Biochimica et Biophysica Acta, 1015 (1990) 415–424
Elsevier

BBABIO 43153

Mono-, di- and trimeric PS I reaction center complexes isolated from the thermophilic cyanobacterium *Synechococcus* sp. * Size, shape and activity

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(Received 28 April 1989)
(Revised manuscript received 4 August 1989)

Key words: Photosystem I; Chlorophyll a_1; HPLC; Reaction center complex; Electron microscopy; (*Synechococcus*)

Photosystem I preparations from the cyanobacterium *Synechococcus* sp. were treated with high concentrations of Tris and octyl glucoside at alkaline pH and elevated temperature. A sucrose density gradient yielded three pigment-protein complexes; these were further purified on a HPLC anion-exchange column. In contrast to SDS-PAGE under non-denaturing conditions, this method produces homogeneous, highly active PS I particles in large quantities using mild solubilization conditions. Gel-filtration HPLC, SDS-PAGE, antenna Chl / Chl a_1 ratio and electron microscopy images suggest that the three complexes represent monomeric, dimeric and trimeric forms of a minimal reaction center I unit. The size of this monomeric complex is 15.3 × 10.6 × 6.4 nm (length × width × height) as determined by electron microscopy. The apparent molecular mass – including 65 antenna chlorophylls per Chl a_1, but excluding the detergent shell – is estimated by three different methods to be (235 ± 25) kDa.

Introduction

During the last decade, much effort has been directed at isolating functional reaction center complexes of PS I from the thylakoid membrane, particularly from cyanobacteria [1–8]. Although functional preparations have been isolated relatively easily using a variety of detergents such as Triton [2,3,8], SDS [6], digitonin [1] and SB 12 [7], there is still much uncertainty concerning the structure of the minimal RC I complex. At the moment, there is no agreement on the number of antenna chlorophylls per reaction center Chl a_1 (P-700), the polypeptide composition or the quaternary structure.

The cyanobacterial PS I complex has been described to consist of two large polypeptides with an apparent mass of about 60–70 kDa and two to four small polypeptides in the range of 8–20 kDa [1–9] as determined by SDS-PAGE. Optical and EPR spectroscopy led to the conclusion, that the Chl a_1 and the early acceptors, A_0, A_1, and F_x are associated with the two large polypeptides [8], although the stoichiometry of F_x and these polypeptides is still controversial [5,10–12,51]. Two additional iron-sulfur centers, F_A and F_B, having a functional role in electron transport, have been shown to be attached to a 9 kDa polypeptide of the chloroplast [13]. For review see Refs. 14–17 and 50.

The quaternary structure of the complex has been investigated using electron microscopy [5,7,18], non-denaturing electrophoresis [1,6] and HPLC [7,19]. In the cases of the thermophilic cyanobacteria *Synechococcus* sp. and *Phormidium laminosum* an oligomeric structure has been detected [6,7]. Electron micrograph image

* Dedicated to Prof. Dr. A. Trebst on the occasion of his 60th birthday.

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Abbreviations: BCA, bichinonic acid; β-DM, β-dodecyl maltoside; Chl, chlorophyll; Chl a_1 (P-700), primary electron donor of PS I; DTE, dithioerythritol; EPR, electron paramagnetic resonance; EXAFS, extended X-ray absorption fine structure; HPLC, high performance liquid chromatography; MES, 2-(N-morpholino)ethanesulfonic acid; MQ, Mono Q-column; OGP, octyl glucopyranoside; PAGE, polyacrylamide gel electrophoresis; PEG, poly(ethyleneglycol) 6000; PS I, Photosystem I; RC I, reaction center of Photosystem I; SB 12, sulfobetain 12; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SG, sucrose density gradient.

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processing of PS I particles from *Synechococcus* sp. gave evidence for a trimeric organization of this complex [7,20], which was also recently found for the PS I complex of *Phormidium laminosum* [18]. On the other hand, electron microscopy has detected monomeric PS I particles in both the mesophilic cyanobacterium *Synechococcus* 6301 [2] and the thermophilic cyanobacterium *Phormidium laminosum* [18], questioning the existence of a PS I trimer in vivo. The molecular mass of these monomeric complexes is also a matter of debate, ranging from 150 kDa [6] to 400 kDa [2] depending on the method used, as is the amount of antenna chlorophyll per reaction center Chl a_1, for which values from 50 [3] up to 130 [2] have been reported.

In this contribution, we report the purification of three different complexes of Photosystem I by an HPLC method. The PS I complexes differ only in apparent mass and surface charge; they show a similar polypeptide composition and comparable numbers of antenna chlorophyll per Chl a_1. From this analysis and averaged electron micrographs of the biggest and the smallest particles it is concluded that the complexes represent monomers, dimers and trimers of the same RC I unit. Using electron microscopy and two further methods to estimate the molecular mass, we conclude that the minimal PS I unit has an apparent mass of (235 ± 25) kDa, including about 65 antenna Chl per Chl a_1. The size and shape of this unit can be estimated from the electron micrographs. The advantage of this HPLC method in comparison to PAGE under non-denaturing conditions is discussed.

