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Investigation of highly stable Photosystem I chlorophyll-protein complexes from the thermophilic cyanobacterium *Phormidium laminosum*

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The photosynthetic complex, Photosystem I, of the thermophilic cyanobacterium *Phormidium laminosum* was found to be highly resistant to denaturation by ionic and non-ionic detergents including SDS. This property was exploited to allow the preparation by sucrose density gradient centrifugation and by SDS-polyacrylamide gel electrophoresis of several protein complexes containing the PS I reaction centre components, chlorophyll *a* and carotenoid. In this report I have focused attention on the three most abundant complexes of estimated molecular masses of 450, 150 and 105 kDa. An initial description of the complexes is presented. The 105 kDa complex resembles plant CP-1 with a very low quantum efficiency for stable charge separation when compared with the 150 and 450 kDa complexes. This deficiency was associated with the loss of two low-molecular-weight polypeptides from the 105 kDa complex. The 450 kDa complex can be separated from the other complexes by centrifugation in a sucrose density gradient, and was found to be the most stable of the complexes in the presence of SDS. All three complexes contain roughly equivalent amounts of the reaction centre component P-700, suggesting that the 450 kDa complex is a multimer of the 150 kDa complex.

Introduction

In photosynthetic organisms, pigment molecules and electron-transfer components are organized in protein complexes. In photosynthetic bacteria, the structure of the photosynthetic unit (photosystem) is relatively well understood, and consists of a reaction-centre complex surrounded by many light-harvesting complexes which absorb and transfer light energy to the reaction centre. A bacterial reaction centre has been purified and crystallized [1] and contains essentially only six pigment molecules, at least four of which are directly involved in electron transfer.

In plants and cyanobacteria (oxygenic organisms) the structure of the two different photosystems is not so well understood. Light-harvesting complexes have been purified [2], but for neither photosystem has a reaction centre been purified that is free of light-harvesting pigments. Even in the most highly enriched reaction centre preparations, a core of about 30–70 chlorophyll molecules is always present [3–6]. It could be argued that the failure to produce a reaction centre free of light-

Abbreviations: Chl, chlorophyll; CP-1, chlorophyll-protein 1; DCIP, 2,6-dichlorophenolindophenol; Hepes, (4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid; PS I, Photosystem I; P-700, primary electron donor of Photosystem I.

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harvesting pigments in oxygenic organisms is a result of increased sensitivity to detergents, and that the conditions required to remove the core of light-harvesting proteins and pigments are sufficiently denaturing to remove the reaction-centre components at the same time. There is, however, evidence that at least one of the plant photosystems (PS I) is organized differently to the bacterial photosystem, and that in this case light-harvesting and electron-transfer functions are located within the same complex. For example, studies of PS I core complexes have shown very few proteins to be present [2,3], and further purification by SDS-polyacrylamide gel electrophoresis resulted in the isolation of a single 110 kDa chlorophyll-containing protein complex termed CP-1 [3]. In addition to about 40 chlorophyll *a* molecules, CP-1 was found to contain electron-transfer components. The primary electron donor chlorophyll (P-700) was detected [3,6] and recently evidence for the presence of early electron acceptors has been presented [7]. One [2] or two [8,9] chlorophyll-free apoproteins of molecular mass 60–70 kDa have been reported after extraction of CP-1 with mercaptoethanol and SDS, and the sequences of genes which probably encode the apoproteins have been published [9]. Thus, CP-1 apparently consists of a relatively simple system with one or possibly two proteins binding both light-harvesting and electron-transfer components. Unfortunately, the study of the functioning of CP-1 has been limited to very short time-scales [7] because the complex lacks electron acceptors (the iron-sulphur centres) which are essential for stable charge separation. A recent report [10] described a lithium dodecylsulphate-solubilized PS I fraction which appeared to have retained an extra electron acceptor (termed X). However, two electron acceptors (A and B) were still absent, and charge recombination occurred in a few milliseconds. The fraction was also unstable, being slow converted into CP-1.

In contrast to the easily denatured plant PS I fractions, PS I isolated from cyanobacteria has often been found to give multiple chlorophyll-containing bands on SDS-polyacrylamide gel electrophoresis [11–16], and stable electron transfer has been observed, most notably in chlorophyll-protein complexes isolated by Takahashi and coworkers from *Synechococcus* sp. [16]. In their report, stable charge separation was associated with the presence of two polypeptides of estimated molecular masses 14 and 10 kDa, and these were postulated to bind the iron-sulphur centres which are termed X, A and B. In *Phormidium laminosum*, two chlorophyll-protein complexes have been identified, termed A and A-1 [11], but the electron-transfer properties of these two complexes were not described.

In this study, I have re-examined the properties of PS I from *P. laminosum*, and have isolated several more chlorophyll-containing complexes. All of the *P. laminosum* complexes will give rise to a light-induced oxidation of the primary electron donor, P-700, but two of the complexes require high light intensities to bring about this reaction. The smallest unit which can efficiently photooxidize P-700 has an estimated molecular mass of 150 kDa. As with *Synechococcus* sp. PS I, efficient charge separation is associated with the presence of two low-molecular weight polypeptides, but I have found that the possibility that at least one of the low molecular weight polypeptides is involved in the binding of carotenoid and long-wavelength absorbing forms of chlorophyll cannot be ruled out.

