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Molecular Weight Determination of an Active Photosystem I Preparation from a Thermophilic Cyanobacterium, *Synechococcus elongatus*†

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ABSTRACT: An active photosystem I (PSI) complex was isolated from the thermophilic cyanobacterium *Synechococcus elongatus* by a procedure consisting of three steps: First, extraction of photosystem II from the thylakoids by a sulfobetaine detergent yields PSI-enriched membranes. Second, the latter are treated with Triton X-100 to extract PSI particles, which are further purified by preparative isoelectric focusing. Third, anion-exchange chromatography is used to remove contaminating phycobilisome polypeptides. The purified particles show three major bands in sodium dodecyl sulfate gel electrophoresis of apparent molecular mass of 110, 15, and 10 kDa. Charge separation was monitored by the kinetics of flash-induced absorption changes at 820 nm. A chlorophyll/P_{700} ratio of 60 was found. When the particles are stored at 4 °C, charge separation was stable for weeks. The molecular mass of the PSI particles, determined by measurement of zero-angle neutron scattering intensity, was 217 000 Da. The PSI particles thus consist of one heterodimer of the 60–80-kDa polypeptides and presumably one copy of the 15- and 10-kDa polypeptides, respectively.

In order to find out which polypeptides are indispensable for constitution of an active PSI[1] complex, preparations of isolated complexes are necessary. Purification schemes have been reported for detergent-solubilized photosystem I from chloroplasts of several higher plants (Vernon & Shaw, 1971; Shiozawa et al., 1974; Bengis & Nelson, 1975; Mullet et al., 1980; Argyroudi-Akoyunoglou & Thomou, 1981; Takabe et al., 1983; Vierling & Alberte, 1983; Bassi & Simpson, 1987) and algae (Nechustai & Nelson, 1981; Hiller et al., 1988) as well as from cyanobacteria (Newman & Sherman, 1978; Nakayama et al., 1979; Bishop & Öquist, 1980; Nechustai et al., 1983; Lundell et al., 1985; Ford, 1987; Ford et al., 1987) and a Chl *b* containing procaryote (Schuster et al., 1985).

For the fractionation of photosynthetic pigment–protein complexes, SDS is more efficient than nonionic detergents, allowing PSI to be purified in only one or two steps. It is, however, more aggressive and in several cases has stripped off the small molecular mass polypeptides of the PSI complex, resulting in a complex lacking the iron–sulfur complexes F_A and F_B that serve as secondary electron acceptors (Bengis & Nelson, 1977; Golbeck & Cornelius, 1986). Some of the procedures use SDS to purify PSI in polyacrylamide gels or in a density gradient (Bishop & Öquist, 1980; Argyroudi-Akoyonoglou & Thomou, 1981; Vierling & Alberte, 1983; Ford et al., 1987). In some of these studies the term CPI is used for a pigmented band containing the PSI polypeptides.

The other procedures use nonionic detergents in combination with centrifugational and chromatographic methods (Satoh & Butler, 1978; Boekema et al., 1987; Bruce & Malkin, 1988).

Density gradient fractionation is time consuming. In the present paper we describe a fast purification procedure that replaces the centrifugation step by isoelectric focusing. The second step is anion-exchange chromatography. Several milligrams of highly active PSI particles containing only four polypeptides and about 60 chlorophyll molecules can be prepared by this method within 2 days.

The polypeptide patterns of the PSI particles purified in various laboratories differ considerably. Nevertheless, all contain two large homologous polypeptides. Vierling and Alberte (1983) and Fish and Bogorad (1986) have reported two bands with apparent masses of 58 and 62 kDa, which in other studies were not resolved and migrated as a single band with an apparent molecular mass of up to 70 kDa (Lundell et al., 1985). Under mild conditions of solubilization and electrophoresis, these two polypeptides remain linked as a heterodimer containing chlorophyll, which migrates as a pigmented band at an apparent mass of approximately 110 kDa (Bruce & Malkin, 1988). This heterodimer contains the

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[1] Abbreviations: PSI, photosystem I; kDa, kilodalton; SB12, 3-(dodecyldimethylammonio)-1-propanesulfonate; SDS, sodium dodecyl sulfate; ET12H, 1-dodecylpropanediol-3-phosphocholine; ES12H, 1-dodecanoylpropanediol-3-phosphocholine; DM, *n*-dodecyl β-D-maltoside; IEF, isoelectric focusing; PEG, poly(ethylene glycol); SANS, small-angle neutron scattering; M_r, relative molecular mass; Chl, chlorophyll; cmc, critical micelle concentration; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

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primary donor P_{700} as well as the first electron acceptors and an iron–sulfur complex (Golbeck & Cornelius, 1986; Golbeck et al., 1988a). Two polypeptides of apparent masses of 9 and 20 kDa also seem to be common to all preparations. The former was found to bind the two iron–sulfur complexes F_A and F_B (Hoj et al., 1987; Wynn & Malkin, 1988), which serve as secondary electron acceptors within PSI (Golbeck et al., 1988b). The latter was shown to be involved in ferredoxin binding (Zanetti & Merati, 1987). Another polypeptide of approximately 20 kDa was reported to serve as a docking protein for plastocyanin binding (Bengis & Nelson, 1977; Wynn & Malkin, 1988).

