THE CHLOROPHYLL–PROTEIN COMPLEXES OF A THERMOPHILIC BLUE-GREEN ALGA

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Received 19 March 1980

1. Introduction

Valuable information about the structure and function of the photosynthetic apparatus in photosynthetic bacteria, blue-green algae and higher plants has been gained from fractionation of the photosynthetic membranes to separate the light-harvesting and reaction-centre pigment–protein complexes which comprise the photosystems.

Many reports describe preparations of the reaction centre pigment–protein complex of PS1 from both blue-green algae and higher plants, and also the higher-plant light-harvesting chl *a*/*b*-protein complex [1–6]. These preparations have normally been obtained by detergent treatment of the photosynthetic membranes followed by techniques such as sucrose gradient centrifugation, ion-exchange chromatography or SDS-PAGE to separate the pigment-protein complexes.

However, only in recent years has comparable progress been made in the purification of the PS2 reaction centre [6–8]. Satoh [8] has now obtained a highly purified preparation from spinach chloroplasts by digitonin extraction, sucrose density gradient centrifugation, ion-exchange chromatography and isoelectric focussing. In addition, the development of much milder procedures for SDS-PAGE has enabled the amount of free chlorophyll generated during electrophoresis to be kept to a minimum [9–12] and several laboratories have now reported finding a band on SDS-polyacrylamide gels which may correspond to the PS2 reaction centre [11–14]. The new band has a higher electrophoretic mobility than the P700-chl *a*-protein complex, and accounts for about 10% of the total chlorophyll. It has been found in chl *b*-less mutants of barley [11,12] and a similar complex has recently been identified in the blue-green algae *Phormidium luridum* and *Anabaena variabilis* [14]. In *A. variabilis* the complex was absent both from PS1-enriched preparations and from heterocyst cells, which lack PS2 activity.

Thus circumstantial evidence supports the assignment of the new chl *a*-protein complex band seen on SDS-polyacrylamide gels to the PS2 reaction centre, but so far more direct evidence has been lacking. Recently [15,16] we reported the fractionation of thylakoid membranes of the thermophilic blue-green alga *Phormidium laminosum* into two fractions highly enriched in PS1 and PS2, respectively. The PS2 fraction retained high rates of O_2 evolution. The present report describes the electrophoretically separated chlorophyll-protein complexes of these fractions and of the original thylakoid membranes of *Phormidium laminosum.*

Abbreviations: chl, chlorophyll; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; LDAO, lauryldimethylamine oxide; P700, the reaction centre of photosystem 1; PS1 and PS2, photosystem 1 and photosystem 2; SDS-PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis; Tris, Tris(hydroxymethyl)aminomethane

2. Materials and methods

Membrane fragments, PS1 and PS2 particles from *P. laminosum* were prepared as in [16] and stored at a concentration of 1.0 mg chl *a*/ml in Buffer C containing 25% (v/v) glycerol, 10 mM $MgCl_2$, 10 mM Hepes/NaOH, 5 mM phosphate buffer (pH 7.5).

Chl *a* concentrations, rates of O_2 evolution and amounts of P700 were measured as in [16].

Samples were prepared for electrophoresis as follows. Membrane fragments (1.0 mg chl *a*/ml in Buffer C) were first pretreated with 0.35% (w/v) LDAO for 30 min at 4°C. 40 μl samples of PS1 particles, PS2

particles and pretreated membrane fragments were then diluted to 100 μl with buffer containing 6.2 mM Tris, 48 mM glycine, and treated at 4°C with 8 μl of 10% (w/v) SDS (SDS:chl = 20:1). 15–25 μl of SDS-treated samples were used immediately for electrophoresis. Electrophoresis of chl-protein complexes was carried out on 1.6 mm-thick slab gels by the method in [14], except that 5 cm of 5% acrylamide resolving gel were overlaid by 1.5 cm of a 3% acrylamide stacking gel. Electrophoresis was carried out at 4°C for 30–60 min, at a constant voltage of 100 V.

Chlorophyll distribution was determined by scanning the unstained gels on a Joyce microdensitometer equipped with a 672 nm narrow-band interference filter (Glen Creston Instruments, London, UK). The scans were photocopied, and the peaks cut out and weighed to determine relative areas.

Absorption spectra of excised gel bands were recorded in situ using a Cary 219 spectrophotometer with a microcell attachment.

