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SOLUBILIZATION AND SPECTRAL CHARACTERISTICS OF CHLOROPHYLL-PROTEIN COMPLEXES ISOLATED FROM THE THERMOPHILIC BLUE-GREEN ALGA *SYNECHOCOCCUS LIVIDUS* *

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The thermophilic blue-green alga *Synechococcus lividus* was grown at 38 and 55°C. The reaction center chlorophyll-protein complexes (CP) of Photosystem (PS I) and PS II, CP a_I and CP a_{II}, were isolated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis at 4°C. SDS solubilization of thylakoids was performed in the temperature range 0–65°C. The low-temperature absorption and fluorescence emission spectral properties of the isolated chlorophyll-protein complexes were analyzed. Only traces of CP a_I were solubilized at temperatures below the lipid phase transition temperature. Instead, a minor PS I component, CP a_I', was obtained that had absorption and fluorescence characteristics similar to those of CP a_I. CP a_I' had a slightly lower mobility than CP a_I in SDS-polyacrylamide gel electrophoresis. The amount of CP a_I in the gel scan profile increased dramatically when solubilization was carried out above the phase transition temperatures, but started to decrease above 60°C. CP a_{II}, on the other hand, could be efficiently extracted even at 0°C and was stable in the scan profile up to extraction temperatures of 30–40°C. Low-temperature absorption and fluorescence emission spectra were typical for CP a_I and CP a_{II} and no specific effects of the two growth temperatures on these properties were observed. The phase transition temperature was considered to be critical for the solubilization of CP a_I, either because of the difficulties of SDS (especially as it forms micelles at low temperatures) in penetrating the solidified membrane lipids at temperatures below that of the phase transition or because the CP a_I monomers of the PS I antennae are so strongly bound to each other that they cannot be dissociated by SDS before thermal agitation has reached a certain level that is achieved above the phase transition temperature. We consider both the difficulties in solubilizing CP a_I at sub-transition temperatures and the heat stability of the two complexes as adaptations which enable *Synechococcus* to grow under extreme high-temperature regimes.

Introduction

Detergent solublization of thylakoids followed by electrophoresis or chromatography has revealed the existence of three major chlorophyll-protein complexes in green platns: CP a_I and CP a_{II} derived from the reaction center Chl *a* antennae of PS I and PS II, respectively, and CP *a/b*, derived from the light-harvesting Chl *a/b*-protein complex [1–5]. Blue-green algea contain only CP a_I and CP a_{II} [6–8]. The suggested origin of CP a_{II} is less well documented than that of CP a_I. In addition, minor bands presumably of aggregated chlorophyll-protein complexes are

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Abbreviations: Chl, chlorophyll; CP, chlorophyll-protein complex; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; PS, photosystem; SDS, sodium dodecyl sulphate.

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often observed in SDS-polyacrylamide gel electrophoresis. It is now quite well established that all chlorophylls are more or less firmly bound to proteins [9]. However, the properties of chlorophyll in vivo are also assumed to be influenced by the ligand potential of chlorophyll with compounds other than proteins, such as water, acyl lipids, carotenoids, or other chlorophyll molecules. It has been shown that the Chl *a* molecules of the reaction center antennae are relatively more hydrophobically bound than are the molecules in the light-harvesting Chl *a/b*-protein complex antenna [10]. Chl *a* of CP a_{II} and the far-red-absorbing Chl *a* fraction of CP a_I appear to be the most hydrophobically associated chlorophyl molecules. These fractions of chlorophyll are readily extracted by non-polar solvents and their weak hydrophobic ligands are easily broken by anionic detergents such as SDS if solubilization is done at room temperatures.