More recently, the genes psaA and psaB of the two high molecular mass polypeptides were sequenced (Fish et al., 1985). The relative molecular masses calculated from the nucleic acid sequence are higher (83 200 and 82 500) than the apparent masses in the gel. Similar masses were calculated from algal (Cushman et al., 1987) and cyanobacterial (Bryant et al., 1987) psaA and psaB genes. Fish and Bogorad (1986) have argued that no extensive processing of these polypeptides takes place.

The molecular mass of the active detergent-solubilized complex has been in dispute. Calculations of the molecular mass have been made from the chlorophyll:protein mass ratio and the chlorophyll:P_{700} ratio. This calculation yields valid results only if all P_{700} donors in the preparation are titratable and if each PSI complex contains one P_{700}. In this way Vierling and Alberte (1983) and Lundell et al. (1985) calculate 350 and 361 kDa, respectively.

In other studies, the molecular mass has been estimated by comparing elution volume in gel filtration chromatography or mobility in SDS–polyacrylamide gels with those of complexes of known mass. Ford (1987) estimates 150 kDa from the migration on a polyacrylamide gel. Ford et al. (1987) report crystals of a PSI complex from a solution of particles with a mass of 450 kDa. Boekema et al. (1987) observe a band at 600 kDa with gel filtration, and electron micrographs of these particles show a trimeric structure. Ford and Holzenburg (1988) harvest particles of similar appearance from a density gradient. The latter authors report another band of PSI particles, different in density from the trimers, which due to their size could be the monomers forming the trimers.

Other estimations of the molecular mass were made from estimations of the stoichiometry of the PSI polypeptides. In some studies ^{14}C labeling is used. Lundell et al. (1985) found a stoichiometry for the 70-, 18-, 17.7-, 16-, and 10-kDa proteins of 4:1:1:1:1 and therefrom estimated a molecular mass of 334 kDa for the complex. Bruce and Malkin (1988) found a ratio of 2:1:1:1:1. Nechustai and Nelson (1981) found 2:1:1:1 for 70-, 19-, 10-, and 8-kDa polypeptides, respectively. The stoichiometry of the PSI polypeptides was also estimated from their staining intensity in SDS gels. Hoj et al. (1987) find for the 65-, 18-, 16-, 14-, and 9-kDa bands a stoichiometry of 4:2:2:1:2 and estimate a molecular mass of 410 kDa for the complex. Fish and Bogorad (1986) have separated the two different polypeptides of apparent molecular mass close to 65 kDa and determined their stoichiometry to be 1:1.

It follows from these contradictory results that a reliable molecular mass determination of the complex in its active form is required for making models of its quaternary structure. The molecular mass of a detergent-solubilized membrane protein can be determined from the sedimentation equilibrium, if the amount of detergent bound to the protein is known (Tanford et al., 1974). This is not required in a modification of the method described by Butler and Kühlbrandt (1988). Unfortunately, the latter is applicable only when the detergent reaches dialysis equilibrium within a reasonable time.

Here we describe a mass determination of the complex using small-angle neutron scattering. With a suitable D_2O/H_2O mixture the scattering-length density of the buffer can be adjusted so that the contribution of the detergent belt surrounding the protein is eliminated. With precise knowledge of the protein concentration, its amino acid composition, and the chlorophyll content in the complex, the molecular mass of the PSI complex can be calculated (Jacrot & Zaccai, 1981). The method has previously been used with the acetylcholine receptor (Wise et al., 1979), a mitochondrial ADP/ATP carrier protein (Block et al., 1982), and the Na/K-ATPase (Pachence et al., 1987).

Materials and Methods

Preparation of PSI-Enriched Membranes. Synechococcus elongatus Näg. f. thermalis Geitl. strain KOVROV 1972/8 was grown in a chemostat at 57 °C in the modified nutrient medium of Kratz and Myers (1955). The cells were grown in cylindrical culture vessels with a diameter of 30 mm, irradiated from all sides by incandescent lamps giving an irradiance on the surface of the culture vessels of approximately 200 W m^{-2}. The specific growth rate varied between 0.15 and 0.25 h^{-1}.