Materials and Methods

Cells of *Synechococcus* sp. were grown and membranes isolated as described in Refs. 21, 22. The preparation of a crude PS I extract using SB 12 as a solubilizing agent has been described in Ref. 19. The extract gave a sharp green band (termed SG 1) in the lower part of a 10–40% (w/w) sucrose gradient in buffer A (20 mM Mes, 20 mM $CaCl_2$, 10 mM $MgCl_2$ (pH 6.5)), from which highly purified PS I particles can be obtained [19]. For disaggregation of this complex, SG 1 was dialyzed against buffer B (0.1 M Tris, 10 mM $MgCl_2$, 20 mM $CaCl_2$, 0.5 M mannitol (pH 8.3)), incubated – unless otherwise indicated – with 1 M Tris (pH 8.3) plus 5% (w/v) OGP for 15 min and heated at 56 °C for 5 min. The resulting crude protein mixture was layered on a 20–40% (w/v) sucrose gradient in buffer C (buffer A plus 0.5 M mannitol) containing 0.045% (w/v) β-DM. Centrifugation was performed in a Beckman VTi 50 rotor at 50000 rpm for 16 h at 4 °C. Three sharp green bands (termed SG 2a, b, c, from top to bottom) with typical PS I absorbance spectra were obtained. Occasionally, a fourth band due to free pigments was observed on top of the gradient.

Further purification of the pigmented sucrose gradient bands was achieved by anion-exchange chromatography on a FPLC Mono Q HR 5/5 column (Pharmacia) using the Waters apparatus as described in Ref. 23. The column was equilibrated with buffer D (buffer C plus 5 mM $MgSO_4$ (pH 6.5)) containing 0.03% (w/v) β-DM at a flow rate of 0.4–0.5 ml/min and PS I fractions were eluted by an $MgSO_4$ gradient, ranging from 5 to 100 mM. Combination of steep concave and convex gradients allowed purification of SG 2a–c to HPLC grade purity even under semi-preparative conditions. The apparent mass of these purified complexes, termed MQa, MQb and MQc, was determined in size-exclusion experiments using a TSK 4000 SW column (Beckman) in buffer C containing 0.03% β-DM, calibrated using an HPLC gel-filtration kit (Pharmacia).

Preparative electrophoresis was carried out on polyacrylamide slab gels (4.2–10.8% (w/v) gradient), containing 0.1% SDS (w/v) or 0.45% (w/v) β-DM for solubilization. Trimeric PS I, obtained from the first sucrose density gradient, was applied onto the gel without further pretreatment. The resulting pigmented bands were excised and either electroeluted or extracted. The molecular weight of these components was determined either by HPLC-size exclusion (see above) or on analytical slab gels, using a high-molecular-weight marker kit (Pharmacia).

Analytical SDS-PAGE was performed according to Ref. 24 either on 1 mm thick 10–15% (w/v) polyacrylamide gradient slab gels or on an automated Phast-System Apparatus (Pharmacia) using a premade 10–15% (w/v) gradient gel. In both cases the samples were incubated with 2% (w/v) SDS and 60 mM DTE for 20 min at 56 °C. The gels were run at room temperature (1 mm gel) or 4 °C (Phast-System), respectively, and protein was visualized by staining with Coomassie brilliant blue R-250.

The ratio of Chl/Chl a_1 for each of the complexes was determined by analysing the amount of chemically induced Chl a_1 oxidation per chlorophyll as described in Refs. 25, 26 and modified according to Ref. 27, as well as by light-induced photooxidation [25,28]. An extinction coefficient of 64000 $M^{-1} \cdot cm^{-1}$ for P-700 was taken as a basis.

The chlorophyll-to-protein ratio was determined using highly purified PS I preparations from Q-Sepharose anion-exchange material (Pharmacia) as previously described [7]. The protein content was performed with BCA-protein-assay reagent (Pierce) in buffer E (40 mM Tris (pH 8.0), 0.03% β-DM), using bovine serum albumin as a standard. The absorbance change at 562 nm was measured and corrected by subtracting the absorbance due to pigments at the same wavelength. Interference of β-DM with the chelating agent used in this method was not observed.

The chlorophyll concentration was determined using

an extinction coefficient of 74000 $M^{-1} \cdot cm^{-1}$ at 679 nm. Electron microscopy was carried out on Philips EM 300 and EM 400 T electron microscopes at 60000–70000 × magnification similar to Refs. 7, 20. After dilution of the purified PS I samples with buffer A + 0.02% (w/v) β-DM, specimens were prepared by the droplet method, using 1% (w/v) uranyl acetate as a negative stain. Selected micrographs were digitized with a Datacopy Model 610F electronic digitizing camera (Datacopy, Long Beach, U.S.A.), using a scanning step of 32 μm. Analysis was performed by means of the IMAGIC software system [29] on a MicroVAX computer. Selected particles were aligned in an iterative way by using improved references [30,31]. All images were band-pass filtered to remove the very high and very low spatial frequencies which represent noise and disturbances during alignments; the band-pass filter was undone after completing the analysis.

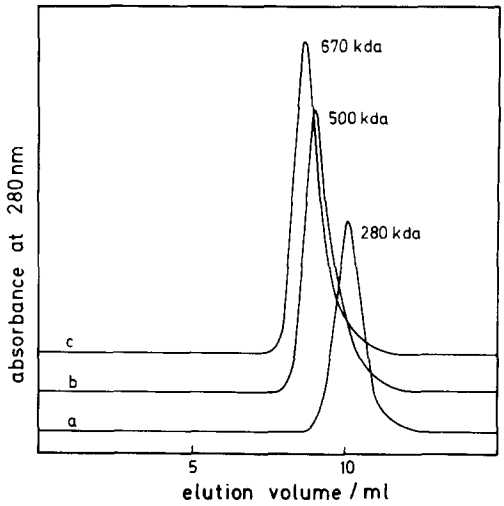

Fig. 2. Elution profiles of highly purified photosystem I complexes on a TSK 4000 SW gel-filtration column in buffer C containing 0.03% β-DM; (a) MQa, (b) MQb, and (c) MQc; masses indicated in kDa; flow rate 0.5 ml/min.

