protein cofactor (bacteriochlorophyll, bacteriopheophytin and carotenoid) interaction. Proc. Natl. Acad. Sci. USA **85,** 7993–7997.