The PSI-enriched thylakoid fragments were prepared according to the procedure of Schatz and Witt (1984) with the following minor modifications: the cells were treated with 0.4% lysozyme for 1 h at 37 °C, the French press treatment was not applied, and 0.5% bovine serum albumin was added during the sulfobetaine treatment. The fragments were frozen in liquid nitrogen with 20% (v/v) glycerol.

Preparation and Purification of PSI Particles. For further purification, the PSI-enriched thylakoid fragments were thawed, and 12 mg of PSI-enriched membrane particles containing 2.4 mg of chlorophyll was treated with detergent for 1 h in the dark at 4 °C. The extract was centrifuged with a desk-top centrifuge at 3000 turns/min for 15 min at room temperature. The following detergents were tried, Triton X-100, SB12, SDS (all from Serva, Heidelberg, FRG), ET12H, ES12H (Berchtold, Biochemical Laboratory, Bern, Switzerland), and DM (Boehringer, Mannheim, FRG), as were different ratios of chlorophyll to detergent. The supernatant (3–4 mL) was mixed with a solution of 2% (v/v) ampholyt (pH range of 3–6, Servalyt, Serva, Heidelberg, FRG) in 20% (v/v) glycerol to give a final volume of 50 mL. This mixture was filled into a cylindrical cell designed for preparative isoelectric focusing (Rotofor, Bio-Rad), cooled to 4 °C by a cooling finger.

After focusing, the PSI particles were harvested from the cell and dialyzed against 20 mM Tris-HCl, pH 8.0, 20% (v/v) glycerol, and 3 mM NaN_3 (buffer A) with 0.03% Triton X-100 as detergent. The proteins were concentrated with an Amicon ultrafiltration cell (Amicon, Witten, FRG) equipped with an XM-10 membrane.

The dialyzed and concentrated material was further purified by anion-exchange chromatography with Q-Sepharose packed into an HR5/5 column (Pharmacia, Freiburg, FRG), with a gel bed volume of 1 mL. The column was kept at 4 °C and equilibrated with buffer A containing 0.03% Triton X-100. After application of the dialyzate, the column was eluted with selected detergents in buffer A (5 mL) to remove contaminating light-harvesting polypeptides and reequilibrated with buffer A containing 0.03% Triton X-100 (15 mL). Finally, a linear $MgSO_4$ gradient (20 mL) was applied, ranging from

0 to 250 mM $MgSO_4$ in buffer A to which 0.03% Triton X-100 was added.

Amino Acid Analysis. The protein solutions (typically 50–100 μL containing 100–200 μg of protein) that had been used for SANS were transferred into tubes, which were used throughout the hydrolysis procedure. The samples were dried in a desiccator connected to a water pump. Care was taken to avoid boiling in these tubes, which may cause losses of protein. The extraction procedure roughly followed the scheme of Scanu et al. (1958). The whole extraction was performed at −20 °C. The dry pellet was resuspended in 500 μL of a 3:1 diethyl ether/ethanol (Merck, Darmstadt, FRG, p.a.) mixture. The tubes were incubated for 14 h and then centrifuged at 1000 rpm for 20 min. About 400 μL of the supernatant was carefully sucked off without disturbing the protein pellet. The extraction was repeated for 4 h and with 500 μL of ether for 1 h. As a control of the extraction process, all supernatants were collected and analyzed for protein and chlorophyll.

For control experiments, 0.5 mL of a standard amino acid mixture (AA-S-18, Sigma, München, FRG) were dried in a desiccator and resolubilized with 2 mL of various buffers [bidistilled water; 50 mM Tris-HCl, pH 8; 50 mM Tris-HCl, pH 8, in 30% (w/v) glycerol)]. For other control experiments a desired amount of norleucine was solubilized with either 2 mL of bidistilled water or 50 mM Tris-HCl, pH 8. The samples were then dried as described above.

For hydrolysis, 6 M hydrochloric acid solutions with norleucine as an internal standard were prepared; 36.6 mL either of Pro Analysi or of Suprapur grade, $d = 1.13$, HCl (Merck, Darmstadt, FRG) was diluted with bidistilled water to give 50 mL of an approximately 6 M hydrochloric acid solution. Crystalline D-norleucine was added to a concentration of 12.5 mM. For some experiments this solution was diluted with 6 M HCl to give norleucine concentrations of 1.25 or 0.625 mM. Finally, 5 μL of mercaptoethanol was added. To each of the dry protein samples, 200 μL of one of these solutions was added. Nitrogen gas was flowed over each tube for 2 min, and the tubes were sealed. Hydrolysis was performed by incubation at 110 °C for 24, 48, or 72 h. The tubes then were opened, and the hydrochloric acid was evaporated in a desiccator with NaOH.