Results

Purification of PS I complexes by HPLC

The PS I fractions separated by sucrose density gradient centrifugation were further purified by a non-linear gradient of $MgSO_4$ on an HPLC anion-exchange column, Mono Q (see Materials and Methods), to yield three PS I complexes, termed MQa, MQb and MQc. In order to check the purity of each of these complexes, they were applied to another Mono Q column and eluted with a linear gradient of $MgSO_4$. The elution profile is shown in Fig. 1. As can be seen, all three complexes could be successfully purified on the first anion-exchange column step. Small amounts of contaminants (as in profile b) could be removed by this second run, since the complexes eluted at substantially different concentrations of $MgSO_4$. It should be mentioned, however, that MQb was more difficult to purify than MQa and MQc due to its greater instability. Evidently, the binding affinities correspond to the size of the complexes in the sucrose density gradient, with the smallest particle from the upper band, SG2a, eluting first. All three complexes – MQa, -b and -c – showed identical absorption spectra typical for PS I (data not shown).

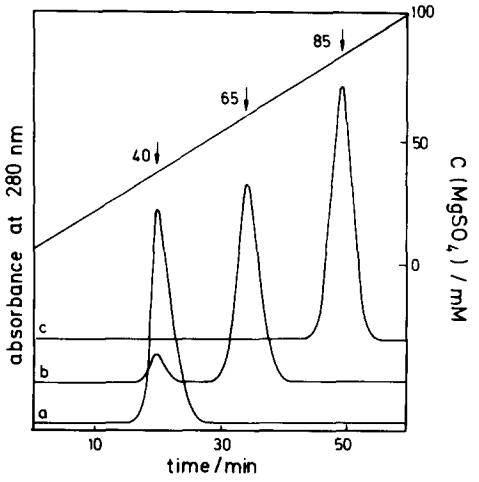

Fig. 1. Elution profiles of (a) MQa, (b) MQb, and (c) MQc preparation from an anion exchange column, Mono Q HR 5/5, upon applying an $MgSO_4$ gradient in buffer D; flow rate: 0.4 ml/min.

As a further check of purity and homogeneity and to determine the masses of these particles, HPLC gel-filtration was performed. Fig. 2 shows the elution profiles of MQa, -b and -c; the elution volumes correspond to apparent masses of about 280, 500 and 670 kDa, respectively. The apparent mass of the latter complex is consistent with that previously determined for the PS I trimer [7,19]. Both Fig. 1 and Fig. 2 show that the particles are pure and homogeneous.

The protein composition of each complex was analysed by SDS-PAGE. Fig. 3 shows a comparison of the densitometer scans of Coomassie blue stained protein profiles of MQa, -b and -c, obtained from a polyacrylamide-gradient gel under denaturing conditions. Each complex is composed of two high-molecular-mass polypeptides of 64 and 59 kDa and two low-molecular-mass ones of about 16 and 12 kDa. The similarity of all three profiles rules out the possibility that the different behaviour of the complexes on anion-exchange chromatography is due to a difference in polypeptide composition.

Activity of the purified PS I complexes

The Chl/Chl a_I ratio was measured for each complex by either using ferricyanide or light to oxidise Chl a_I (see Table I). Both methods are complementary. While in the chemical assay Chl a_I is oxidized directly in the photochemical assay, only those reaction centers

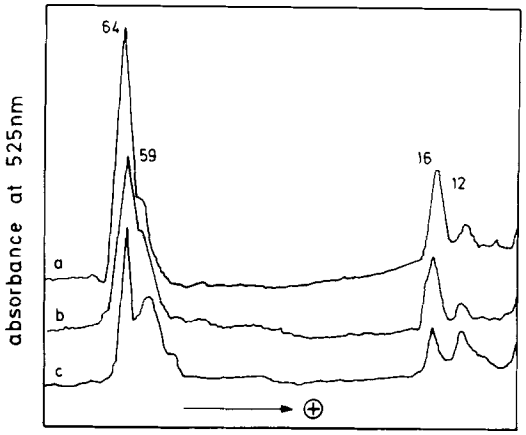

Fig. 3. Protein composition of the PS I complexes MQa (a), MQb (b) and MQc (c). Samples containing equal amounts of chlorophyll were electrophoresed through 10–15% polyacrylamide gels on a Phast-apparatus and stained with Coomassie blue. Densitometer scans at 525 nm are shown.

showing an active electron transport from P-700 to P-430 are detected. (The millisecond time resolution of the apparatus used does not allow the detection of fast nanosecond recombination reactions which occur in centers lacking the terminal acceptors.)

All three complexes, MQa–c, show the same ratio of about 66 Chl/Chl a_1, consistent with previous observations [1,4]. The slightly higher values obtained in the photo-oxidation assay may be due to some loss of the electron acceptor components.

Data from the crude SG 1 preparation of PS I, shown for comparison, demonstrate that the additional purification steps reported here considerably reduce the Chl/Chl a_1 ratio.

Purification of PS I complexes by preparative gel electrophoresis

To compare the quality of the PS I particles isolated by the HPLC procedure with the electrophoretic methods reported by other groups [1,6], the PS I trimer of the first sucrose density gradient was applied to a preparative gradient gel under non-denaturing conditions. For partial dissociation of the trimer, the gel

TABLE I

Apparent mass and Chl/Chl a_1 ratio of different photosystem I complexes obtained by anion-exchange chromatography (HPLC)

The apparent masses were determined by HPLC gel-filtration.