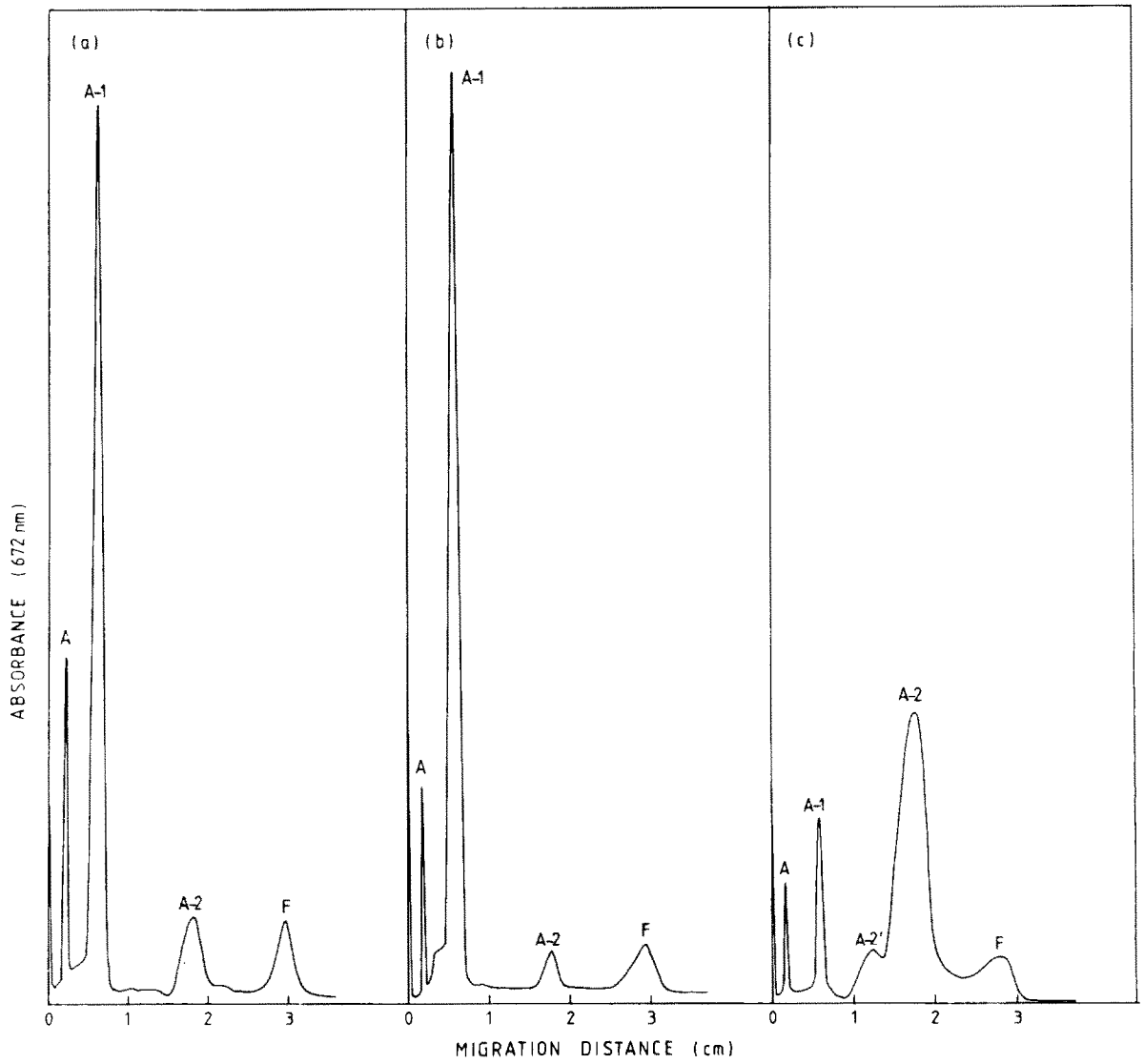

Fig.1. Densitometer scans showing electrophoretic separation of chl-protein complexes in *P. laminosum* (a) membrane fragments (b) PS1 particles (c) PS2 particles. Membrane fragments were pre-treated with LDAO at a ratio of LDAO:chl = 3.5:1, and all samples were treated with SDS at a ratio of SDS:chl = 20:1.