Heat inactivation of photosynthesis may be related to the breakage of short-range bonds of hydrophobic or hydrophilic character in PS II, as manifested in the functional disorganization of PS II at high temperatures [11]. It was of interest therefore to investigate whether adaptation to high temperature in plants leads to an increased strength of the chlorophyll-protein interactions as reflected in the heat stability of SDS-solubilized CP a_I and CP a_{II}. We used the thermophilic blue-green alga *Synechococcus lividus* because it contains only CP a_I and CP a_{II} (no CP *a/b* which overlaps CP a_{II}) and also because considerable physiological data are already available on the photosynthetic properties of this alga [12]. It was found that extracted CP a_{II} of *Synechococcus* grown at 38 or 55°C had a remarkable heat stability and that temperatures above that of the phase transition were needed in order to solubilize CP a_I efficiently.

Materials and Methods

The thermophilic blue-green alga *S. lividus* (strain SY-4 from Mercedes Edwards) was grown at 38 and 55°C as described previously [12]. Thylakoids were isolated using a lysozyme treatment (modified according to the techniques reported by Ono and Murata [13]). Algea were washed in growth medium and suspended in 0.5 M sorbitol, 10 mM Hepes-NaOH buffer, pH 7.5, 5 mM sodium/potassium phosphate buffer, pH 7.8, 12.5 mM EDTA, 0.1% lysozyme (Sigma L6876). The chlorophyll content was adjusted to 0.4 mg/ml. The suspension was incubated for 1 h at 37°C, with the use of a magnetic stirrer, after which the algae were collected by centrifugation at 12 000 × *g* for 5 min. The algae were suspended in 50 mM Hepes-NaOH buffer, pH 6.8, 0.15 M KCl, 0.01 M NaCl and 5 mM $MgCl_2$ and the suspension was then passed through a French pressure cell at 77 kg/cm^2. Unbroken algae were centrifuged, suspended in the breaking medium and again passed through the pressure cell. The thylakoids from the combined supernatants were collected by centrifugation at 48 000 × *g* for 40 min. The thylakoids were solubilized in 0.1 M Tris-acetate, pH 6.8, 0.5 M urea, 0.035 M SDS, 0.3 mM EDTA, 0.13 M 2-mercaptoethanol, 0.5 M sucrose and 0.8 mM bromphenol blue. The SDS : Chl ratio was adjusted to 31 : 1 (mol : mol). The solubilization was performed for 5 min at temperatures between 0 and 65°C and, after centrifugation, the supernatant was subjected to electrophoresis at 4°C for about 1 h on gel tubes (3 mA/tube; 8% acrylamide, 0.28% bis-acrylamide) in a buffer of 0.5 M urea, 3.5 mM SDS, 0.3 mM EDTA, 0.02 M thioglycolic acid and 0.1 M Tris-acetate, pH 9.0. For more details on SDS-polyacrylamide gel electrophoresis see Refs. 10, 14 and 15. The polyacrylamide gels were scanned for chlorophyll-protein complexes at 677 nm in a Beckman DU spectrophotometer with a gel scanner attachment (Gilford). The proportion of chlorophyll in the different bands of the gel was calculated by planimetry.

Low-temperature (89 K) absorption spectra were measured with a Cary 17 spectrophotometer equipped with a scattering transmission attachment The disc of a chlorophyll-containing band was cut out from the gel and positioned in an aluminium sample holder in contact with liquid N_2. For a description of the use of this instrument see Ref. 16. Low-temperature (77°K) fluorescence emission spectra of chlorophyll-containing gel discs were measured with a microprocessor-based spectrofluorimeter described earlier [17]. Both absorption and fluorescence spectra were deconvoluted by Resol [16,18].

Results

When SDS-polyacrylamide gel electrophoresis was performed on SDS extracts made at temperatures well below or above the phase transition temperature

of *Synechococcus*, different relative distributions between the chlorophyll-protein complexes were obtained. Fig. 1 shows gel scan profiles of extracts obtained at 20 and 55°C from thylakoids of *Synechococcus* grown at 55°C, which has a phase transition temperature near 43°C [12]; Similar profiles were obtained from algae that were grown at 38°C (phase transition temperature near 37°C [12]) (not shown). The profiles obtained from thylakoids that were solubilized above the phase transition temperature had bands of CP a_I and CP a_{II} (apparent molecular weights of 85 000 and 43 500, respectively), whereas extracts made below the phase transition temperature showed only traced of CP a_I. Instead, a new band, CP a_I', appeared that had a slightly lower mobility (apparent molecular weight 90 000–98 000) than CP a_I. In fact, CP a_I' was used to denote two very closely moving bands (Fig. 1).