Complex	Apparent mass (kDa)	Chl/Chl a_1	
		chemical oxidation	photo-oxidation
MQa	280 ± 20	68 ± 4	88 ± 5
MQb	500 ± 25	64 ± 4	87 ± 5
MQc	670 ± 35	68 ± 4	85 ± 5
SG1	670 ± 35	100 ± 10	105 ± 8

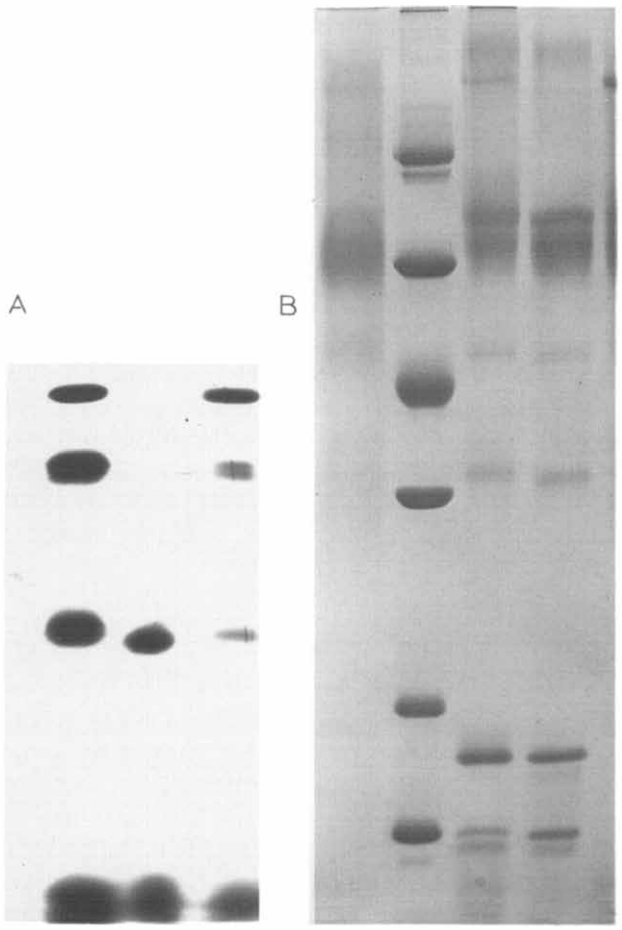

Fig. 4. (A) Preparative SDS-PAGE of different PS I complexes under native conditions: 4.2–10.8% polyacrylamide gradient containing 0.1% SDS. Lane 1. PS I complexes originating from a sucrose density gradient centrifugation; lane 2, reelectrophoresis of complex EII; lane 3, reelectrophoresis of complex EI; for details see text. (B) Analytical SDS-PAGE of different PS I preparations under denaturing conditions: 10–15% polyacrylamide gradient containing 0.1% SDS. Lane 1, protein EIII; lane 2, mass marker kit; lane 3, protein EI; lane 4, protein EII; for details see text.

contained 0.1% SDS (according to Refs. 1, 6). Fig. 4A, lane 1, shows three chlorophyll-containing protein complexes, EI–EIII, originating from the trimer. They were eluted from the gel and the mass of the two larger complexes was determined by HPLC gel-filtration, yielding masses corresponding to 650 kDa (EI) and 280 kDa (EII) (data not shown); however, the preparations were not as pure as the ones shown in Fig. 2.

Lane 2 and 3 of this gel were obtained by re-electrophoresis of complex EI and EII under the same conditions. While EII is transformed into EIII (lane 2), EI splits into three bands. Calibration with marker proteins yields masses of 540 kDa (EI), 200 kDa (EII) and 110 kDa (EIII).

If SDS was replaced by 0.45% (w/v) β-DM, similar results obtained; however, the run-time increased by more than a factor of 2 and much less of complex EIII was formed.

TABLE II

Apparent mass and Chl/Chl a_I ratio of different Photosystem I complexes obtained by preparative gel electrophoresis

The apparent masses were determined by HPLC gel-filtration, the values in brackets were obtained by SDS-PAGE.

Complex	Apparent mass (kDa)	Chl/Chl a_I	
		chemical oxidation	photo-oxidation
EI	670±35 (540±25)	40±3	108± 7
EII	280±20 (200±10)	49±3	104± 7
EIII	220±10 (110± 6)	43±3	190±12

Fig. 4B shows the polypeptide composition of EI, EII and EIII following analytical SDS-PAGE under denaturing conditions. Complex EI and EII (lane 3 and 4, respectively) show an identical polypeptide profile of five subunits with apparent masses of 70, 64, 16.6, 12.8 and 12.4 kDa. Two additional polypeptides with apparent masses of 33 and 45 kDa are contaminants. MQa and MQc, run under the same conditions, yield an identical polypeptide pattern concerning the PS I subunits, but with fewer contaminants (data not shown). This analysis indicates that the band of about 12 kDa in Fig. 3a–c is composed of two unresolved polypeptides. EIII (lane 1) seems to have lost all small polypeptides; it is therefore very likely identical to CP-1 in Ref. 8, the 105 kDa complex in Ref. 6 and the so-called CP1-e in Ref. 1.

Table II summarizes the apparent mass and the Chl/Chl a_I ratios of the three PS I complexes isolated by preparative PAGE (all from the same extract). There are considerable differences between the ratios as determined by chemical oxidation to those by photooxidation. This holds especially for complex EIII (see Discussion).

A comparison of the molecular weights of Table II with those of Table I (both determined by HPLC gel-filtration) suggests that complex EI can be equated with MQc and EII with MQa. No particles with a mass identical to MQb could be seen under the conditions used for native PAGE.