Materials and Methods

P. laminosum (strain OH-1-p.C1. 1) was grown at 42° C in half-filled 1 l conical flasks containing Castenholz medium [17] in a shaking water bath set at 130 r.p.m. Light was provided by four 40 W fluorescent tubes positioned 40 cm above the water bath. Cells were harvested by low-speed centrifugation about every 5 days. Sphaeroplasts and photosynthetic membranes were prepared as described by Stewart and Bendall [4]. The membranes were treated with 0.35% (w/v) lauryldimethylamine oxide for 40 min on ice. Centrifugation at $100\,000 \times g$ for 1 h resulted in a pellet enriched in PS I and a supernatant containing mostly Photosystem II, as described earlier [4]. The pellet was resuspended in 10 mM Hepes, 5 mM K_2HPO_4, 10 mM $MgCl_2$, 25% glycerol (pH 7.5) (buffer C), and then separated into fractions by two centrifugation steps: $800 \times g$, 5 min; $100\,000 \times g$, 1 h. The pellet from the first step contained mostly intact cells and was discarded.

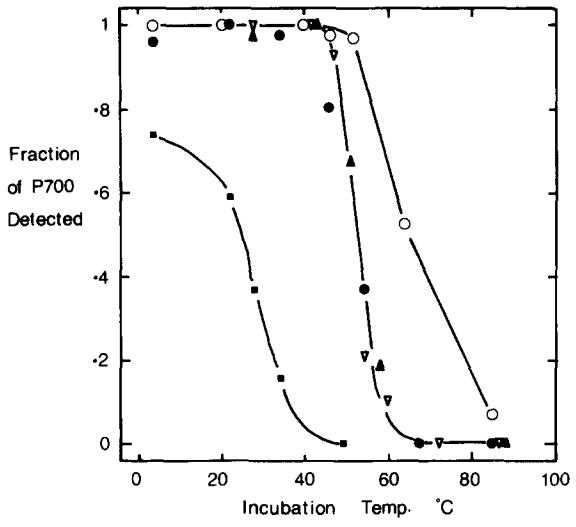

Fig. 1. Samples were incubated for 5 min at various temperatures and then transferred to ice. After dilution into the pH 6.3 medium, the P-700 content was determined by the method described in Ref. 22. Open circles: PS I-enriched membrane fragments. Closed circles: as above, but membranes were incubated in the presence of 5% (w/v) SDS (SDS/Chl = 150:1). Open triangles: the 450 kDa complex. Closed triangles: the 105 kDa complex. Squares: spinach PS I plus 5% (w/v) SDS (SDS/Chl = 50:1). The data are presented as a fraction of the P-700 detected in control samples which were diluted and assayed immediately.

The second pellet contained mainly PS I, and after resuspension at about 2 mg Chl per ml the P-700 content on a chlorophyll basis was determined. Three different detergent treatments were tested. Triton X-100 (5% w/v) at 42°C was found to solubilize the membranes effectively (at 1 mg Chl per ml), and SDS (2% w/v) at 20°C or 37°C gave similar results. Incubation with SDS (2% w/v) at 20°C was adapted as a routine procedure. After 5 min of incubation with the detergent, the sample was returned to ice, polyethylene glycol 6000 was added to a final concentration of 28% w/v, the $MgCl_2$ concentration was increased to 25 mM and then the sample was pelleted in an Eppendorf centrifuge (10000 × *g*, 5 min). The sample was resuspended in a small volume of 20 mM Mes (NaOH), 5 mM $MgCl_2$, 15 mM NaCl, 0.2% w/v Triton X-200 (pH 6.3) (the pH 6.3 medium) and layered onto sucrose density gradients (0.2–1 M) made up with the same buffer. Centrifugation was at 45000 r.p.m. in a Kontron TST 60 rotor (approx. 200000 × *g*) for 16 h.

SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli [18] with the modifications in sample preparation detailed later in the text. Bands cut from the gels were ground in a small pestle and mortar and extracted twice with the pH 6.3 medium. The slurry was pelleted by centrifugation (10000 × *g*, 1 min), and the supernatant was removed and then concentrated by dialysis against solid sucrose (BioRad, electrophoresis purity).

Chlorophyll was determined by the method of Arnon [19], and protein according to Ref. 20 with the pre-treatment to remove chlorophyll described in Ref. 21.

Optical difference spectra and light-induced absorption changes were measured with an Aminco DW-2 spectrophotometer. Actinic illumination was provided by a 150 W tungsten-filament lamp focused onto the cuvette. The sample was illuminated through a Balzers 447 nm interference filter and the photomultiplier was protected by a Schott RG645 long-pass filter. Light-induced absorption changes due to P-700 were measured at 698.5 nm using 735 nm as a reference wavelength. An extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ was used [6].

Results

The resistance of *P. laminosum* PS I fractions to denaturation by detergent and high temperature was investigated and the data are shown in Fig. 1. The P-700 content of the various fractions was determined by chemical oxidation and reduction according to the method of Markwell et al. [22] after incubation at various temperatures for 5 min. Purified PS I-containing membrane fragments isolated as described in the Materials and Methods section were found to lose slowly P-700 with a mid-point of about 70°C (Fig. 1, open circles), but the presence of SDS (5%, w/v) caused a downward shift in the mid-point by about 10°C (Fig. 1, closed circles). Purification of the SDS solubilized material did not restore extra thermostability (triangles), but also did not cause any further destabilization of the sample. In contrast, spinach PS I was found to be easily denatured in the presence of high concentrations of SDS (squares). Even incubation on ice for 5 min was found to cause some loss of P-700, and the mid-

point temperature was about 20 °C. In other experiments it was found that the assay method of Markwell et al. [22] was essential for the detection of P-700 in spinach PS I fractions treated with SDS, but not required for the *P. laminosum* PS I fractions. When the Markwell protocol was not employed to measure P-700 in SDS-treated spinach fractions, large absorption changes due to the oxidation of free chlorophyll completely obscured any absorption changes due to P-700. The absence of this effect in *P. laminosum* fractions suggests that the release of light-harvesting chlorophyll by SDS is considerably reduced. The presence of SDS caused a shift in the absorption spectrum of P-700 to shorter wavelengths, so that the maximum bleaching was at 697 nm instead of 700 nm. This effect was observed in both spinach and *P. laminosum* fractions. An increase in the bleaching by about 30% was also observed after the addition of SDS to *P. laminosum* PS I membrane fractions. The blue-wavelength shift and absorption increase by P-700 in the presence of SDS have been discussed in Ref. 22.