Fig. 2 shows the relative distribution of CP a_I', CP a_I, CP a_{II} and SDS-solubilized chlorophyll in the scan profiles obtained after SDS-polyacrylamide gel electrophoresis of SDS extracts made at temperatures between 0 and 65°C for *Synechococcus* grown at 38°C (A) and 55°C (B). Clear bands of CP a_I were obtained from SDS extracts made above 40°C, near the phase transition temperature of the 55°C-grown cells (Fig. 2B). There was a tendency for Cp a_I to appear at temperatures a few degrees lower in 38°C-grown algae (Fig. 2A). CP a_I of both algal cultures started to deteriorate when the solubilizing temperature ranged above 60°C. CP a_I', unlike CP a_I, was solubilized at temperatures between 0 and 40°C. Above this temperature it started to disappear. Thus, CP a_I' disappeared from the scan profile as CP a_I appeared. Although under optimal conditions boht CP a_I' and CP a_I made up 40–50% of the chlorophyll on the gel, the chlorophyll content of CP a_I' never exceeded one-third to one-half of the chlorophyll content in CP a_I (cf. Fig. 1). The reason for this discrepancy between the presentations in Figs. 1 and 2A and B is that much less chlorophyll entered the gel when the extracts were obtained below the phase transition temperature rather than above. Extracts obtained below the transition temperature always gave rise to considerable amounts of chlorophyll, which showed a tendency to enter and remain at the very top of the gel, whereas no (or only very little) residue was obtained with extracts solubilized above the phase transition temperature.

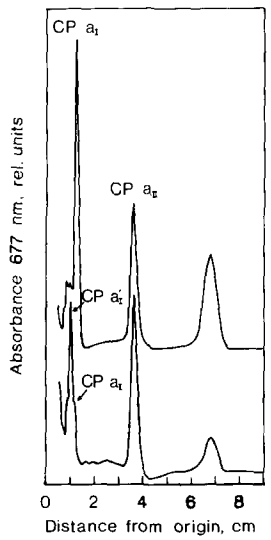

Fig. 1. Densitometer tracings (677 nm) of chlorophyll-protein complexes isolated by SDS-polyacrylamide gel electrophoresis from thylakoids of *S. lividus* grown at 55°C and solubilized with SDS at 20°C (lower curve) and 55°C (upper curve) for 5 min.

CP a_{II}, unlike CP a_I, was solubilized at high yield even at 0°C and started to decrease in the scan profile above about 40°C. It could hardly be isolated from extracts solubilized at 55–65°C. The relative proportion of SDS-solublilized cholophyll stayed at 15–25% as long as the solubilization was performed below about 40°C, and with increasing solubilization temperature it began an exponential rise while CP a_{II}, and later CP a_I, started to decrease in the scan profiles.

Absorption spectra were recorded for the SDS extracts, for all of the isolated chlorophyll-protein complexes, as well as for the residue which was found (in the case of low solibilization temperatures) at the top of the gel after electrophoresis had been completed. CP a_I' and CP a_I had identical absorption properties as shown in Fig. 3A and B and Table I, which gives the band positions, half-band widths and relative proportion of the band components. A comparison between CP a_I and CP a_{II} shows the well known differences in the absorption properties of the two photosystems [19]. The long-wavelength components at 684, 693–694 and 707–708 nm were