Determination of the chlorophyll-to-protein ratio

The chlorophyll-to-protein ratio of PS I was determined with the pure trimeric complex. A graph of protein (w/v) vs. chlorophyll (w/v) shows linearity up to about 0.2 mg chlorophyll/ml. The slope indicates a ratio of 1:3.1 for the weight of antenna chlorophyll relative to the weight of protein. Hence, an average PS I preparation, which possesses a chlorophyll-to-Chl a_I ratio of 65 (without SDS treatment, see Table I), contains approx. 58 kDa of chlorophyll and 180 kDa of

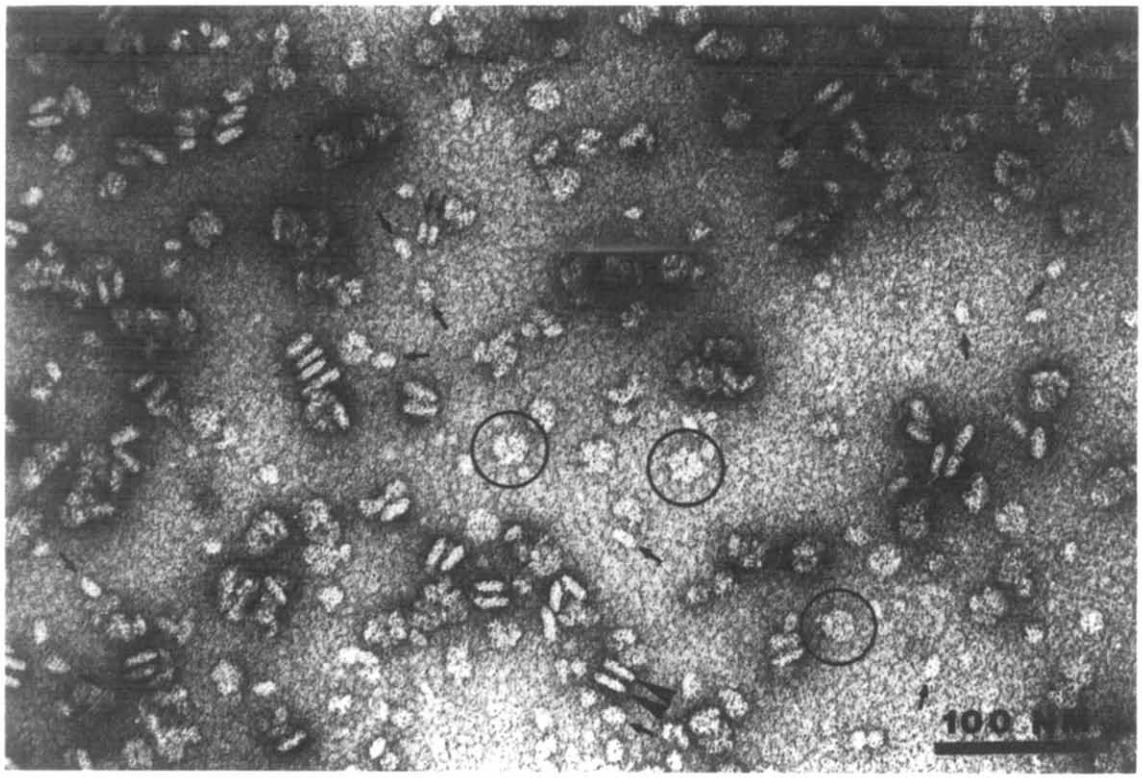

Fig. 5. Electron micrograph of a mixture of isolated MQa (monomeric) and MQc (trimeric) PS I particles, negatively stained with 1% uranyl acetate in the presence of 0.02% dodecyl maltoside. Monomeric top- and side-view projections are indicated by small arrows; trimeric side views are shown by thick arrows and some trimeric top views are encircled.

protein per Chl a_1. In total, this represents a value of approx. 238 kDa per Chl a_1.

Structural investigation of PS I complexes by electron microscopy

Electron micrographs of MQc show the presence of homogeneous trimeric PS I particles as already observed in previous work [7,20]; the MQa particles are also homogeneous, but much smaller (data not shown). In order to compare the shapes of MQc and MQa under identical conditions, we prepared specimens for electron microscopy, in which both MQa and MQc were solubilized in β-DM and mixed in a ratio of about 1 : 1 (w/w).

Fig. 5 shows an electron micrograph of such a mixture, following negative staining. From the trimeric top and the staggered side views of the big particles it is evident that MQc is identical to the previously characterized PS I trimer. Some of the small MQa particles also appear in a staggered configuration with about the same width as the trimeric one. By analogy to our previous reports [7,21,23] we attribute this view to the side view of MQa. In comparison to this, the top view of MQa shows a larger area and separate particles. Their size and shape corresponds roughly to the monomeric form of the PS I trimer and therefore will be termed 'monomer' from now on.

From this and five other micrographs, we selected trimeric and monomeric top- and side-view projections which were brought into equivalent positions by repeated alignments on improved references [30]. Fig. 6A shows the sum of 120 aligned trimeric top views; they have an average diameter of 21 nm. From monomeric particles, 423 top-view and 88 side-view projections were selected and aligned. The sum of the best 160 top-view images, Fig. 6B, yields a length and a width of 15.3 and 10.6 nm, respectively, for the isolated monomer. Fig. 6C shows the sum of the best 34 (out of 88) monomeric side views. They yield a similar length, 15.0 nm, as the monomeric top views (Fig. 6B) and a width of 6.4 nm.

From the comparison of Fig. 6A and B it can be concluded that the dimensions of the monomeric top view correspond to those of one third of a trimer particle.

Due to the small amount of PS I dimers obtained during the preparation and their instability, we were unable to obtain sufficient electron micrographs to perform the image processing.