As described in the previous section, high concentrations of two different detergents were employed to solubilize the *P. laminosum* PS I-containing membrane fragments, and then isopycnic centrifugation was used to purify the material. Two clearly separated PS I-containing bands were observed with all the membrane-solubilization protocols employed. The lower of the two bands contained about 70–90 chlorophyll molecules per P-700 and was located at a sucrose concentration of about 0.8 M assuming a linear gradient. The other band was slightly broader and was located at a sucrose concentration estimated at 0.6 M. The ratio of chlorophyll to P-700 in this band was approximately the same as in the lower band. In samples treated with 5% (w/v) Triton X-100 at 42 °C, the upper, broader band contained about 10-times as much material as the lower band, but in the SDS-solubilized fractions, the distribution of chlorophyll was approximately even. With both detergents, protein-free chlorophyll and carotenoid were found at the top of the sucrose density gradient. SDS-solubilization (2% w/v at 20 °C for 5 min) was adopted as a routine procedure, and the experiments described in Figs. 3, 4 and 5 were performed with material prepared in this fashion. Solubilization with Triton X-100 was found to produce fractions which were contaminated with small amounts of phycobiliproteins.

Further purification of the sucrose density gradient fractions was attempted by using SDS-polyacrylamide gel electrophoresis. Fig. 2 shows two gradient gels (5–10% polyacrylamide (w/v)) which were used to probe the fractions. The lower band from the sucrose gradient produced a major green band of estimated molecular weight 450 kDa when analysed on SDS-polyacrylamide gel electrophoresis. (Fig. 2A, track 1). With more denaturing conditions (Fig. 2A, track 2), a band closely corresponding to plant CP-1 was also observed and after staining, the P-700 apoprotein was disclosed. In these experiments about one-fifth of the total chlorophyll migrated to the bottom of the gel, and this fraction was later determined to be protein-free. Fig. 2B shows the analysis of the

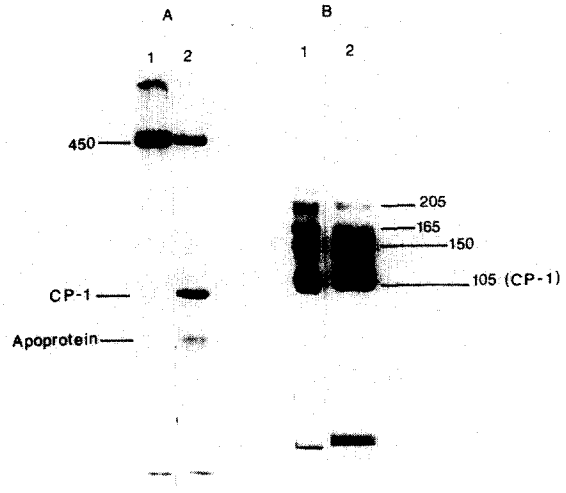

Fig. 2. SDS-polyacrylamide gel electrophoresis of the lower and upper PS I-containing bands from the sucrose density gradient. (A) 5–10% polyacrylamide gradient gel of the lower band run at room temperature for 5 h. Track 1 – membranes initially solubilized with 2% (w/v) SDS at 20 °C, 20 μg Chl was loaded. Track 2 – as in track 1, but membranes initially solubilized at 37 °C and 6 μg Chl was loaded. (B) as above, but with the upper sucrose band. Solubilization was with track 1: 2% (w/v) SDS at 20 °C, 8 μg Chl loaded; track 2: 5% (w/v) Triton X-100 at 42 °C, 15 μg Chl loaded. Gels were stained with Coomassie Brilliant Blue R-250. The numbers represent the estimated molecular masses in kDa, as determined with the following molecular-weight-marker proteins: ferritin (440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDA), bovine serum albumen (67 kDa). The CP-1 apoprotein (approx. 67 kDa) was only observed after the staining procedure.

upper sucrose gradient band. The migration pattern was completely different to that shown in Fig. 2A, and four major chlorophyll-containing bands of molecular mass 105, 150, 165 and 205 kDa were resolved. Weaker green bands of approximate molecular mass 230 kDa (track 1) and 140 kDa (track 2) could also be distinguished. Protein-free chlorophyll also represented about 20% of the total chlorophyll in these experiments. Additional contamination by low-molecular-weight polypeptides in the Triton X-100 solubilized fraction is indicated at the bottom of track 2 in Fig. 2B. On other gels, the *P. laminosum* PS I fractions were run next to spinach PS I, and the 105 kDa band was found to migrate in an identical fashion to spinach CP-1 (unpublished results).