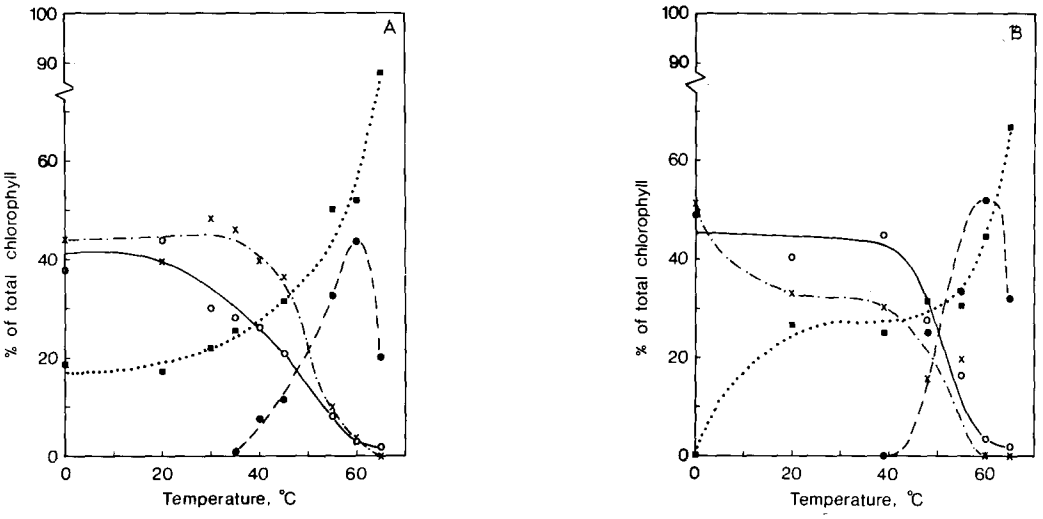

Fig. 2. Relative distribution of chlorophyll-protein complexes after SDS-polyacrylamide gel electrophoresis of SDS extracts obtained by solubilizing thylakoids for 5 min at temperatures between 0 and 65°C. *S. lividus* was grown at 38 (A) and 55°C (B). (o———o) CP a_I', (•- - - - -•) CP a_I, (X· — · — ·X) CP a_{II}, (■· · · · · ■) SDS-solubilized chlorophyll.

only found in CP a_I (Table I) and the peak of CP a_{II} was shifted to shorter wavelengths in comparison with that of CP a_I (Fig. 3A and C); the 669–670 nm band was twice as strong in CP a_{II} as in CP a_I, whereas the two chlorophyll-protein complexes showed equal relative amounts of the 677–678 nm band. No significant variations were found in the absorption properties that depended upon the solubilization temperature or upon the growth temperature of *Synechococcus*. Analyses of absorption spectra of CP a_I or CP a_I' isolated by SDS-polyacrylamide gel electrophoresis (Fig. 3A and B; Table I) showed that they were like the antenna Chl *a* of the PS I reaction centers solubilized by Triton X-100 from *Pisum sativum* chloroplasts [16]. Similarly, curve analyses revealed the same Chl *a* components in CP a_{II} isolated by SDS-polyacrylamide gel electrophoresis as in the light-harvesting Chl *a*/*b*-protein complex solubilized from pea by Triton X-100 [16].