For the determination of the polypeptide composition of the chl-protein complexes, the green gel bands were excised and incubated for 40 min at 30°C in 100 μl of buffer containing 62.5 mM Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.001% bromophenol blue [17]. The incubated gel segments were placed directly on a second gel for re-electrophoresis. Electrophoresis, for 2 h at 150 V, was carried out as in [17], except that the 1.5 cm stacking gel was 5% in acrylamide and the 5 cm resolving gel contained 15% acrylamide. Chlorophyll-containing samples were not boiled before re-electrophoresis, because boiled samples apparently aggregated, and were retained at the top of the gel. This phenomenon has been previously [5,8]. Apparent MW were determined by co-electrophoresis with denatured proteins of known MW [18]. The protein markers used were β-galactosidase (135 000), bovine serum albumin (68 000), ovalbumin (43 000), carbonic anhydrase (30 000) and lysozyme (14 300).

Gels were stained for 2 h with 0.125% Coomassie Blue R-250, 45% methanol and 10% acetic acid, and destained in 10% acetic acid.

All chemicals used for preparing gels were of the highest degree of purity available from BDH.

3. Results

Fig.1a shows the electrophoretic pattern obtained for unfractionated membrane fragments of *P. laminosum*. Four green bands were observed, corresponding to bands A, A-1, A-2 and F reported for membrane fragments of *P. luridum* [14]. Bands A and A-1 together accounted for 75–80% of the total chlorophyll, but the relative amounts of chlorophyll in bands A and A-1 varied according to the method of sample preparation, with milder conditions (omission of pretreatment with LDAO, and lowering the SDS:chl ratio to 10:1) giving relatively more chlorophyll in band A. Band A-2 contained about 12–15% of the total chlorophyll, and about 8–12% ran as free pigment.

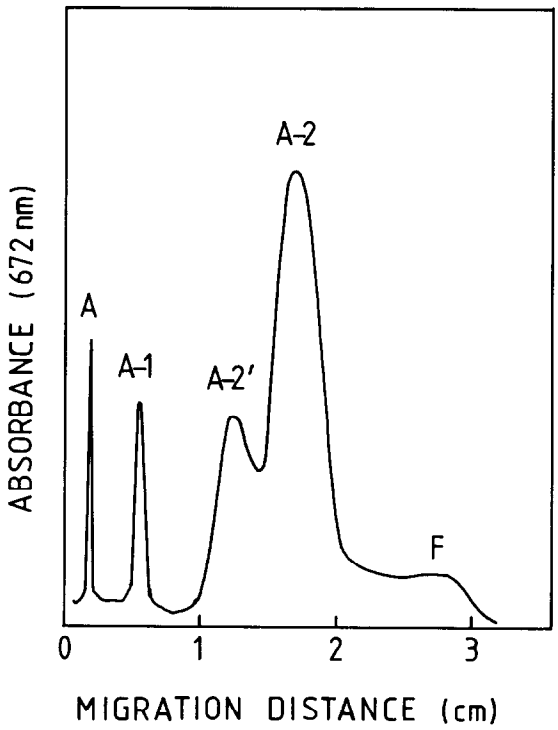

Fig.2. Densitometer scan showing separation of chl-protein complexes in *P. laminosum* PS2 particles solubilised with SDS at a ratio of SDS:chl = 10:1.

Table 1
Electron transport activities of membrane fragments, PS1 and PS2 particles from *P. laminosum*, and distribution of chlorophyll in their chl-protein complexes separated by SDS-PAGE

	Membrane fragments	PS1 particles	PS2 particles
Chl *a* in bands A + A-1 (%)	75–80	85–90	10–15
Chl *a* in bands A-2 + A-2′ (%)	12–15	2–6	70–80
Chl *a* in band F (%)	9–12	8–10	12–15
Rate of O_2 evolution (μmol O_2/mg chl *a*/h)[a]	180–250	30–50	900–1300
P700 (nmol/mg chl *a*)	6.2–6.9	7.2–7.4	1.0–1.2

[a] With 1 mM DMBQ and 2 mM $K_3Fe(CN)_6$ as electron acceptors

Strikingly different electrophoresis patterns were obtained for PS1- and PS2-enriched preparations from *P. laminosum* (fig.1b,c). The properties of these preparations, and of unfractionated membrane fragments, are summarised in table 1. In PS1 particles, 85–90% of the chl *a* was found in band A-1, while band A-2 was barely detectable; in contrast, in PS2 particles, which were highly enriched in O_2 evolution, two major green bands in the region of band A-2 (denoted A-2 and A-2′), accounted for up to 80% of the chlorophyll, with much smaller amounts of chlorophyll in bands A, A-1 and F. A-2′, which may be an oligomer of A-2, was generally more marked when lower SDS:chl ratios were used in the treatment of samples (fig.2), when the 3% stacking gel was omitted, or when gels were run for shorter times. Band A-2 was reasonably stable for up to an hour's electrophoresis, but did gradually break down to yield free pigment, so that band F for PS2 particles appeared as a rather diffuse zone of pigment between band A-2 and the electrophoretic front.