It should be noted that in Fig. 2A the gel was run at room temperature, whereas in Fig. 2B the gel was run in a cold room at approx. 6 °C. When the upper sucrose gradient band was run on SDS-polyacrylamide gel electrophoresis at room temperature the migration pattern was different to that shown in Fig. 2B. The intensity of the 105 kDa band was increased and the 150 kDa band was absent. The migration pattern of the lower sucrose band was not significantly altered by electrophoresis temperature, emphasising the stability of the 450 kDa complex which is discussed later.

Each band was excised, extracted and concentrated before further analysis was carried out. Table I shows the results of the assays for P-700 in the various chlorophyll-protein complexes. When measured chemically [22], the 450, 150 and 105 kDa complexes were found to contain roughly similar amounts of P-700 based on the total chlorophyll concentration. The 165 and 205 kDa complexes were found to be slightly less enriched in P-700. Much larger differences between the complexes were observed when the P-700 content was determined by measuring light-induced absorption changes. Low-intensity actinic light (0.4 $W \cdot m^{-2}$ at 447 nm) was used to probe for an efficient charge separation. Only the 150 and 450 kDa complexes had values for Chl/P-700 which were comparable to the chemical assay. The 105 and 165 kDa complexes displayed very little light-induced absorption changes due to P-700 photooxidation under these conditions. The 205 kDa complex showed intermediate properties.

TABLE I

P-700 ENRICHMENT IN THE VARIOUS CHLOROPHYLL-PROTEIN COMPLEXES ISOLATED BY SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS, AS DETECTED BY CHEMICAL OXIDATION AND REDUCTION (AS IN FIG. 1), AND BY PHOTOOXIDATION WITH LOW LIGHT INTENSITY (0.4 $W \cdot m^{-2}$, SEE FIG. 3)

The values given are a ratio (Chl/P-700), the average of two separate preparations is shown.

Approximate molecular mass of the complex (kDa)	Chl/P-700 chemical assay	Chl/P-700 photo-oxidation
105	54	555
150	57	75
165	129	> 2000
205	85	260
450	54	60

Fig. 3 shows examples of the assays used in Table I for the 450, 150 and 105 kDa complexes. The chemically oxidised minus reduced spectra of P-700 were approx. the same in all three complexes (Fig. 3A), with the maximum bleaching occurring at about 697 nm. Fig. 3B shows light-induced absorption changes in the three different complexes, but in this case high-intensity actinic illumination (20 $W \cdot m^{-2}$ at 447 nm) was also used to probe the samples. The magnitude of P-700 photooxidation was approximately equal when either high or low intensity light was used to excite the 450 and 150 kDa complexes. However, large differences were observed with the 105 kDa complex. As expected from Table I, low-intensity illumination produced only a very small absorption change with the 105 kDa complex which was difficult to distinguish from slow baseline drifting. In contrast, high intensity light produced a much larger absorption change, although the photooxidation appeared to be slower than in the 450 and 150 kDa complexes under the same conditions. Point-by-point spectra of the light-induced absorption changes were typical of the P-700 spectrum (data not shown).

From these results it would appear that an inefficient charge separation can occur in the 105 kDa complex, and therefore with a low turnover rate (low-light-intensity illumination) production