Fluorescence emission spectra were measured for the SDS extracts, for all of the isolated chlorophyll-protein complexes and, when present, for the residue on the top of the gel. Both CP a_I' and CP a_I showed the strong far-red emission peak at 726 nm that is typical for the antenna of PS I (Fig. 4A and B and Table II) [20]. However, CP a_I' had a much stronger peak at 684 nm than did CP a_I. This peak is thought to be due to a free-moving chlorophyll because a similar emission peak was obtained when fluorescence was measured on a gel disc that was cut out between CP a_I and CP a_{II}. The reason why the 684–685 nm band is relatively stronger in CP a_I' than in CP a_I is therefore explained by CP a_I' solubilized at 20°C being weaker than CP a_I solubilized at 55°C (cf. Fig. 1). When detectable, CP a_I' and CP a_I always had the fluorescence characteristics shown here and adaptation of *Synechococcus* to 38 or 55°C did not make any difference. When solubilized at temperatures below about 40°C, CP a_{II} revealed a main fluorescence peak at 687 nm (Fig. 4C, Table I). Curve analyses also revealed quite a strong emission band at 693–697 nm. Bands with these approximate positions are generally typical for PS II [20]. However, solubilization at temperatures above 40°C caused a change in the emission properties of CP a_{II} so that the main peak shifted to 684 nm and a new band at 700–705 nm becamer stronger as the solubilization temperature increased (Fig. 4D and Table II). These changes occurred in the high-temperature range where CP a_{II} gradually disappeared from the gel. The reason for the appearance of this far-red emission band of CP a_{II} solubilized at high temperatures is not known. The residue from the extract made at low solubilization temperatures entered only the very top of the gel

