Biochimica et Biophysica Acta, 681 (1982) 263–272
Elsevier Biomedical Press

BBA 41121

THE INFLUENCE OF CAROTENOIDS ON THE CONFORMATION OF CHLOROPHYLL-PROTEIN COMPLEXES ISOLATED FROM THE CYANOBACTERIUM *PLECTONEMA BORYANUM*

ABSORPTION AND CIRCULAR DICHROISM STUDY

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(Received October 26th, 1981)
(Revised manuscript received May 4th, 1982)

Keywords: Chlorophyll-protein complex; Carotenoid; Thylakoid membrane; Photosynthesis; (Plectonema boryanum)

Thylakoid membranes of the cyanobacterium *Plectonema boryanum* solubilized with Triton X-100 can be resolved into three fractions of pigment-protein complexes (Hladík, J. and Sofrová, D. (1981) Photosynthetica 15, 490–503). Fraction I contained relatively the highest amount of carotenoids as well as monomeric forms of chlorophyll *a*, Fractions II and III contained chlorophyll-protein complexes with a characteristic exciton-split circular dichroism in the red region. It has been shown that fraction III is an oligomeric form of the chlorophyll-protein complex of fraction II. Circular dichroism spectra indicate that, different from fraction II, fraction III contains specifically oriented and space-fixed molecules of carotenoids. Thermal dissociation of fracion III to fraction II is accompanied by disappearance of the positive circular dichroism effect of carotenoids in the 500–550 nm region, thus causing deorganization of the carotenoids, proceeding in parallel to the geometrical rearrangement of chlorophyll molecules. Extraction of the carotenoids of fraction III with heptane is acompanied by dissociation of fraction III. We assume that the observed effects are due to binding of the two pigments to the protein component of the complex and that carotenoids can mediate a part of the interactions which stabilize the structure of pigment-protein complexes. Thus, besides the light-harvesting and protective functions, carotenoids can also play a structural role.

Introduction

Being relatively primitive organisms, cyanobacteria appear to be a suitable model for studying the relationship between the structure and function of the thylakoid membranes of photosynthetic organisms [1–5]. It is only in recent years that more information has been collected concerning the organization of cynobacterial thylakoid membranes.

The best known is the P-700-Chl *a*-protein complex (CP I), first isolated from *Phormidium* by chromatography on hydroxyapatite from membranes solubilized with SDS [6]. Development of separation techniques enabled more chlorophyll-protein complexes to be obtained from thylakoid membranes of cyanobacteria. Electrophoresis on a polyacrylamide gel allowed Reinman and Thornber [7] to prove the existence of two new complexes –

Abbreviation: Chl, chlorophyll.

one with a molecular weight less than that of the P-700-Chl *a*-protein complex, the other with considerably larger molecules. Similar results have been reported by Rusckowski and Zilinskas [8] and Stewart [9]. The electrophoretically more mobile complex is very unstable and decomposes easily. It has been ascribed, in terms of its function on the basis of indirect evidence and in analogy with higher plants, to Photosystem II, probably to its reaction centre. The second complex of lower electrophoretic mobility can be prepared under slightly more drastic conditions. Its apparent molecular weight is about 255000 [7,10] and in its properties it is analogous to CP I. This complex also has an analogy in higher plants [3], where it is frequently denoted CP Ia.

The high sensitivity of circular dichroism (CD) spectra to details of geometry and its changes for optically active molecules have made this technique a widely used tool in studies of entire photosynthetic systems and their components [11]. CD spectra of light-harvesting complexes of higher plants were studied at low temperatures by Canaani and Sauer [12]. Gregory and Raps [13] used this method to study the influence of detergents on chlorophyll-protein complexes. Besides the Cotton effect ascribed to porphyrins, CD bands were also observed in bacteria under certain conditions in the absorption region of complementary pigments – carotenoids [14]. Cogdell et al. [14] observed relatively intense Cotton effects corresponding to carotenoids in reaction centre complexes of the bacterium *Rhodopseudomonas sphaeroides*. Since these bands are not present in CD spectra of pigments extracted from the complex with organic solvents, the above authors interpreted them as being a manifestation of a chiral arrangement of individual carotenoid molecules, due to their bonding to the protein of the reaction centre complex. An increasing number of recent studies, in which different techniques were employed [15], led to similar conclusions, confirming the specific orientation of complementary pigments with respect to chlorophyll.

The aim of our work was a detailed study of the structure of two chlorophyll-protein complexes isolated from the thylakoid membranes of the cyanobacterium *Plectonema boryanum* with the use of Triton X-100, followed by chromatography on a DEAE-cellulose column. It has already been shown earlier that these complexes contain the reaction centre P-700 and, therefore, are linked in some way to Photosystem I [10,16]. We used a combination of physical and chemical techniques to study the course of thermal dissociation of the higher structure of these complexes and the possible role of carotenoids in the aggregation of these complexes.

Methods

The cyanobacterium *P. boryanum* 594 (Gomet) was cultivated in liquid medium as described by Kratz and Meyers [17] in a cultivation tube of 500 ml volume, continuously agitated with CO_2-enriched air. The tube was illuminated with an incandescent lamp of 75 W; the temperature of the suspension was 15–18°C.