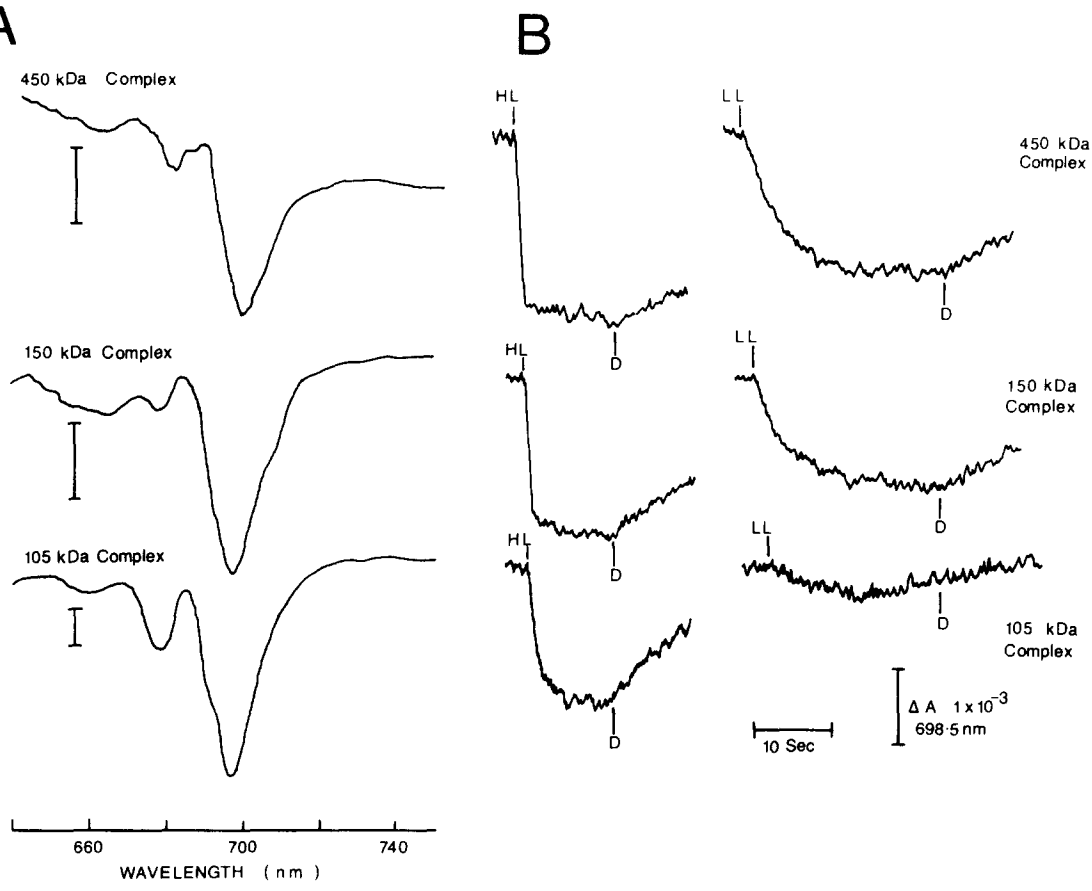

Fig. 3. (A) Oxidised-minus-reduced difference spectra of P-700 in the 450, 150 and 105 kDa complexes as in Fig. 1. The vertical bar represents an absorption of $1 \cdot 10^{-3}$. Chl concentrations were 1.6, 2.0 and 4.0 μM, respectively. (B) Light-induced absorption changes due to P-700 at 698.5 nm in the three complexes. LL, low-light-intensity illumination (0.4 $W \cdot m^{-2}$); HL, high-light-intensity illumination (20 $W \cdot m^{-2}$); D, light off (dark). Chl concentrations were 2.3 μM (450 kDa complex), 2.1 μM (150 kDa complex), 2.0 μM (105 kDa complex). Samples contained 10 mM sodium ascorbate and 0.1 mM DCIP in the pH 6.3 medium.

of $P\text{-}700^+$ proceeds at approximately the same rate as electron donation, and thus $P\text{-}700^+$ is not observed. Similar results were obtained with the 165 kDa complex. An inefficient charge separation has been observed in plant CP-1 complexes [13,23] and this deficiency has been associated with the loss of the iron-sulphur centres A and B [23,24].

The polypeptide composition of the chlorophyll-protein complexes was investigated, and Fig. 4 shows the results for the 450, 150 and 105 kDa complexes. The complexes were dissociated by incubating with 6% (v/v) 2-mercaptoethanol, 5% (w/v) SDS, 12% (v/v) glycerol, 90 mM Tris-HCl (pH 6.8), and heating at 70 °C for 10 min. Two additional low-molecular-weight proteins were stained in the extracts of the 450 and 150 kDa complexes. A strongly stained protein of apparent molecular mass of 15 kDa could be observed with a weaker staining band below it. The estimated molecular weight of this protein was approx. 10 kDa. The broad band at approx. 180 kDa was present in the extract of all three complexes, and may be an aggregation artefact. Lowering the temperature of incubation with the solubilizing buffer to 50 °C was found to remove mostly the 180 kDa band, but the 450 and 150 kDa chlorophyll-containing bands were not fully dissociated at this temperature. Increasing the incubation temperature to 100 °C was found to cause complete aggre-

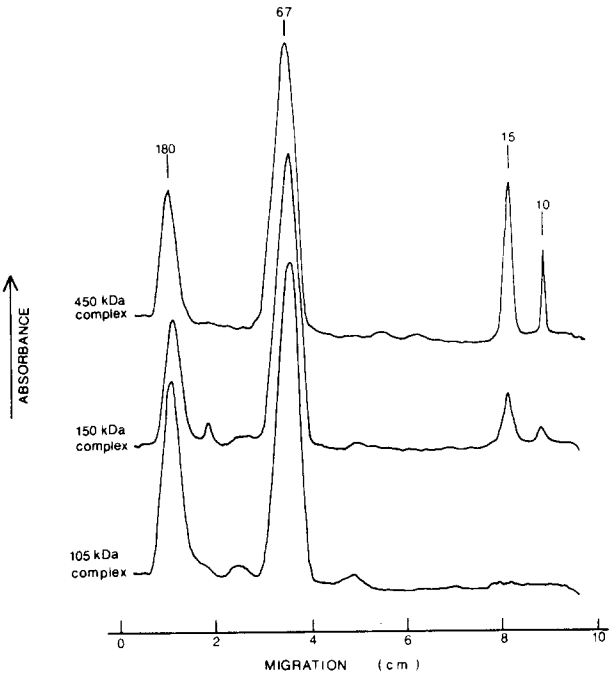

Fig. 4. Densitometer scans of different tracks on a 10%–15% polyacrylamide gradient gel after staining with Coomassie Brilliant Blue R-250. The figures represent the estimated molecular weights of the various bands in kDa, as determined with the marker proteins details in Fig. 2 with two additional low-molecular-weight markers, Cytochrome *c* (12.5 kDa) and trypsin inhibitor (20 kDa). For the 450 and 150 kDa complexes 5 μg Chl was loaded. For the 150 kDa complex 2.5 μg Chl was loaded, and for this track the vertical scale is doubled.

gation of the samples so that no proteins entered the polyacrylamide gel. A similar effect has been reported in plant PS I fractions [2]. Further resolution of the bands did not occur when SDS-polyacrylamide gel electrophoresis was carried out with 2 or 3 M urea in the gels, nor when samples were solubilized in the presence of 8 M urea according to Ref. 16.