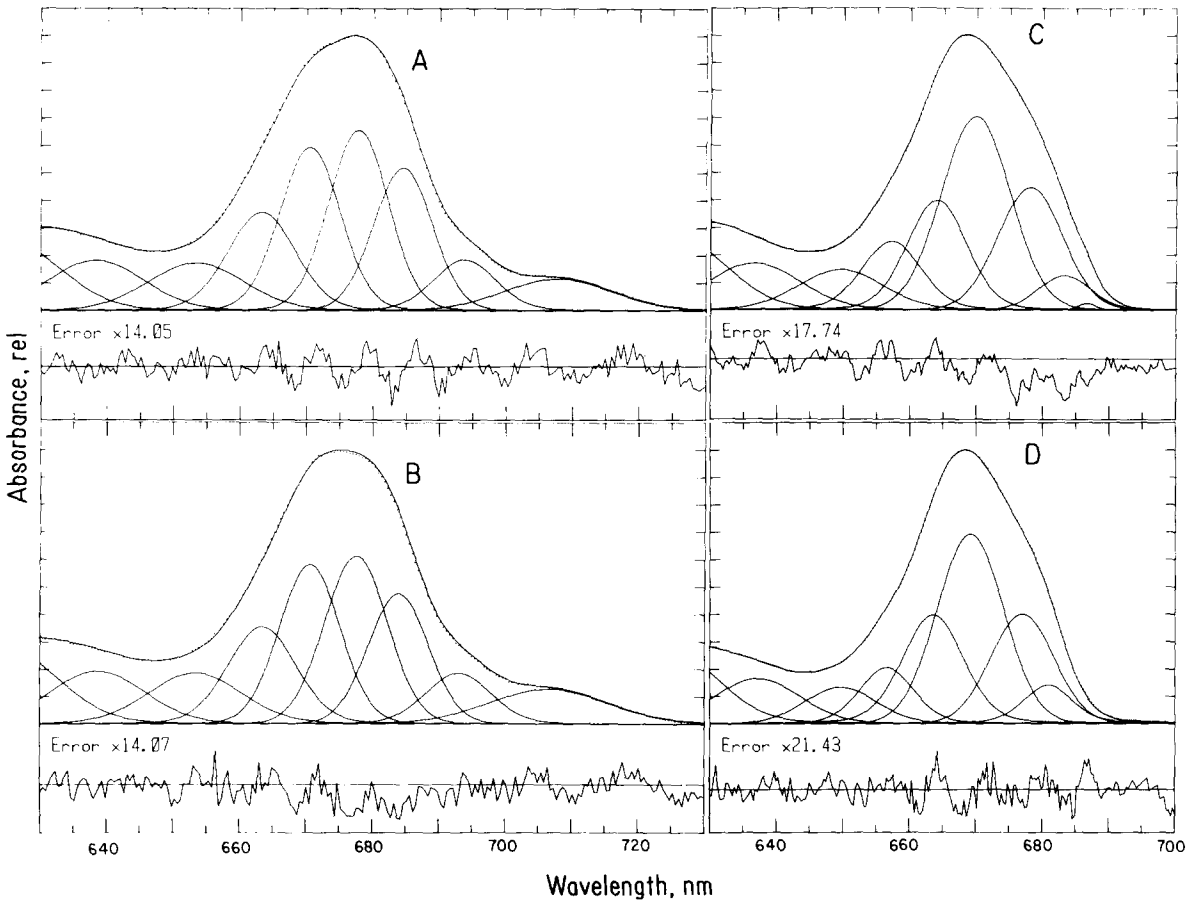

Fig. 3. Absorption spectra (89 K) of chlorophyll-protein complexes isolated from *S. lividus* grown at 55°C. (A) CP a_I' from thylakoids solubilized at 38°C, (B) CP a_I from thylakoids solubilized at 48°C, (C) CP a_{II} from thylakoids solubilized at 40°C and (D) Cp a_{II} from thylakoids solubilized at 55°C. For identification of individual bands obtained by RESOL see Table I. The measured curve is given by the dotted line while the solid line through the dots is the sum of the bands. The error gives the differentce between the measured and calculated spectra.

TABLE I

Curve analyses of absorption spectra measured at 89 K for (A) CP a_I' solubilized at 38°C, (B) CP a_I solubilized at 48°C and (C and D) CP a_{II} solubilized at 40 and 55°C, respectively. The chlorophyll-protein complexes were isolated by SDS-polyacrylamide gel electrophoresis from *S. lividus* grown at 55°C. The percentage of each band was calculated from the sum of the areas of the chlorophyll bands. Components with maxima below 660 nm were not included because they are considered to belong to secondary vibration bands. S.E., standard error (in per cent) of a peak height which has been normalized to 1000 relative units [16]. Half-band widths (HW) are given. Gaussian curves make up 85–100% and Lorentzian curves the rest.

	Band component (nm)														S.E.
	663–664		669–671		677–678		681–683		684		693–694		707–708		
	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW	
CP a_I	16	12	23	11	25	10	–	–	20	10	8	11 [a]	8	19	0.28
CP a_I	16	12	23	11	25	11	–	–	16	11	8	12 [b]	10	21	0.31
CP a_{II} (40°C)	23	11	44	12	26	11	6	9 [c]	–	–	–	–	–	–	0.27
CP a_{II} (55°C)	25	11 [d]	44	12	24	11	8	9	–	–	–	–	–	–	0.18

[a] 81% Gaussian.
[b] 84% Gaussian.
[c] 59% Gaussian.
[d] 76% Gaussian.

TABLE II

Curve analyses of fluorescence emission spectra measured at 77 K for (A) CP a'_I solubilized at 20°C, (B) CP a_I solubilized at 55°C and (C and D) CP a_{II} s
t 20 and 55°C, respectively. The chlorophyll-protein complexes were isolated by SDS-polyacrylamide gel electrophoresis from *S. lividus* grown at 55°C
entage of each band was calculated from the sum of the areas of the bands. S.E. (see Table I) and half-band widths (HW) are given. Gaussian curves mak
00% and Lorentzian curves the rest.

	Band component (nm)																		S.E.
	676		683		684–685		691–693		700–704		726		738		747–748		757–761		
	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW	
CP a'_I	1	6	–	–	19	9	5	10	4	14	43	19	13	16	7	16	8	26	0.43
CP a_I	–	–	3	12	3	8	1	9	2	13	52	19	17	16	8	17	14	35	0.22
	676		681		684–685		688		693–697		703		713–719		743–747				
	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW	%	HW			
CP a_{II} (20°C)	–	–	9	9	21	9	35	10	14	15	–	–	6	24	15	31			0.40
CP a_{II} (55°C)	1	5	–	–	44	10	–	–	11	11	27	16	4	16	12	34			0.35