Cyanobacteria were harvested by centrifuging at $5000 \times g$ for 20 min and suspended in isotonic mannitol/phosphate medium, pH 6.8 (0.33 M mannitol, 30 mM phosphate, 10 mM $MgCl_2$). Spheroplasts were prepared according to the method of Biggins [18]. They were destroyed by osmotic shock. Membrane fragments were washed twice with 36 mM sodium borate buffer, pH 8.9, containing 1 mM EDTA and 0.1% 2-mercaptoethanol as described earlier [10,16]. Chlorophyll-protein complexes were obtained by solubilizing with 1% Triton X-100 in sodium borate buffer, pH 8.9. The Triton/chlorophyll ratio was 25 : 1 (w/w). The suspension was agitated for 30 min at 4°C in the dark and then centrifuged at $100000 \times g$, at 0°C for 15 min.

The supernatant was immediately applied to a column of DEAE-cellulose. Chromatography was carried out by the method of Bengis and Nelson [19] as described earlier [10].

Electrophoresis on polyacrylamide gel was done in an electrophoretic system described by Markwell et al. [20] in tubes of 0.5×8 cm on 5% gel. During electrophoresis (90 min) a constant voltage of 65 V was maintained.

The chlorophyll content was determined by spectrophotometric means, using an extinction coefficient of $E = 60\ mM^{-1} \cdot cm^{-1}$ at 677 nm [6] or, after extraction with methanol according to the method of Ogawa and Vernon [21]. The absorp-

tion spectra of the complexes were measured with a Specord ultraviolet-visible double-beam spectrophotometer (Karl Zeiss, Jena) at room temperature. CD spectra were measured with a Mark III Dichrographe (ISA-Jobin Yvon) instrument in quartz cells of 1 cm optical path length at a sensitivity of $1.0–5 \cdot 10^{-6}$ dichroic absorbance units. When measuring time-temperature curves, cells were placed in a thermostatically controlled block located in the sample compartment of the instrument and the automatic repetitive scanning mode was employed. Temporal data were derived from the rate of shift of the monochromator, controlled by the stepping motor of the Dichrographe. Curves were recorded at 12 nm/min; the rate of the return motion was 120 nm/min.

Carotenoids were extracted as follows: isolated fractions of chlorophyll-protein complexes (fractions II and III) were mixed with Amberlite XAD-2 globules so that Triton had been removed (0.6 g globules/2 ml solution) and agitated in a refrigerator in the dark at 4°C for 120 min. Amberlite was then removed by filtering and the solution was lyophilized. Lyophilized chlorophyll-protein complexes from individual fractions or isolated thylakoid membranes were extracted with redistilled *n*-heptane (10–15 ml heptane/mg Chl) in a glass homogenizer in the dark at room temperature for 5 min. This step was repeated until the extraction solution remained colourless (about five to six times). The accumulated extracts were condensed in vacuo to a volume of 10 ml and joined with one-half of the original amount of extracted particles. Thus, reactivation was done with double the amount of carotenoids with respect to the chlorophyll, as compared to the original preparation.

The heptane extract of carotenoids was condensed in a vacuum evaporator and chromatographed on a thin silica gel layer (Silufol). The development mixture was petroleum ether/isopropanol/water at a ratio of 100:12:0.25 (v/v/v). Carotenoid zones were cut out and eluted with 2.0 ml petroleum ether (*β*-carotene) or 0.5 ml ethanol (xanthophylls). Absorption spectra were measured and individual carotenoids were identified by comparison with standards [22]. Quantitative analysis was carried out with the use of extinction coefficients ($E_{1\,cm}^{1\%}$) reported by Davies [23].

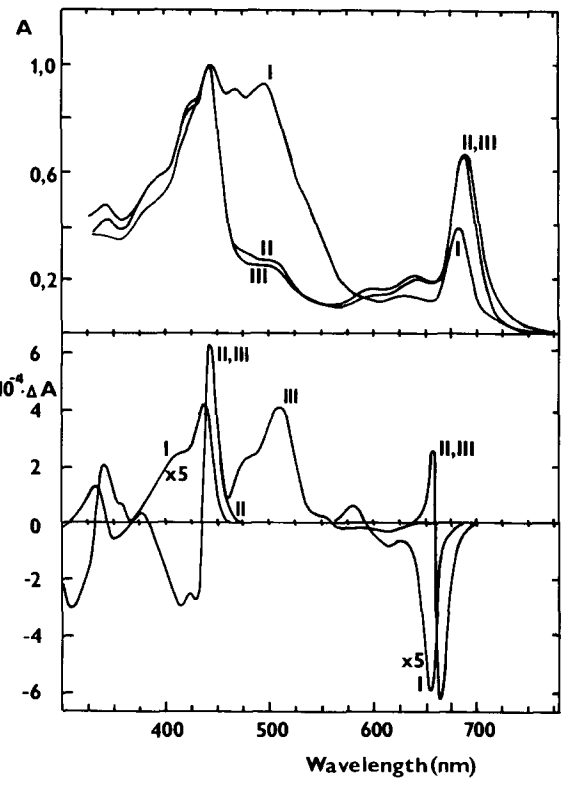

Fig. 1. The room-temperature spectral characterization of pigment-protein complexes, obtained by chromatography on a DEAE-cellulose column. (A) Absorption spectra, path length 1 cm; (B) CD spectra. I, II, III fractions I, II, III respectively; the curves of fractions II and III are identical with the exception of the 480–540 nm region. Path length 1 cm, Chl concentration 15 μg/ml.