Differences in the absorption spectra of the chlorophyll-protein complexes were also detected (Fig. 5). The difference spectra show features between 640 and 750 nm which probably result from longer-wavelength absorption by chlorophyll in the 450 kDa complex, and to a lesser extent, in the 150 kDa complex (Fig. 5A). A peak and trough in the difference spectra around 430 nm may also be due to a long-wavelength shifted chlorophyll absorption in the 450 and 150 kDa complexes. The most noticeable feature in the 400–500 nm wavelength region of the difference spectra is a broad absorption indicative of carotenoid. Extraction of the pigments of the three complexes with 80% (v/v) acetone [18] was found to remove the differences in the 650–750 nm range, but the broad feature in the 400–500 nm region was preserved (Fig. 5B). This feature, attributed to carotenoid, was slightly blue-shifted by acetone extraction, and peaks around 500 and 470 nm were no longer clearly resolved. These experiments seem to suggest that additional carotenoid is bound in the 450 and 150 kDa complexes, but that differences in the 650–750 nm range of the absorption spectra are probably due to protein-chlorophyll interactions which induce a longer-wavelength absorption by light-harvesting chlorophylls. In other experiments, the 165 kDa complex was found to have a very similar absorption spectrum to the 105 kDa complex, whilst the 205 kDa complex had a spectrum more closely resembling that of the 150 kDa complex (unpublished data).

The chlorophyll-to-protein ratio (on a weight basis) was found to be approx. 3.0 for the 105 kDa complex, and about 3.5 for the 450 and 150 kDa complexes. The value of 3.0 for a CP-1-type complex is comparable with earlier work [3].

Discussion

There are several reports of the observation of PS I-containing chlorophyll-protein complexes isolated from cyanobacteria using SDS-polyacrylamide gel electrophoresis. Chlorophyll-containing bands migrating more slowly than CP-1 (110 kDa) have often been described, but in many cases, only one or two bands in addition to CP-1 were resolved [11–13]. In these earlier studies, electrophoresis was usually carried out rapidly (in less than 1 h), and concentrated samples were loaded onto the gels (SDS/Chl ratios: 10:1 to 20:1). It seems possible that with these electrophoresis conditions – which are suitable for isolating CP-1 from plants – the multiple bands reported here and in other reports [14–16] were not resolved. It seems likely that the different electrophoresis conditions explains the discrepancy between this report and an earlier investigation of *P. laminosum* chlorophyll-protein complexes [11]. It

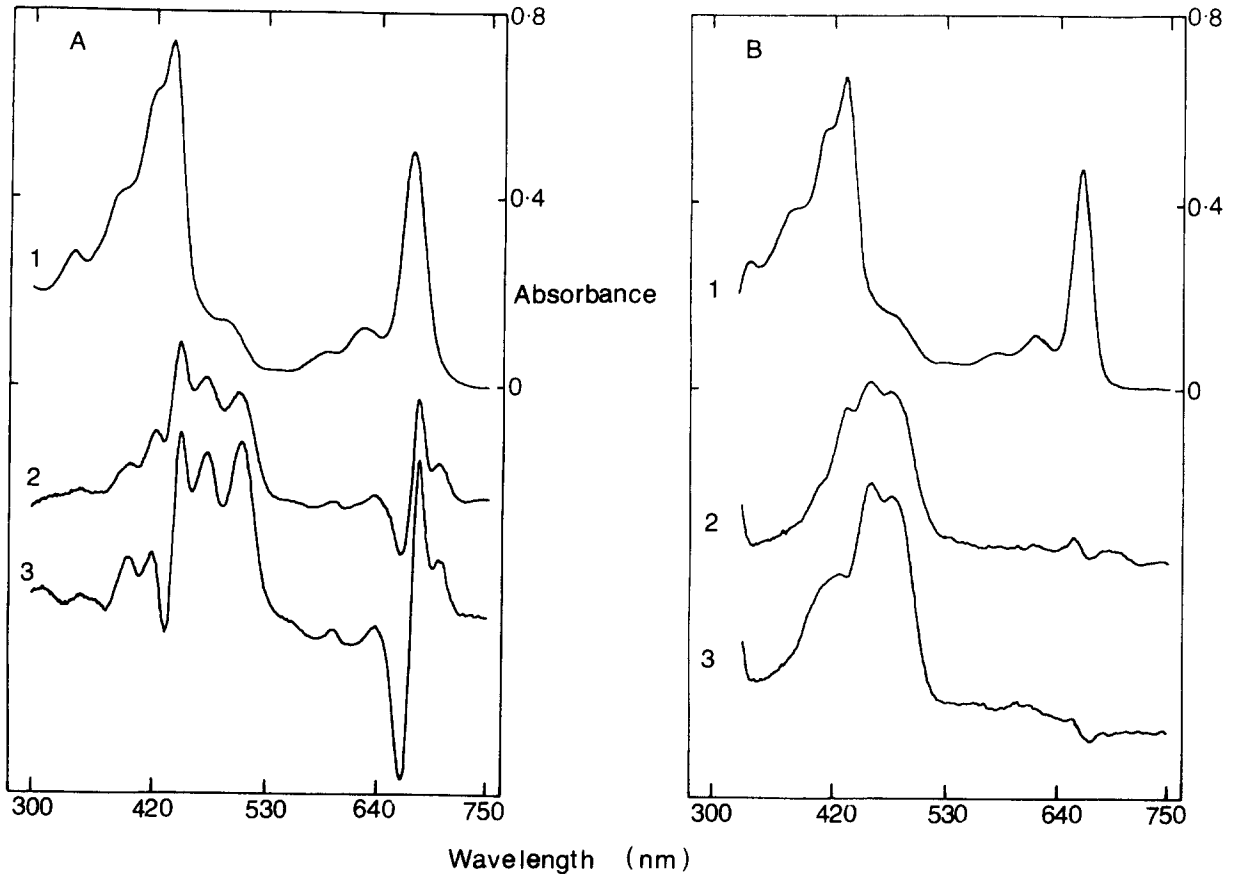

Fig. 5. (A) Trace 1 shows the absorption spectrum of the 105 kDa complex in the pH 6.3 medium. Traces 2 and 3 show the difference spectra obtained by subtracting the 105 kDa spectrum from the spectra of the 150 and 450 kDa complexes, respectively. The difference spectra 2 and 3 have been enlarged 10-fold relative to the upper trace. Chl concentration was 7.5 $\mu g\ ml^{-1}$. (B) As in (A), except that the pigments from the three complexes are shown after extraction into 80% (v/v) acetone. The difference spectra 2 and 3 were enlarged 14-fold relative to the upper scale. Chl concentration was 6.5 $\mu g \cdot ml^{-1}$.