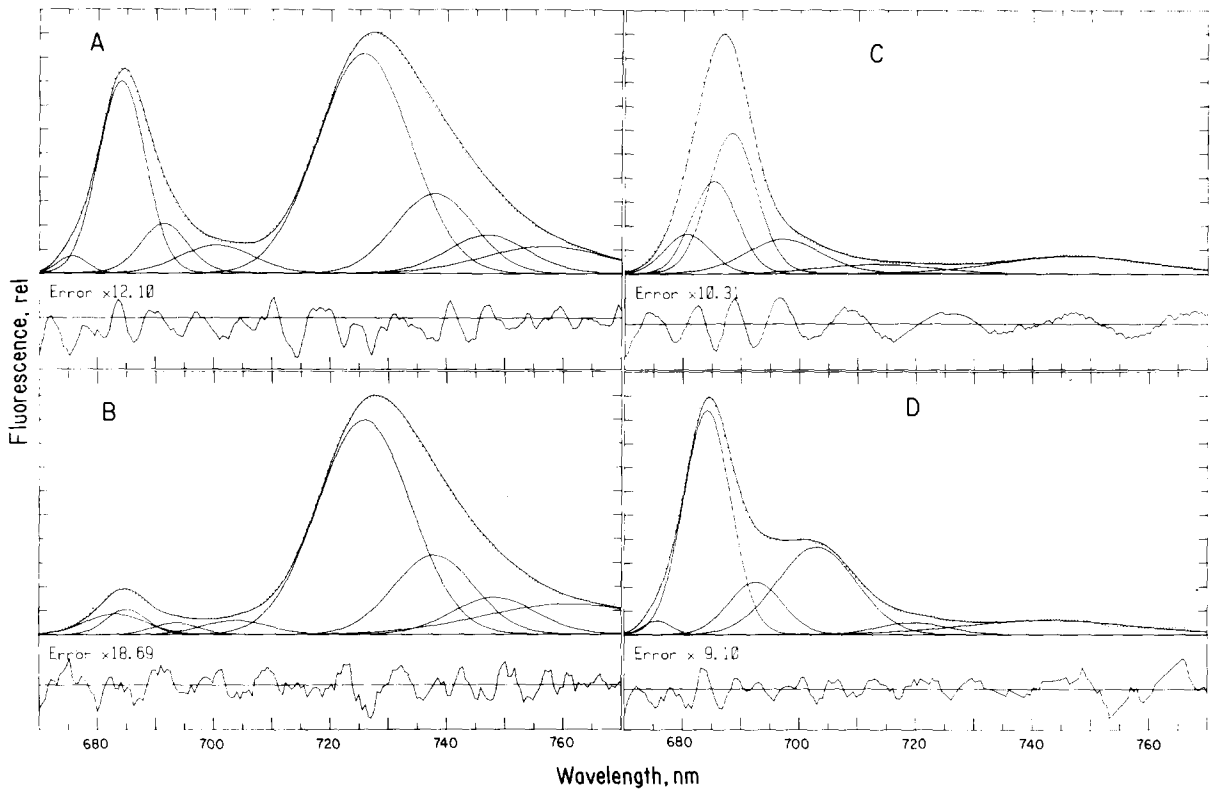

Fig. 4. Fluorescence emission (77 K) spectra of chlorophyll-protein complexes isolated from *S. lividus* grown at 55°C. (A) CP a_I' from thylakoids solubilized at 20°C, (B) CP a_I from thylakoids solubilized at 55°C, (C) CP a_{II} from thylakoids solubilized at 20°C and (D) CP a_{II} from thylakoids solubilized at 55°C. For specification of individual emission bands obtained by RESOL see Table II. The measured curve is given by the dotted line while the solid line through the dots is the sum of the bands. The error gives the difference between the measured and calculated spectra.

and had an emission spectrum essentially like that of CP a_I. This would be expected since CP a_{II} can be solubilized and isolated with a high yield at these low temperatures, while CP a_I is solubilized only above 40°C.