Fig.3 shows the in situ absorption spectra for bands A-1, A-2 and F. The spectra of bands A and A-2′ were identical to those of A-1 and A-2, respectively. The complexes had red absorption maxima at 678.5 nm (band A-1), 671 nm (band A-2) and 669.5 nm (band F). Both A-1 and A-2 contained carotenoid, indicated by shoulders in the absorption spectra around 460–500 nm. Carotenoid was also present in the free pigment band. The spectra presented in fig.3 are very similar to spectra for the electrophoretically isolated PS1- and presumed PS2-complexes reported for higher plants [11] and the blue-green alga *P. luridum* [14].

Chemical difference spectra showed the presence of P700 in bands A and A-1 but not in bands A-2, A-2′ or F (results not shown).

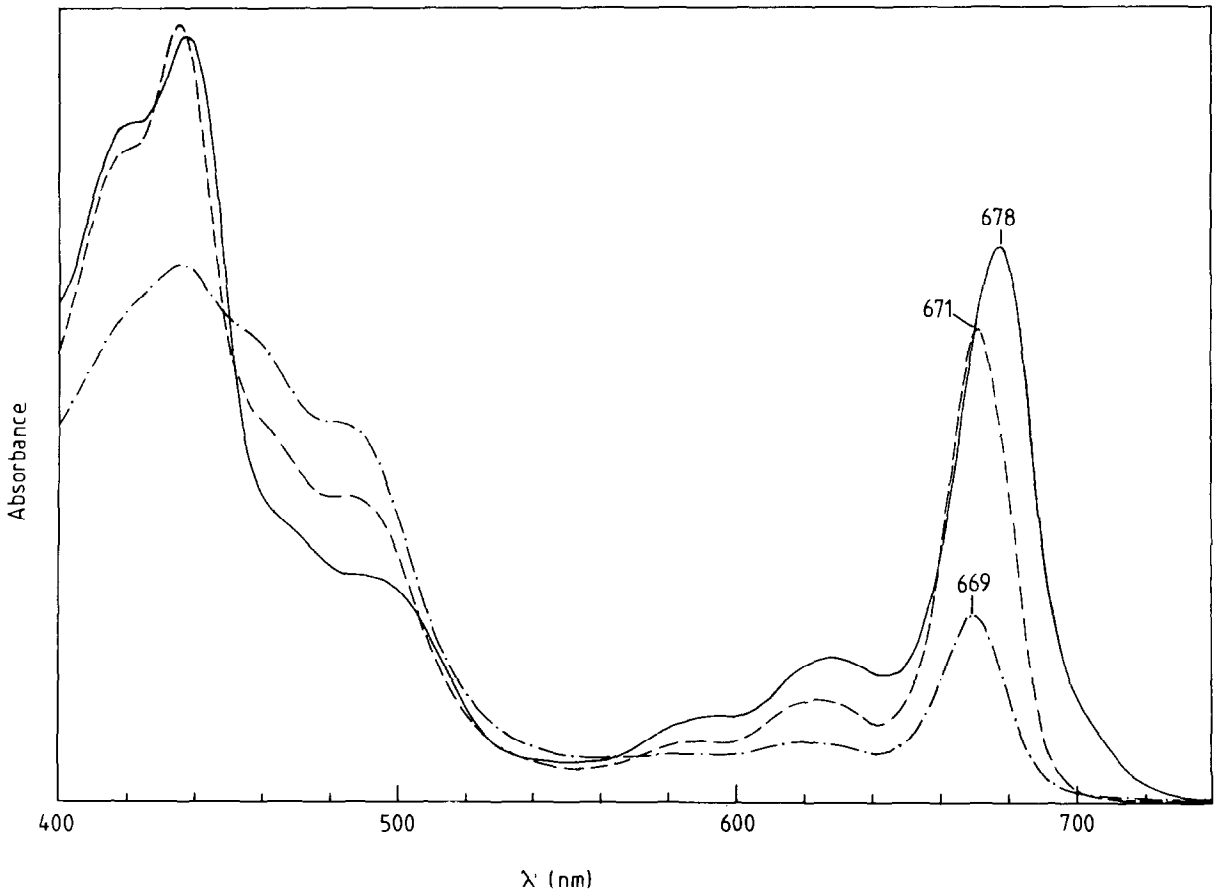

Fig.3. Absorption spectra of electrophoretically separated chl-protein complexes of *P. laminosum*. Band A-1 (——), band A-2 (– – –), band F (—·—·—).

When band A-2 was excised and re-electrophoresed, two major polypeptide bands were observed, with apparent MW of 46 000 and 40 000. Several previous reports have indicated that polypeptides in the 40–50 000 range may be associated with the PS2 reaction centre both in higher plants [7,8,19,20] and in blue-green algae [14,21].

A-1 on re-electrophoresis gave a major band at 110 000 (examination of the gel before staining showed that this band retained some chlorophyll) and minor bands at 60 000, 17 400, 16 400, 13 700 and 12 300. When the gel segment was treated at a higher temperature (40°C) before re-electrophoresis more chlorophyll was released, the 110 000 band became fainter and the 60 000 band more intense. The polypeptide profile then closely resembled that reported for purified PS1 particles from the blue-green alga *Synechococcus cedrorum* [22], though other investigations with blue-green algae have revealed somewhat different results for the constituent polypeptides of the P700-chl *a*-protein complex [3]. Different methods of sample preparation and electrophoresis may be responsible for the discrepancies.

4. Discussion

The constituent chlorophyll-protein complexes of *Phormidium laminosum* membranes were very similar to those found in other blue-green algae [14] and therefore have been denoted here as in [14]. The results presented in this paper support the assignment of complexes A and A-1 to PS1, with the former being probably an oligomeric form of the latter.