Results

Pigment-protein complexes of *P. boryanum*, solubilized with Triton X-100, can be separated into three fractions by chromatography on DEAE-cellulose [10]. Fraction I, eluted with equilibrating buffer solution, contains the major part of the carotenoids and a lesser part of the chlorophyll and proteins. Two green chlorophyll-protein fractions are eluted with a linear NaCl gradient. Absorption and CD spectra of fractions I–III in the visible region are shown in Fig. 1. It can be seen that the largest amount of carotenoids is contained in fraction I. The absorption spectra of fractions II and III are very similar, except that the red absorption band of fraction III is shifted 2 nm toward longer wavelengths, and the 495 nm

absorption of fraction II indicates a greater content of carotenoids relative to chlorophyll. The CD spectrum of fraction I differs substantially from those of fractions II and III. It has a single negative band in the long-wavelength region and two positive overlapping bands in the Soret region. The character of this CD spectrum corresponds to the curves of monomeric free Chl *a* forms, known from measurements of this pigment in polar organic solvents [24]. The CD spectra of fractions II and III are characterized by the presence of intense double Cotton effects in the main chlorophyll absorption bands. In the region of the red absorption band, the long-wavelength component of the split non-conservative Cotton effect is negative, in the Soret region the long-wavelength component is positive. In the CD spectrum of fraction III, moreover, we find a pair of intensive positive Cotton effects between 500 and 550 nm, i.e., in the absorption region of carotenoids.

Electrophoresis on polyacrylamide gel has shown that the composition of fractions II and III is homogeneous, each containing a single chlorophyll-protein complex, denoted in analogy as Complex II and Complex III: these differ in molecular weights. The apparent molecular weight of the first is about 112000, that of the second 260000. As already shown earlier [10], the two complexes have very similar polypeptide and amino acid compositions and, under re-electrophoresis conditions, Complex III is converted into Complex II. The conditions for dissociation of Complex III

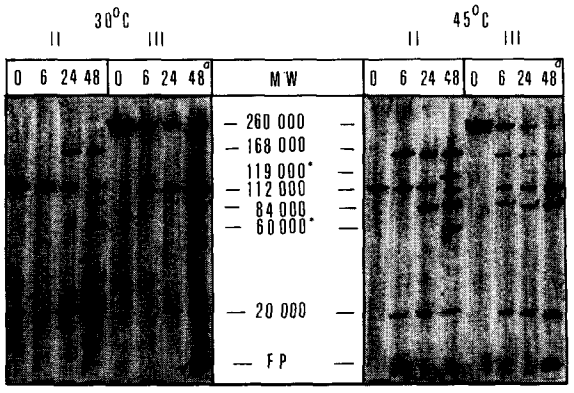

Fig. 2. Thermal changes of chlorophyll-protein complexes (fractions II and III) resolved by SDS-polyacrylamide gel electrophoresis. (a) Time (min) of heating to given temperature in the presence of 30% glycerol; *, polypeptide contained no chlorophyll; FP free pigment.

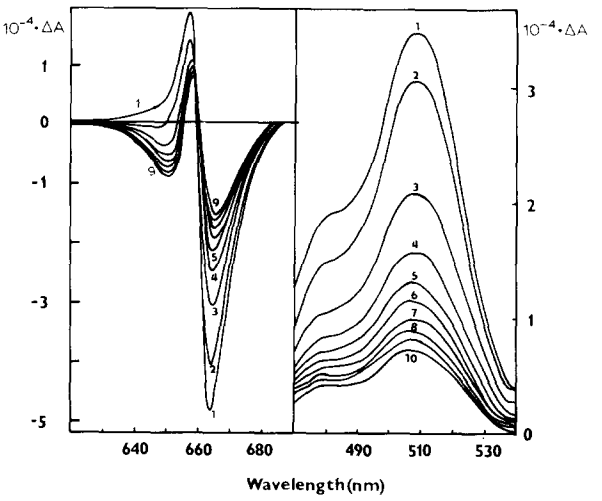

Fig. 3. Time-temperature changes in CD spectra of fraction III in the red and carotenoid-absorption regions. Fraction III was heated to 45°C for periods of: 1–0, 2–6, 3–12, 4–19, 5–25, 6–32, 7–38, 8–44, 9–51 and 10–57 min. Chl concentration 15 μg/ml.

to Complex II have also been described previously [16]. One condition is heating to 50°C in the presence of 30% sucrose or glycerol. The results of electrophoresis indicate that this temperature does not yet lead to liberation of chlorophyll from the protein matrix, while dissociation of the complex to subunits is already possible (Fig. 2). To analyze the mechanism of the degradation process in more detail, we employed the distinct temperature rela-