Discussion

The findings that CP a_I did not appear in the scan profile when the temperatures used for SDS extraction were below 40°C is interpreted to show that CP a_I extractability is dependent upon the physical state of the thylakoid membrane lipids. *Synechococcus* grown at 38 and 55°C has phase transition temperatures near 37 and 43°C, respectively [12], and these differences in transition temperatures may explain why CP a_I was somewhat more efficiently extracted near 40°C in the 38°C-grown algae than in the 55°C-grown algae (Fig. 2A and B). The fatty acids of the thylakoid membrane lipids of the thermophilic alga *Synechococcus* are characterized by being very saturated, particularly so for the 55°C-grown algae [12]. Below the phase transition temperature these lipids would be in a more ordered state and in combination with SDS forming micelles at low temperatures [21], SDS may not be capable of penetrating efficiently the more compact and solid thylakoid membrane lipids and solubilize the proteins. Above the phase transition temperature, thermal movements would make the lipids more fluid and cause the membranes to become more permeable [22], thus allowing monomers of SDS to penetrate the thylakoid membranes more easily. However, the resistance of CP a_I to extractions by SDS below the phase transition

temperature may also be related to the nature of the PS I antenna. We know that, after electrophoresis of the extracts obtained at temperatures below about 40°C, there was a residue with PS I emission characteristics left at the very top of the gel. It is possible that the antenna of PS I in this thermophilic alga is composed of CP a_I monomers, the tertiary structures of which are more resistant, and the CP a_I monomers are held together in oligomers having much stronger bonds than what is usual for mesophilic plants. Proteins of thermophilic bacteria are known to be stabilized by strong, hydrophilic salt bridges [23], which are much more resistant to high temperatures than are short-range hydrophobic ligands or hydrogen bonds. The latter bonds are more abundant in stabilizing and aggregating proteins of mesophilic plants. Perhaps the antenna of PS I cannot be dissociated by SDS until thermal agitation has reached a certain level that is equivalent to the phase transition temperature.

The similar absorption and fluorescence emission characteristics of CP a_I and CP a_I' indicate that they are both derived from the reaction center antennae of PS I. Because of its low apparent molecular weight, CP a_I' is not identical with the reaction center complex CP 1a or A, which has been isolated from spinach [4] and blue-green algae [6], respectively, and which has a molecular weight of about 200 000. The reason why CP a_I and CP a_I' were extracted at different temperatures may be that CP a_I' is extracted from a certain population of the PS I antenna, which is accessible to SDS at temperatures below that of the phase transition. Another possibility would be that CP a_I' represents a fraction of a homogeneous PS I antenna, which can be solubilized at temperatures below the phase transition temperature. Perhaps the mobility of CP a_I' is lower than that of CP a_I because the former has different surface properties and/or because some lipids may adhere to the protein. The rather similar apparent molecular weight of CP a_I and CP a_I' make it unlikely that their different migration rates would depend upon different amounts of low molecular weight polypeptides which, together with a 70 000 molecular weight protein, are supposed to make up the reaction center antenna of PS I [4,24].

The CP a_{II} isolated from *Synechococcus* was remarkably heat resistant, since it could be isolated with high yield between 0 and 40°C (Fig. 2A and B). In other plants (spinach, pea), CP a_{II} can only be isolated using SDS-polyacrylamide gel electrophoresis if the SDS solubilization is performed near 0°C, probably because preferentially weak, short-range hydrogen bonds or hydrophobic ligands link the protein and the chlorophyll of CP a_{II} [10]. This suggests that the remarkable heat stability of CP a_{II} isolated from *Synechococcus* is produced by an increase in the strength of protein-chlorophyll interactions.

We consider the increased heat resistance of the SDS-extracted chlorophyll-protein complexes and the difficulties of solubilizing the PS I antenna into monomers at low temperatures to be an expression of the adaptation of *Synechococcus* to grow under extremely high temperatures. This alga is a useful tool for investigating the mechanisms of heat tolerance in photosynthesis.

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