Direct evidence is also provided here that the chl-protein complex A-2 (which appears to correspond closely with band 'CPa' found in higher plants [11, 12]) is the PS2 reaction centre. Previous attempts to find a PS2 reaction centre band on SDS-polyacrylamide gels by using enriched PS2 preparations for electrophoresis have failed, apparently because the PS2 complex in most organisms is particularly labile to SDS, and rapidly loses all chlorophyll to the free pigment band during electrophoresis [7,8,14]. However *P. laminosum,* a thermophilic blue-green alga, has been shown in previous work to be highly resistant to the denaturing effects of detergents [15,16,23]. Active, O_2-evolving PS2 particles could be prepared from the alga by detergent treatment of the membranes [16], and in the present investigation these particles were subjected to normal electrophoretic procedures without large-scale breakdown of the chl-protein complexes. Thus for the first time it has been possible to show that a high degree of enrichment in PS2 activity was associated with similar enrichment in amounts of band A-2 on SDS-polyacrylamide gels, thus strengthening considerably the evidence that this complex is indeed associated with the reaction centre of PS2. Band A-2 from *P. laminosum* also had a similar polypeptide composition to those reported for PS2 preparations obtained by other techniques [7,8, 19–21].

The complex A-2′, which has not been described previously, appeared to be an oligomer of A-2, since it had an identical absorption spectrum to that of A-2 and was more intense under milder conditions of sample preparation and electrophoresis, while the total amount of chlorophyll in A-2 plus A-2′ remained constant. It has been suggested [11] that the oligomeric forms of the P700-chl *a*-protein complex and the light-harvesting chl *a*/*b*-protein complex, which are also observed when mild electrophoretic procedures are used, may be more similar to the in vivo state of these complexes than the monomers previously observed; the same may be true of the oligomer of A-2.

The thermophilic blue-green alga *Phormidium laminosum* offers the advantage that active PS2-enriched particles can be prepared by a simple procedure [16]. These particles can then be used to obtain relatively large amounts of the PS2 reaction centre by the rapid technique of SDS-PAGE. A more detailed characterisation of the blue-green algal PS2 reaction centre, and comparison of its properties with those of the purified higher plant complex [8], may now be possible.

Acknowledgements

I am grateful to Dr D. S. Bendall for helpful discussions and critical reading of the manuscript, and to King's College, Cambridge, for a Research Fellowship.

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