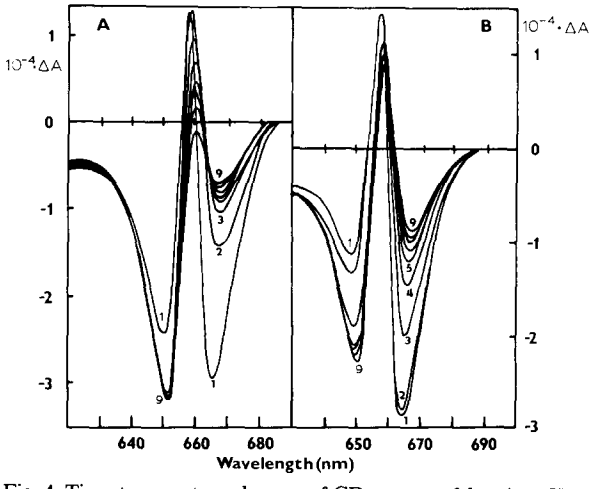

Fig. 4. Time-temperature changes of CD spectra of fractions III (A) and II (B) in the red region. After prior incubation to 45°C as in Fig. 3, the temperature was raised to 55°C (curve 1). The recording time periods of incubation at 55°C are as in Fig. 3. Chl concentration 15 μg/ml.

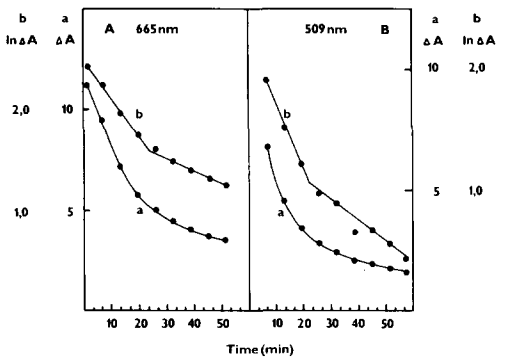

Fig. 5. Kinetics of thermal changes of the CD intensity of fraction III. Changes in the negative peak at 665 nm (A) and in the positive peak at 509 nm (B) were measured at 45°C and are expressed relative to the initial dichroic absorbance..

tionship of chlorophyll CD bands in the long-wavelength region and, in the case of fraction III, Cotton effects in the absorption region of carotenoids as well. The measured curves are shown in Fig. 3 and 4.

The kinetics of intensity changes of CD in the negative peak at 665 nm or in the positive peak at 509 nm are shown in Fig. 5. The set of CD curves, obtained by incubating a sample of fraction III at 45°C for 32 min is characterized by the pseudo-isodichroic point at 659 nm. The original form of the Cotton effect, which can be interpreted as the sum of two intense bands of opposite signs with mutually slightly shifted peaks, alters during this time to give a more complicated three-band spectrum which is gradually converted with increasing temperature to a considerably less intense, simple negative Cotton effect, corresponding to the CD spectrum of chlorophyll in fraction I. In dichroic bands in the absorption region of carotenoids we observe, under the same conditions, only decreasing intensity down to total disappearance, proceeding in time parallel to changes in the CD bands of chlorophyll. In the red region, Complex II exhibits exactly the same course of time-temperature changes in the chlorophyll CD spectrum as we observe with Complex III.

Electrophoretic separation of samples of Complexes II and III, incubated for 6–48 min at 30°C, is shown in Fig. 2. It is clear that in the first 6 min the polypeptide pattern remains the same under these conditions as in the case of Complex II. After this time, a smaller chlorophyll-protein complex of apparent molecular weight 20000 is separated from Complex II and an aggregate of apparent molecular weight 168000 is formed. After 48 min of heating, two chlorophyll-free polypeptides appear: it has been proved earlier for the smaller of these, mol.wt. 61000, that it is the apoprotein of CP I, i.e., of Complex II [8–10]. Complex III disintegrates quickly under the same conditions and, after 24 min of heating, roughly equal amounts of the decomposing complex and of the complex formed by decomposition are present. After 48 min the larger Complex III has virtually totally dissociated, Complex II and a smaller amount of chlorophyll-protein complex of apparent mol.wt. 20000 being formed.

Fig. 2 also shows the temperature changes of the polypeptide pattern of Complexes II and III after incubation at 45°C. After 6 min, Complex II already aggregates distinctly. Moreover, dissociation takes place at the same time and a chlorophyll-protein complex of apparent mol. wt. 84000 appears. Two pigment-free polypeptides are also found on the gel – the apoprotein (mol. wt. 61000) and probably its dimer (mol.wt. 119000). Under these conditions, Complex III likewise decomposes very quickly, forming Complex II or its aggregate (mol.wt. 168000). After 48 min, dissociation of chlorophyll from the protein matrix is quite distinct and at the same time, the content of the polypeptide of mol.wt. 84000 increases, most probably due to decomposition of the aggregate of mol.wt. 168000. In all the cases described, the smallest chlorophyll-protein complex of mol.wt. 20000 is also found in the sample.

The time correlation between the disappearance of dichroism in the absorption region of carotenoids (500–550 nm) and dissociation of Complex III to Complex II becomes evident when the course of thermal degradation of pigment-protein complexes is studied with the aid of CD spectra and electrophoresis.

At a later stage of our experiments, we therefore wanted to determine the influence of carotenoid extraction on the integrity of these complexes. Carotenoids were extracted by the technique described in Methods.