Attempts to reassociate the isolated complexes

In contrast to earlier investigations [1], re-association of monomers into trimers has not been achieved. Slow precipitation of monomers using PEG as the precipitating agent and microdialysis against detergent-free buffers (for up to 1 week) did not induce the formation of larger aggregates. Incubation of purified monomers with 1% SB 12 and at elevated temperatures (37 °C), i.e., under conditions similar to the extraction of the *Synechococcus* membranes, does not result in the formation of larger aggregates. On the other hand, careful analysis of the upper pigmented band of SG 1, which follows the membrane extraction with SB 12, revealed a small amount of monomer (< 5%). About the same amount of monomer is obtained, if isolated trimers are incubated under conditions identical to the extraction procedure [19] and separated by the SG 1 gradient (see Material and Methods).

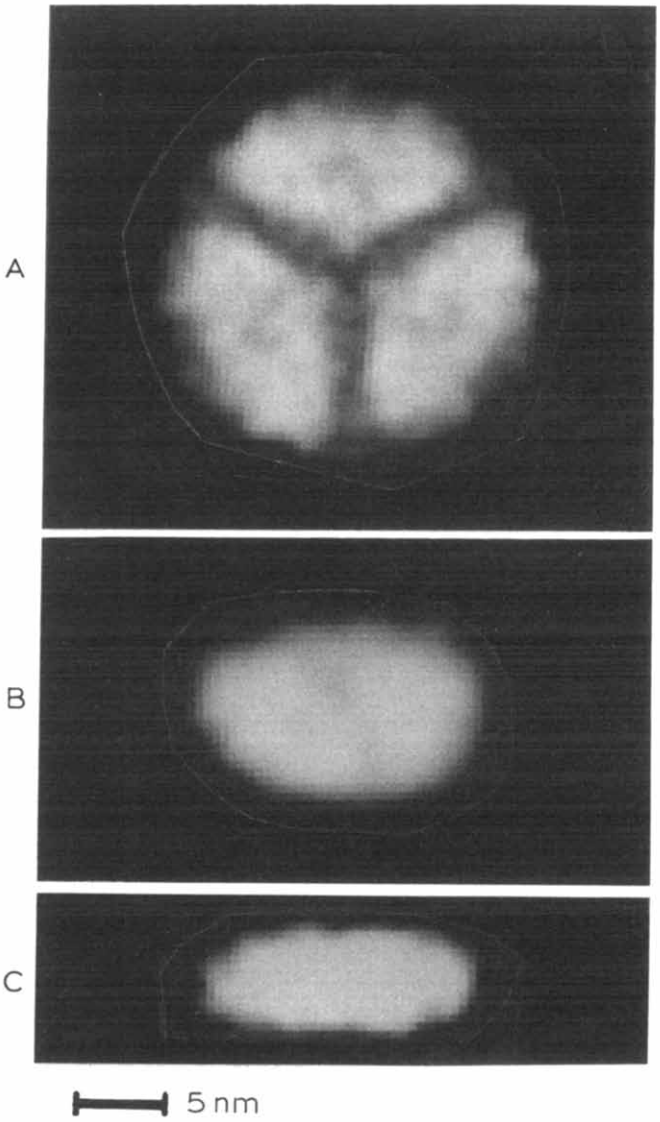

Fig. 6. Image analysis of monomeric and trimeric PS I particles solubilized with 0.02% dodecyl maltoside. (A) Average image of 120 trimeric top-view projections, summed after repeated alignments. (B) Average image of 160 monomeric top-view projections (C) Average image of 66 monomeric side-view projections.

Discussion

Characterization of the PS I complexes

We have shown that it is possible to dissociate the well-characterized PS I trimeric complex [7,19,20] into highly active subcomplexes. Several characteristics of these subcomplexes strongly suggest that they are the monomeric and dimeric particle of the PS I trimer.

(a) HPLC gel-filtration shows that each of the three isolated complexes – MQa, MQb and MQc – is homogeneous. The ratio of their apparent molecular weights is 1 : 1.8 : 2.5, indicating monomeric, dimeric and trimeric particles. The molecular weight of the heaviest particle is, within the errors associated with this technique, identical to that of the previously characterized PS I trimer. The deviation from the exact 1 : 2 : 3 ratio predicted on the basis of their being oligomers can be attributed to the ability of smaller particles to bind relatively more detergent than larger ones. This has recently been shown with monomeric and dimeric PS II particles from the same organism [21,23].

The apparent molecular weight has also been determined by electrophoresis of the PS I monomer and trimer under non-denaturing conditions. However, due to the problems of applying this method to partially denatured proteins and the known underestimation of mass for hydrophobic proteins by electrophoresis [1,32], these values are unreliable and inferior to those determined by HPLC gel-filtration.

(b) Electron microscopy shows obvious similarities between the top view of the monomer and the monomeric part of the trimer (Fig. 6A + B). The diameter of the PS I trimer dissolved in β-DM, 21.0 nm, is larger than the one previously observed, when these particles were solubilized in OGP, 19.0 nm [7,20]; this is consistent with the difference in chain length of these detergents and confirms our previous suggestion [7] that this is the top view of the particle. Both the monomeric part of the trimer and the isolated monomer have an elongated shape of approximately equal size. The isolated monomer, however, has a larger width (10.6 vs. 8.5 nm), which can be explained by a different contribution of the boundary layer to the particle dimensions. It is supposed to have a β-DM boundary layer around all the outer regions in the top view position, whereas the monomer in the trimeric arrangement will have no boundary layer on the inner sides. On the other hand, irrespective of the type of detergent used for solubilization, the width of the shortest axis of both monomer and trimer is identical, consistent with a transmembrane orientation.