could be argued that the additional bands observed with harsher electrophoresis conditions were break-down products, but it seems probable that slow denaturation would produce a smear on the gel rather than the clearly defined bands shown in Fig. 2. It seems more likely that in cyanobacteria there is a range of multimeric forms of the PS I complex and that the interactions between the subunits are relatively strong, since they are maintained in high detergent concentrations. Until the actual subunit composition of the various PS I complexes is elucidated, it will be difficult to avoid confusion in the identification or the naming of the bands, and it is for this reason that I have used the apparent molecular weights of the various complexes, even though these are likely to be inaccurate [9,16]. The estimation of 450 kDa for the complex migrating the smallest distance is likely to be particularly inaccurate. The marker protein ferritin (440 kDa) migrated very close to the position of the 450 kDa complex, but it must be admitted that the migration of a water-soluble marker protein is likely to be different to an integral membrane complex of the same size. A separate determination of molecular weight using analytical ultracentrifugation is at present being carried out.

The question of whether there is any physiological role for the apparent multimeric nature of PS I in cyanobacteria remains to be investigated.

A direct comparison of PS I isolated from different cyanobacterial sources would help to identify which complexes are a common feature of the cyanobacterial photosynthetic machinery. It is worth noting that the most stable of the complexes was the 450 kDa complex, and thus a role in adaptation to high temperatures could be envisaged. It also seems feasible that several PS I units could associate to allow the collective sharing of their light-harvesting pigments in a similar fashion to that envisaged for the Photosystem II 'α-centres' in plants [25]. Indeed this feature could compensate for the relatively small number of light-harvesting chlorophylls per PS I reaction-centre in cyanobacteria (in *P. laminosum* PS I-enriched membrane fragments, the Chl/P-700 ratio is about 110:1, compared to about 300:1 for plant PS I).

There have been many studies of low-molecular-weight proteins in PS I fractions [26–28] and a variety of proteins of molecular mass ranging from 8 to 19 kDa have been associated with the binding of the iron-sulphur centre acceptors X, A and B. In the study of Takahashi and co-workers [16], and now in this work, the polypeptide composition of the most purified complexes appears to be very simple. Two low molecular mass polypeptides of 10 and 15 kDa can be identified, and these two proteins are only found in complexes where efficient photosynthetic charge separation can occur. A protein of apparent molecular mass 67 kDa appears to be a common feature of all the chlorophyll-protein complexes.

Inefficient charge separation in the 105 and 165 kDa complexes probably occurs because of the presence of a poor electron acceptor which must compete against a fast charge recombination within the depleted complex. Thus, many turnovers are required in order to trap the electron on the weak electron acceptor. Kinetic measurements of charge recombination within the 105 kDa complex which support this hypothesis will be presented in a future report (Ford, R.C., Sétif, P. and Brettel, K., unpublished observations). The presence of the iron-sulphur centres A and B is necessary for stable charge separation [29], and therefore it seems reasonable to propose that these centres are located on the 10 and 15 kDa polypeptides. The recent work of Golbeck and Cornelius [10] would tend to place the iron-sulphur centre X on the 67 kDa protein, although the presence of centre X in a CP-1 fraction prepared by polyacrylamide gel electrophoresis remains to be demonstrated.

As a qualification to the previous paragraph, however, it should be acknowledged that the low-molecular-weight proteins could be involved in the binding of carotenoid or long-wavelength absorbing forms of chlorophyll. The absence of the 10 and 15 kDa polypeptides in the 105 kDa complex is associated with a change in the absorption spectrum (Fig. 5). It may be possible to explain the loss of carotenoid and the shift towards blue in the absorption spectrum by proposing an increased access of SDS to the light-harvesting pigments in the 105 kDa complex. In this way, the 10 and 15 kDa proteins could be thought of as blocking the denaturing action of SDS by their presence in the complex. Further experiments are required in order to rule out the possibility that the 10 and 15 kDa proteins are involved in pigment binding.

One of the aims of this and many other studies was to obtain a bacterial-type photosynthetic reaction centre of PS I which was free of light-harvesting pigments. Despite the use of high detergent concentrations and elevated incubation temperatures, this has not proved possible. Estimates of the chlorophyll-to-protein weight ratio in the *P. laminosum* complexes suggest that about 30 chlorophyll molecules are present per reaction centre. This figure is approximate, and will depend on many factors, in particular the accuracy of the molecular-weight determination by SDS-polyacrylamide gel electrophoresis, which is likely to be an under-estimate [9,16]. The values obtained by measuring the P-700 content on a chlorophyll basis give a higher figure of 50–60 chlorophylls per reaction centre. Thus despite the rather simple polypeptide composition of the *P. laminosum* complexes, a large amount of light-harvesting chlorophyll is still present.