Chromatographic separation of chlorophyll-protein complexes obtained after extraction of carotenoids from whole native membranes of *P.*

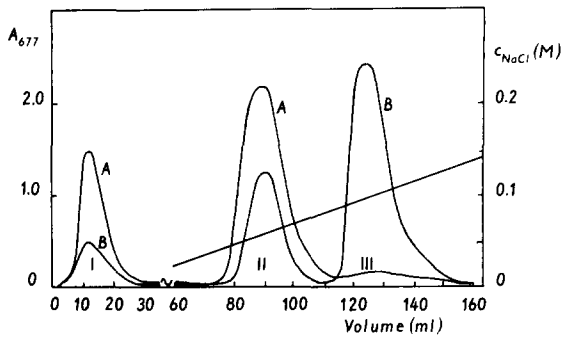

Fig. 6. Elution curve of chlorophyll-protein complexes eluted from a column of DEAE-cellulose (50 mM Tris-HCl, pH 8.0, +NaCl gradient). Thylakoid membranes were (A) or were not (B) extracted with *n*-heptane before solubilization with Triton X-100.

boryanum is shown in Fig. 6. When membranes, modified in this way were treated with Triton X-100 and chromatographed on DEAE-cellulose, only fraction II was observed at the normal value of the NaCl gradient of 60 mM. Fraction III was totally absent and all the chlorophyll was contained in the single fraction II.

Extraction of carotenoids from the isolated chlorophyll-protein complexes II and III was not total, but the carotenoid content was decreased considerably (Fig. 7). The ratio (mol/mol) of carotenoids extractable with heptane to Chl *a* was determined to be 1.47 for fraction I, 0.35 for fraction II and 0.28 for fraction III.

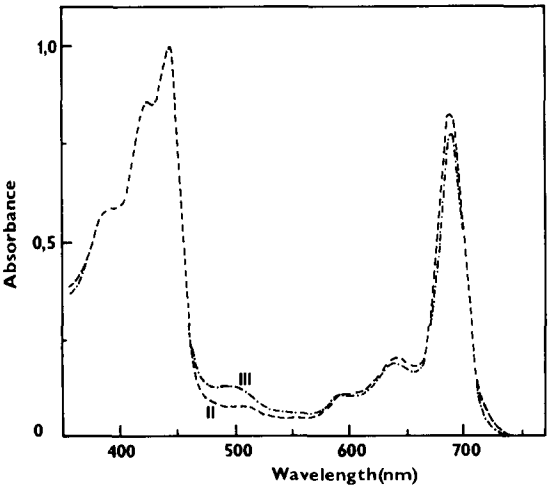

Fig. 7. Absorption spectra of fraction II (— — —) and III (— · —) after heptane extraction of carotenoids, path length 1 cm.

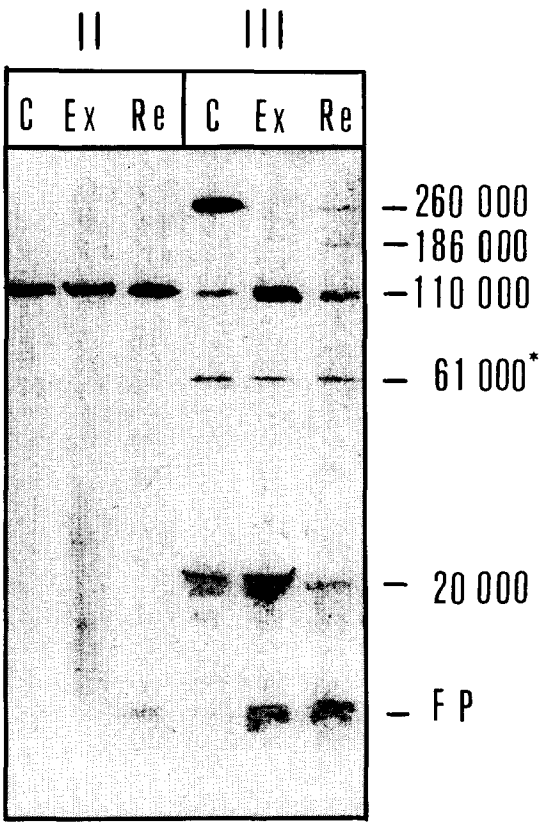

Fig. 8. Electrophoretic pattern of chlorophyll-protein complexes of fractions II and III after heptane extraction of carotenoids (Ex) and after recombination of complexes with heptane extract (Re). C control; FP free pigment; *, polypeptides containing no chlorophyll.

It was proved by chromatographic study that all fractions contain several types of carotenoids. Besides β-carotene, which is dominant in all fractions, myxoxanthin, crypto- and zeaxanthin were found in a fraction I. The molar ratio of these four carotenoids is 20:3:3:2.5. The composition of the heptane extract of fractions II and III is the same; both contain β-carotene, myxoxanthin and cryptoxanthin at a molar ratio of 10:4:1.

Electrophoretic separation of chlorophyll-protein complexes of fractions II and III after extraction is shown in Fig. 8. Lyophilized preparations were used as controls, and the excess Triton X-100 was removed, with Amberlite XAD-2. No changes were observed in Complex II under these conditions. On the other hand, lyophilized fraction III already contains slightly dissociated pigment-pro-

tein structures and a small amount of Complex II appears, as well as the polypeptide of mol.wt. 61000 and a chlorophyll-protein complex of apparent mol.wt. 20000.