The resolution of the image analysis on isolated monomers (Fig. 6B) is inferior to that of the trimers [7,20], since the accuracy of the alignments is much better for larger particles. Moreover, no attempts to classify the monomeric top views have been made. Therefore, the sum of Fig. 6B may contain a mixture of projections that differ in handedness and/or in their position (180 degree ambiguity).

(c) Analytical PAGE shows an identical polypeptide composition for the PS I monomer (MQa), dimer (MQb) and trimer (MQc), which in turn is very similar to the one of the PS I trimer used as the starting material [7] and in agreement with the results from other cyanobacteria [1,3,6]. While the two big subunits can be attributed to the products of the *psaA* and the *psaB* genes [32,33], the function of the remaining three small subunits is uncertain. Certainly, none of them represents cytochrome *c*, which is lost during the isolation procedure [34]. However, since the optical measurements of Chl a_1 activity in all the samples tested were performed at levels of the acceptor benzyl viologen (100 μM), which have previously been shown to require the presence of the Fe-S center F_A and F_B (Ref. 35; Golbeck, J., personal communication), we conclude that the three isolated PS I complexes possess these centers. Recently, it has been shown that a chloroplast gene, *psaC*, codes for a 9 kDa protein [36], which binds both these centers [13]. We suspect, therefore, that the lowest-molecular-weight polypeptide of Fig. 4B corresponds to this protein.

(d) The Chl/Chl a_1 ratio of the PS I monomer, dimer and trimer produced by sucrose density/Mono Q column and of the PS I monomer and trimer generated by native gel electrophoresis are virtually identical within each group. This suggests that each of the two procedures yields particles with an identical set of components of the electron transport chain. However, comparing the difference of the Chl/Chl a_1 ratios as determined by chemical and photochemical assay, a much larger difference is found for the PAGE PS I particles (Table II, factor > 2) than for the MQ PS I particles (Table I, factor about 1.3). The small differences for MQ PS I in both assays (Table I) show that the detergent β-DM keeps the preparation in their native, fully active state (see Results). On the other hand, the significant discrepancy between high Chl/Chl a_1 values obtained by photo-oxidation of PS I and the respectively low values by chemical oxidation (Table II) indicate a partial disaggregation of the photosystem and/or electron transport by SDS. While the very low values obtained by chemical oxidation reflect the loss of additional antenna chlorophyll, the rather high values by photo-oxidation indicate the loss or inactivation of electron transport components at the acceptor site. This is especially true for the CP I particle, EIII, which has lost all its small subunits. However, when SDS is substituted by β-DM in the electrophoresis method, the particles show similar Chl/Chl a_1 values of about 80 in both the chemical and the photochemical assay. This suggests that it is the detergent and not the separation method which is responsible for the above-mentioned dis-

crepancies. Regarding these results, we consider a Chl/Chl a_1 ratio of about 65 as obtained for complexes solubilized by β-DM as representative for particles with fully retained components at the acceptor site and minimal amount of antenna chlorophyll. Obviously, the Chl/Chl a_1 values reported may indicate the quality of the separation as well as the destructive influence of the detergent. This may partly explain the very different Chl/Chl a_1 values reported in literature.

Evaluation of oligomeric PS I complexes and preparation procedures

Up to now, the preparation of oligomeric PS I particles has only been reported for thermophilic cyanobacteria. Using SDS-PAGE, Takahashi et al. [1] have previously isolated from the same organism two PS I particles, which they suggested were monomeric and dimeric forms. Since the PS I dimer is less stable even under mild conditions and no data were produced concerning their molecular weights, our work suggests that these two complexes are instead the monomer and the trimer.

This is in line with mono- and trimeric particles in PS I preparations of *Phormidium laminosum* [18]. Comparison of these particles produced by native PAGE with the PS I particles produced in this work by HPLC demonstrate that the latter method is a much milder isolation procedure. This is indicated by the occurrence of the labile dimeric PS I complex as well as by the fact that both PS I monomer and trimer are stable even if the HPLC anion-exchange step is repeated. Besides this, HPLC yields complexes of higher purity in comparison to native PAGE. Material gained by electrophoresis still contains a significant amount of residual free pigments and low-molecular-weight proteins when applied on an HPLC gel-filtration column.

The trimeric PS I particle showed extreme stability during these investigations, in line with the finding that PS I is the most stable component of the photosynthetic electron transport chain in the thylakoid membrane of *Synechococcus* [37]. Considering the treatment necessary to dissociate this complex – OGP, Tris and heat treatment – the interaction between the PS I monomers seems to be a combination of both hydrophilic and hydrophobic forces. Despite these extremely strong interactions, trimers can not form from monomers.

This raises the question of whether it is the PS I monomer or trimer which represents the PS I complex in vivo. While Ford and co-workers conclude from their results that the PS I trimer is an artifact induced by the presence of detergent [18], our results leave this question open. It should be pointed out, however, that our procedure for preparing PS I particles deviates substantially from the one of Ford et al. [47]. For extraction of PS I, they choose conditions, i.e., Tris (pH 8.3) and SDS, which in the case of PS I from *Synechococcus* sp. contribute considerably to the dissociation of trimers into monomers (Rögner, M., unpublished). In contrast to these conditions, we extracted and solubilized PS I from the membrane using SB 12 and β-DM, detergents which are known to keep proteins in their native state [38,39] and stabilize PS I activity [18]. Under these conditions, increasing the detergent concentration did not cause aggregation but further dissociation of the trimer into the monomer. For comparison, we also extracted PS I of *Synechococcus* by Triton X-100 according to Ref. 2. In contrast to this report, which shows electron micrographs with ellipsoid monomeric PS I particles for *Synechococcus* 6301, we obtain trimeric PS I particles for *Synechococcus* sp., based on HPLC anion-exchange and gel-filtration (data not shown).