If we reject the hypothesis that a bacterial-type reaction centre is obtainable from PS I, and accept the hypothesis that in PS I, light harvesting and electron-transfer functions are located within the same unit, then we are faced with a conceptual problem. It is difficult to envisage how so many large and rigid pigment molecules could be bound by relatively little protein. For the 105 kDa (CP-1)

complex, it would seem a though at most two 80 kDa polypeptides are present [9], and these proteins must bind roughly one-third of their weight of pigment molecules as well as at least two electron-transfer components. A possible explanation for the remarkable properties of the CP-1 complex seems to be that many of the light-harvesting pigments can be readily removed by detergents [3,30] or by extraction with organic solvents [31,32]. These observations suggest that many of the light-harvesting pigments are not very tightly bound within the complex. As far as I am aware, the preparation giving the least accessory chlorophyll involved an ether extraction of plant PS I [32]. The chlorophyll-to-P-700 ratio was reported to be as low as 6:1, but the procedure caused protein aggregation, and this may be why the technique has not been widely employed in the study of PS I. Clearly, the interaction of so many light-harvesting pigments and electron transfer components within a single complex will be very complicated, and the elucidation of the structure of the PS I reaction centre seems likely to provide new insights into the mechanisms of photosynthesis.

In conclusion, PS I from the thermophilic cyanobacterium *P. laminosum* seems to be resistant to high concentrations of the ionic detergent SDS, allowing a simple purification procedure for obtaining photosynthetically active chlorophyll-protein complexes. When compared with PS I from the extremely thermotolerant species *Synechococcus* sp. [16], the *P. laminosum* complexes appear to be at least as resistant to denaturation by SDS, and in the case of the larger complexes, the polypeptide composition is simpler. The use of an initial purification by sucrose density gradient centrifugation allows the separation of PS I into two fractions which differ in the size of the chlorophyll-protein complexes that they contain.

Note added in proof

Since this manuscript was submitted, the reaction centre of Photosystem II has been isolated free of light-harvesting pigments (Namba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112), and the 450 kDa complex described in this report has been crystallized (Ford, R.C., Picot, D. and Garavito, R.M. (1987) EMBO J. 6, 1581–1586).

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References

1 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624
2 Williams, R.S., Shaw, E.K., Sieburth, L.E. and Bennett, J. (1986) Methods Enzymol. 118, 338–352
3 Bengis, C. and Nelson, N. (1975) J. Biol. Chem. 250, 2783–2788
4 Stewart, A.C. and Bendall, D.S. (1979) FEBS Lett. 107, 308–312
5 Satoh, K., Nakatani, H.Y., Steinback, K.E., Watson, J. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 142–150
6 Mathis, P., Sauer, K. and Remy, R. (1978) FEBS Lett. 88, 275–278
7 Sétif, P., Bottin, H. and Mathis, P. (1985) Biochim. Biophys. Acta 808, 112–118
8 Lagoutte, B., Sétif, P. and Duranton, J. (1980) Photosynth. Res. 1, 3–16
9 Fish, L.E., Kuck, U. and Bogorad, L. (1985) J. Biol. Chem. 260, 1413–1421
10 Golbeck, J.H. and Cornelius, J.M. (1986) Biochim. Biophys. Acta 849, 16–24
11 Stewart, A.C. (1980) FEBS Lett. 114, 67–72
12 Reinman, S. and Thornber, J.P. (1979) Biochim. Biophys. Acta 547, 188–197
13 Hoarau, J., Remy, R. and Leclerc, J.C. (1977) Biochim. Biophys. Acta 462, 659–670
14 Guikema, J.A. and Sherman, L.A. (1980) Biochim. Biophys. Acta 637, 189–201
15 Huang, C., Berns, D.S. and Guarino, D.U. (1984) Biochim. Biophys. Acta 765, 21–29
16 Takahashi, Y., Koike, H. and Katoh, S. (1982) Arch. Biochem. Biophys. 219, 209–218
17 Castenholz, R.W. (1970) Schweiz. Z. Hydrol. 32, 538–551
18 Laemmli, U.K. (1970) Nature 227, 680–685
19 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
20 Lowry, O.H., Rosenbrough, N.M., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
21 Ford, R.C., Chapman, D.J., Pedersen, J.Z., Cox, R.P. and Barber, J. (1982) Biochim. Biophys. Acta 681, 145–151
22 Markwell, J.P., Thornber, J.P. and Skrdla, M.P. (1980) Biochim. Biophys. Acta 591, 391–399
23 Sétif, P., Acker, S., Lagoutte, B. and Duranton, J. (1980) Photosynthesis Res. 1, 17–27
24 Malkin, R., Bearden, A.J., Hunter, F.A., Alberte, R.S. and Thornber, J.P. (1976) Biochim. Biophys. Acta 430, 389–394

25 Anderson, J.M. (1982) Photobiochem. Photobiophys. 3, 225–241
26 Bonnerjea, J., Ortiz, W. and Malkin, R. (1985) Arch. Biochem. Biophys. 240, 15–20
27 Guikema, J. and Sherman, L. (1982) Biochim. Biophys. Acta 681, 440–450
28 Lagoutte, B., Sétif, P. and Duranton, J. (1984) FEBS Lett. 174, 24–29
29 Evans, M.C.W. (1982) in Iron Sulphur Proteins (Spiro, T.G., ed.), Vol. 4, pp. 250–284, J. Wiley, London
30 Nechustai, R., Muster, P., Binder, A., Liveanu, V. and Nelson, N. (1983) Proc. Natl. Acad. Sci. USA 80, 1179–1183
31 Sane, P.V. and Park, R.B. (1970) Biochem. Biophys. Res. Commun. 41, 206–210
32 Ikegami, I. and Katoh, S. (1976) Biochim. Biophys. Acta 376, 588–592