After heptane extraction, Complex III dissociates completely, the amount of minor products increasing in relation to the control and free pigment appearing. After recombination of chlorophyll-protein complexes with heptane extract, reassociation takes place in part. The chlorophyll-protein complex of mol.wt. 260000 is again formed, and moreover a chlorophyll-protein complex of mol.wt. 186000 appears. The remaining amount of Complex II is greater, however, than that in the control sample, and total reassociation is not achieved even when the incubation time is prolonged and the carotenoid/chlorophyll ratio is raised.

The CD spectrum of fraction III after extraction of carotenoids shows that the procedure considerably alters the original asymmetric arrangement of Chl *a* molecules. We found a complex three-band spectrum for this sample, in which the intensities of both negative maxima are comparable. The CD curve of this shape has been observed for unextracted fraction III after 51 min exposure to a temperature of 45°C (see Fig. 3). The three-band character of dichroism in the red region was maintained also in the sample after recombination, but the intensity ratio of the above-mentioned negative peaks was altered: higher negative intensity was found in the short-wavelength band. Compared to spectra of non-extracted fraction III, this spectrum corresponds to a transition state in which after 57 min at 45°C the temperature was raised to 55°C (see curve 1 in Fig. 4A). From this it follows that the extraction procedure destroys the native conformation of the complex to a large extent. The recombination described also did not lead to restoration of the carotenoid Cotton effects in the 500–550 nm region.

Discussion

The results presented confirm the suitability of the method of separating detergent extracts on DEAE-cellulose for studying chlorophyll-protein complexes of thylakoid membranes, as shown earlier [10,19,25,26]. Of the two electrophoretically homogeneous chlorophyll-protein complexes, obtained by the above method from thylakoids of *P. boryanum*, Complex II can be considered to be identical with complex CP I [8] as well as the chlorophyll-protein Al [7.9] (i.e., P-700-Chl *a*-protein complex, originally isolated and described by Thornber [6]) on the grounds of those characteristics which up to now have been studied – amino acid composition [10], pigment content and size.

On the basis of similar amino acid and polypeptide compositions, as well as dissociation caused by external influences, the larger Complex III which we isolated has been proved bo be an oligomer of Complex II. The CD spectra of the complexes should be considered in this respect. The observed increase in intensity and exciton splitting of Cotton effects in chlorophyll-protein complexes as opposed to CD spectra of the free pigment (measured, e.g., in fraction I or in model chlorophyll solutions in organic solvents) is a result of interaction of pigment molecules, bound in a certain way to the protein matrix of the complex. Signs and intensities of the observed bands are determined by details of the geometry of the resulting specifically asymmetric chlorophyll arrangement.

The assumption of the importance of the chlorophyll-protein bond is also confirmed by conclusions which can be drawn from the time-temperature relationship of CD spectra of Complex III, as will be discussed later. A comparative study by Sauer and Austin [27], carried out with pigment-protein complexes of bacteria and higher plants, shows that with the exception of specially purified preparation of reaction centres, chiroptical properties of the samples studied are determined by dichroism of the antenna systems. The signs and ratios of intensities of Cotton effects measured with Complexes II and III correspond to published CD spectra of the antenna systems of barley, and its mutant deficient in Chl *b*, as well as CD curves of antenna bacteriochlorophyll-protein complexes. Minimal differences in CD spectra of Complex II and its aggregated form (oligomer) – Complex III – are manifestations of the fact that the relative geometric arrangement and interactions of the antenna chlorophyll remain practically unaffected when two Complexes II are joined to form a higher-level structure. We assume, there-

fore, for Complexes II and III, that in the antenna the chlorophyll is enclosed in a protein matrix in such a way that it forms groups with a certain geometric arrangement, supported or directly determined by interactions with suitable groups of the polypeptide chain, in analogy with the well known three-dimensional structure of the bacteriochlorophyll-protein complex reported by Matthews et al. [28].

Complex III differs markedly from Complex II in the Cotton effects in the 500–550 nm region, ascribed to the carotenoids present. These bands were found neither in fraction II, which contains somewhat more carotenoids relative to chlorophyll, nor in fraction I with the highest carotenoid content. This means that flexible polyene chains of carotenoids are fixed in Complex III by interaction with the arranged protein matrix, due to which their chiral conformations cannot be rotationally averaged. This assumption agrees with the conclusions of Cogdell et al. [14] based on similar observations in systems isolated from photosynthetic bacteria.

Participation of carotenoids in the formation of the structure of Complex III is illuminated by experiments in which dissociation of this complex was studied. Dissociation can be initiated by heating in glycerol to 30 or 45°C. Elevation of the temperature has a marked influence on the shape of CD bands of chlorophyll as well as of carotenoids. This means that changes take place in the geometric arrangement of pigment molecules in the complex. The kinetics of this process include two components – when the sample is exposed to a temperature of 45°C, a rapid process takes place in the region of the red chlorophyll band, which is replaced by a slower process after 10–15 min. The half-times of the two processes are 18–20 and 50–80 min, respectively, assuming first-order kinetics. Changes in Complex III, which are manifested in the carotenoid band in 509 nm and, when the sample is exposed to the same temperature, in the negative peak at 665 nm of the CD spectrum, proceed by similar kinetics (see Fig. 5).