We therefore disagree with the opinion that the formation of oligomers is induced by detergent. This contention is further supported by the fact that under the same conditions of solubilization, PS I from the mesophilic cyanobacterium *Synechocystis* PCC 6803 is extracted mainly as a monomer (Rögner, M., unpublished data).

As electron micrographs of negatively stained membrane fractions of *Phormidium laminosum* [18] are either obscured by surface-bound proteins or the membranes have already undergone a detergent treatment, i.e., are no longer necessarily in their native state, the question of whether the PS I trimer represents the native state can only be solved by freeze-etch/freeze-fracture electron microscopy. Using this approach, it has been shown that the PS II dimer [23] represents the in vivo state in *Synechococcus* sp. [40].

Size and mass of the minimal PS I unit

The results of this work suggest that the MQa particle, obtained by HPLC, and the EII particle, obtained by SDS-PAGE, represent the 'minimal PS I unit' for photo-oxidation of Chl a_1. Three approaches have been used to estimate the molecular weight of this particle.

(1) The molecular mass as determined by HPLC gel-filtration corresponds to the one of a mixed protein/detergent micelle. As β-DM forms large micelles with a molecular weight of about 50 kDa [41], the molecular mass of the PS I monomer without the contribution of the detergent can be estimated to be (230 $\pm$ 20) kDa.

(2) This value is supported by an analysis of the electron micrographs of the PS I monomer: By subtracting a boundary layer of 16.7 Å, previously determined as the chain length of β-DM [42], from its top view area (Fig. 6B), a volume of (470 $\pm$ 50) nm^3 can be calculated for the PS I protein complex. Assuming a value of (0.5 $\pm$ 0.1) kDa/nm^3 [43], a net molecular mass of (235 $\pm$ 50) kDa can be calculated.

(3) The protein/chlorophyll ratio of 3.1 suggests a

molecular mass of 238 kDa for the PS I monomer (see Results). Since this value is based on 65 Chl per photoactive reaction center, the presence of any photo-inactive centers will result in a lower apparent molecular mass of the PS I monomer.

Averaging our results, the mass of the minimal PS I unit is about (235 ± 25) kDa. This value differs considerably from the 150 kDa as determined by SDS-PAGE [6] and 300–400 kDa as estimated from protein/chlorophyll ratios, electron microscopy and sucrose density gradient centrifugation for other cyanobacterial PS I complexes [2,5]. However, the underestimation of mass by SDS-PAGE and the overestimation of volume, when the particle is dissolved in bulky detergents like Triton X-10 [2], should not be overlooked.

Our results are in good agreement with molecular weights deduced from the sequences of the cyanobacterial PS I genes *psaA–psaE* (Ref. 33; Rhiel, E. and Bryant, D. personal communication), which presumably correspond to the five PS I subunits of our preparation. Adding their molecular mass plus the contribution of 65 Chl yields a mass of about 260 kDa for the PS I core complex, based on a 1:1 stoichiometry of all subunits as recently shown for *Dunaliella salina* [44].

A molecular mass of 235–260 kDa clearly rules out the possibility of four large subunits per Chl a_1, which has been inferred from ^{14}C-labeling [5] and EPR experiments [8,29]. More recent EPR and EXAFS results are, however, in agreement with our data (Ref. 52, McDermott, A.E. and Golbeck, J., personal communication), as well as recent data concerning the mass of the chloroplast PS I [44,45,52].

The location of these two big subunits within the monomer can be inferred from a close analysis of the top view (Fig. 6B). The highest stain-excluding density (which is interpreted as being protein) is close to the outer ends of the projection. A similar, but more pronounced density distribution was found for the monomeric part within the trimer (Fig. 6A; Ref. 20). We tentatively attribute these regions to the two big subunits, as has been outlined in Ref. 20.

Our EM results are in good agreement with the electron microscopy analysis of other monomeric cyanobacterial PS I complexes. Both for the mesophilic *Synechococcus* sp. PC-7002 (18 × 8 nm) and for the thermophilic *Phormidium laminosum* (15 × 9 × 6 nm) ellipsoid particles have been detected.

This investigation gives a detailed characterization of the mass, size, shape and activity of the monomeric PS I particle (as summarized in Table III). A more detailed understanding of the structure–function relationship within this monomer can be gained by X-ray analysis of PS I crystals. Up to now, PS I has been crystallized in two different types of crystal; in both cases the building block of the crystal is the trimer [47–49].

TABLE III

Stoichiometry of reaction center chlorophyll, Chl a_1, antenna Chl and corresponding (apparent) mass for the purified monomeric PS I complex

Values for the apparent mass derived from the size exclusion and electron microscopy experiments have been modified to take into account the contribution of the detergent shell (see Discussion)

Monomeric RC I	
Oxidizable Chl a_1 per complex	1
Chlorophyll attachment per complex	65 ± 5
Total mass based on protein estimation	238 ± 20
Apparent mass based on size exclusion	230 ± 20
Apparent mass based on the volume (EM)	235 ± 50

Acknowledgements

We thank Dr. S. Gerken and Dr. K. Brettel for help in performing the Chl a_1 activity measurements, Prof. E. Zeitler for his support of this work and Ms. D. DiFiore, Ms. I. Geisenheimer and Ms. M. Gerdsmeyer for excellent technical assistance. We are further indebted to Dr. P. Nixon and Dr. B. Diner for reading the manuscript. The financial support of the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 312) is gratefully acknowledged.

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