It was proved by electrophoretic means that after prolonged incubation or after treatment at higher temperatures, dissociation of Complex III is accompanied by re-aggregation of Complex II which was originally formed. Aggregation of chlorophyll-protein complexes or, of the polypeptide of thylakoid membranes at elevated temperature, can be understood when we consider the high content of hydrophobic amino acids [6,10]. The newly formed aggregate has a lower apparent molecular weight compared to Complex III. This obviously does not exclude the possibility that this aggregate is also a dimer of Complex II and, therefore, in principle corresponds to Complex III. The reason is that aggregation may lead to a different spatial orientation of the two subunits, so that the newly formed aggregate has a slightly different conformation, which may alter its electrophoretic properties. It seems, however, from our results that the problem of dissociation and re-aggregation of Complex III is more complicated.

We conclude (see Fig. 9) that the arrangement of carotenoids in Complex III is caused by their incorporation in a specific binding site of the protein, probably localized in the contact region of subunits in the oligomer. This interpretation of the results is supported by several arguments:

(a) We may expect a similar course in the kinetics of temperature-induced reorientation of thermodynamically widely different systems, i.e., chlorophyll and carotenoid molecules, when both pigments are bound to the same protein matrix, controlling it by their conformation changes.

(b) From the identity of CD spectra measured for Complexes II and III in the absorption region of chlorophyll, it follows that at least those parts of protein molecules which determine changes in the asymmetric arrangement of this pigment have very similar conformations in both complexes. Nevertheless, the specific chiral form of carotenoids, observed in oligomeric Complex III, is missing in Complex II.

(c) Dichroism of carotenoids decreases in parallel with decreasing concentration of oligomeric units (mol.wt. 260000) and growth of the amount of conformation-altered subunits of Complex II (mol.wt. 112000) when Complex III is thermally dissociated.

(d) According to CD spectra, extraction of carotenoids with heptane has a substantially equal effect. In addition, irreversible changes in protein conformation damage the original geometry of the binding site of carotenoids in this case. These molecules are then unable to resume their original

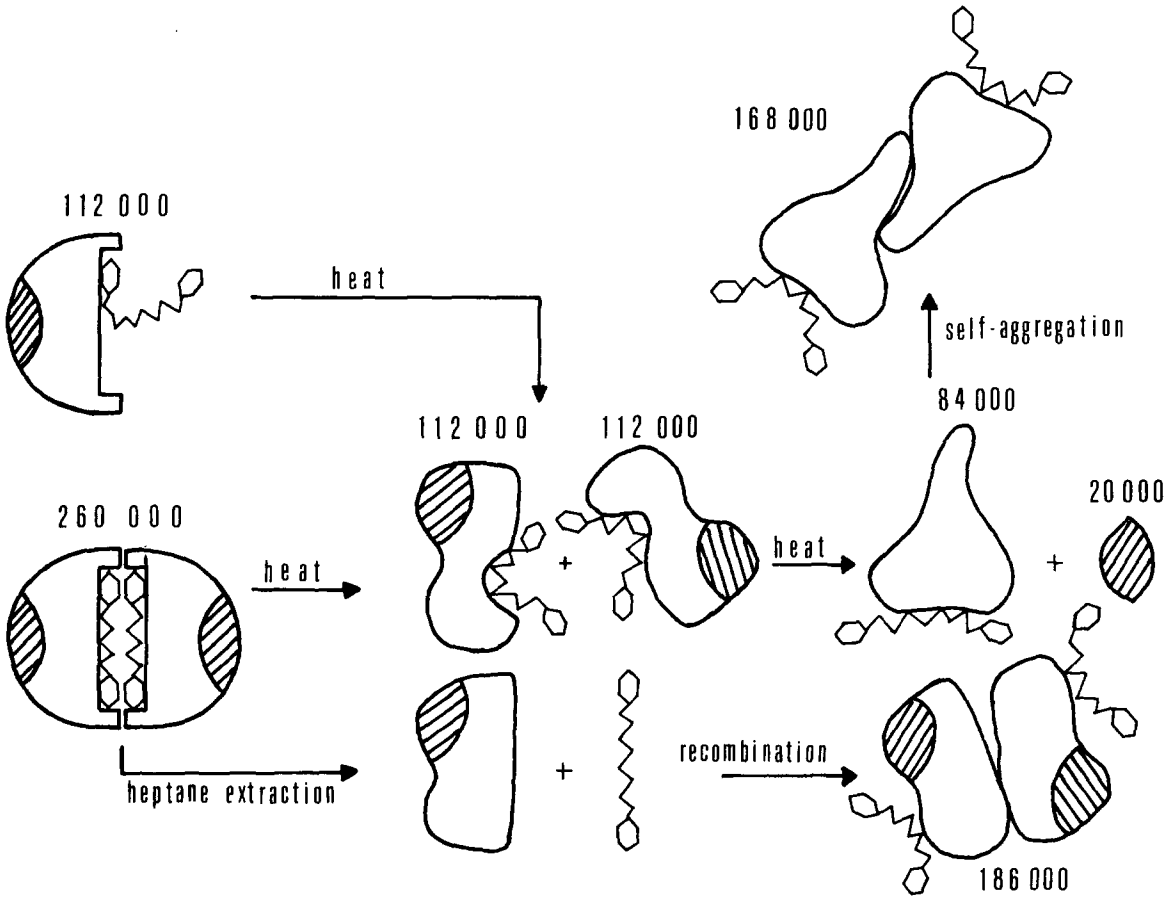

Fig. 9. Schematic interpretation of changes of chlorophyll-protein complexes, induced by heating or by heptane extraction of carotenoids. Apparent molecular weights of complexes are given.

fixed chiral arrangement either in later phases of the process, or when we attempt to replace them.

To summarize: In Complex III the chiral form of carotenoids disappears either after splitting of the second subunit by the detergent or after splitting of the multi-subunit complex and damaging of its protein conformation by heat or heptane treatment, i.e., when the subunit-subunit interactions are altered. Extraction of carotenoids from whole native thylakoid membranes has a similar effect.

It may be assumed on the basis of the above facts, that carotenoids may mediate a part of the interactions which stabilize chlorophyll-protein structures within thylakoid membranes, taking part in specific binding of pigment-protein subunits. In addition to the light-harvesting and protective function, the carotenoids therefore probably have a structural function in photosynthetic material in vivo. A supramolecular aggregate of this type could be the high molecular weight chlorophyll-protein complex of apparent mol.wt. close to 500000 which we have observed earlier [16], or the CP I oligomers described by Hoarau et al. [29].

The further functional consequences of this structural characteristic of chlorophyll-protein complexes of cyanobacteria are now under study.

References

1 Anderson, J.M. (1975) Biochim. Biophys. Acta 416, 191–235
2 Thornber, J.P. (1975) Annu. Rev. Plant Physiol. 26, 127–158
3 Thornber, J.P., Markwell, J.P. and Reinman, S. (1979) Photochem. Photobiol. 29, 1205–1216

4 Boardman, N.K., Anderson, J.M. and Goodchild, D.J. (1978) Cur. Top. Bioenerg. 8, 35–109
5 Thornber, J.P., Alberte, R.S., Hunter, F.A. Shiozawa, J.A. and Kan, S.-A. (1977) Brookhaven Symp. Biol. 28, 132–148
6 Thornber, J.P. (1969) Biochim. Biophys. Acta 172, 230–241
7 Reinman, S. and Thornber, J.P. (1979) Biochim. Biophys. Acta 547, 188–197
9 Rusckowski, M. and Zilinskas, B.A. (1980) Plant Physiol. 65, 392–396
9 Stewart, A. (1980) FEBS Lett. 114, 67–72
10 Hladík, J. and Sofrová, D. (1981) Photosynthetica 15, 490–503
11 Olson, J.M., Ke, B. and Thompson, K.H. (1976) Biochim. Biophys. Acta 430, 524–537
12 Canaani, O.D. and Sauer, K. (1978) Biochim. Biophys. Acta 501, 545–549
13 Gregory, R.P.F. and Raps, S. (1974) in Proceedings of the 3rd International Congress on Photosynthesis (Avron, M., ed.), pp. 1977–1982, Elsevier, Amsterdam
14 Cogdell, R.J., Parson, W.W. and Kerr, M.A. (1976) Biochim. Biophys. Acta 430, 83–93
15 Paillotin, G., Vermeglio, A. and Breton, J. (1979) Biochim. Biophys. Acta 545, 249–264
16 Sofrová, D. and Hladík, J. (1980) Photosynthetica 14, 86–89
17 Kratz, W.A. and Meyers, J. (1955) Am. J. Bot. 42, 282–287
18 Biggins, J. (1967) Plant Physiol. 42, 1442–1455
19 Bengis, C. and Nelson, N. (1977) J. Biol. Chem. 252, 4564–4569
20 Markwell, J.P., Reinman, S. and Thornber, J.P. (1978) Arch, Biochem. Biophys. 190, 136–141
21 Ogawa, T. and Vernon, L.P. (1971) Biochim. Biophys. Acta 226, 88–97
22 Karrer, J. and Jucker, E. (1950) Carotenoids, Elsevier, Amsterdam
23 Davies, B.H. (1976) in Chemistry and Biochemistry of Plant Pigments (Goodwin, T.W., ed.), Vol 2, Academic Press, London
24 Wolf, H. and Scheer, H. (1973) Ann. N.Y. Acad. Sci. 206, 549–567
25 Nakayama, K., Yamaoka, T. and Katoh, S. (1979) Plant Cell Physiol. 20, 1565–1576
26 Hladík, J. and Sofrová, D. (1980) Photosynthetica 14, 532–544
27 Sauer, K. and Austin, L.A. (1978) Biochemistry 17, 2011–2019
28 Matthews, B.W., Fenna, R.E., Bolognesi, M.C., Schmid, M.F. and Olson, J.M. (1979) J. Mol. Biol. 131, 259–285
29 Hoarau, J., Remy, R. and Leclerc, J.C. (1977) Biochim. Biophys. Acta 462, 659–670