For chromatographic separation of the amino acids, the dry samples were solubilized with 5–10 mL of buffer B [60 mM trisodium citrate hydrochloride (Merck, Darmstadt, FRG), pH 2.0, 10 mM phenol, and 160 mM thiodiglycol (Pierce) in bidistilled water] by shaking and ultrasonication for 1 min. The separation of the amino acids was done with Biotronik chromatography system LC6000E on a Dionex DC6A column with diameter of 0.6 cm and a gel bed height of 22 cm. The column was equilibrated with buffer B at 33 °C and was eluted at a rate of 29 mL/h. Between 10 and 250 μL of sample was injected so as to obtain an appropriate signal. After the sample was injected, the column was operated according to the instruction manual supplied by the manufacturer. For detection, the fluorescence reaction of amino acids with *o*-phthaldehyde was used (Roth & Hampai, 1973; Benson & Hare, 1975). Fluorescence was detected with a Gilson fluorometer with excitation and emission wavelengths at 340 and 450 nm, respectively. Fluorometric response was calibrated by injection of a test mixture containing 400 pmol of each amino acid. There is no fluorescence from Pro, and the fluorescence response for Cys is smaller by a factor of 20 as compared to those of the other amino acids (Benson & Hare, 1975). The peak areas were integrated with a Spectraphysics SP4270 integrator. All separations were repeated twice, and the results were averaged.

Miscellaneous Analytical Methods. Specimens for electron microscopy were prepared and stained with uranyl acetate as described by Ford and Holzenburg (1988).

SDS–PAGE was carried out according to Laemmli (1970) with modification for the Pharmacia PhastSystem. Acrylamide gradient gels (8–25%) from Pharmacia were equilibrated in 4 M urea overnight prior to use. Following electrophoresis, the gels were stained for protein with Coomassie blue. Spectra were taken with a Perkin-Elmer Lambda 17 spectrophotometer. Chlorophyll was determined by the method of Arnon (1944). Protein was determined with a Coomassie Brilliant Blue G-250 assay (Bio-Rad Protein Assay, Bio-Rad Laboratories, München, FRG). Redox titration to measure P_{700}^{+} minus P_{700} difference spectra was carried out according to Setif and Mathis (1980), with modifications taken from Melis and Brown (1980). To calculate the Chl/P_{700} ratio, an extinction coefficient of 64 000 M^{-1} cm^{-1} at 700 nm was used for P_{700}.

The time course of the absorption at 820 nm was measured with a "home-made" instrument after illumination with a flash of a dye laser (pulse duration 10–20 ns, emission peak at 710 nm). Absorption was measured with light from a tungsten–iodine lamp, which passed through a monochromator and an RG610 filter. The light transmitted through the sample was passed through BP820 and RG760 filters and was measured with a silicon diode detector. The signal was stored in a transient recorder (Nicolet 2090A). The time resolution of the instrument was 1 μs. For calculation of the P_{700} content from the initial absorption change at 820 nm, an extinction coefficient of 6500 M^{-1} cm^{-1} was assumed (Setif et al., 1981).

Small-Angle Neutron Scattering. For determination of the Triton X-100 matchpoint, detergent solutions were prepared from a 10% (w/v) stock solution made up in H_2O/20% glycerol (v/v). This solution was diluted 10-fold with H_2O/D_2O mixtures each containing 20% (v/v) glycerol and 0, 20, 40, 60, and 80% (v/v) D_2O. By 10-fold dilution of a H_2O/20% (v/v) glycerol solution with the H_2O/D_2O mixtures, corresponding solutions for background measurement were made.

Solutions of pure PSI particles were dialyzed against buffer A containing 0.03% Triton X-100 and concentrated with an Amicon ultrafiltration cell equipped with an XM-10 membrane (Amicon GmbH, Witten, FRG) to a concentration of 9 mg/mL as determined with the Bio-Rad assay.

D_2O (≥99.8%, Fluka GmbH, Neu-Ulm, FRG), H_2O (bidistilled), and glycerol (99.5% p.a., Roth, Karlsuhe, FRG) were mixed at desired ratios by weighing. To these solutions, Tris (Merck, Darmstadt, FRG), sodium azide, and Triton X-100 were added to give final concentrations of 20 mM, 3 mM, and 0.03% (w/v), respectively. The calculated final D_2O/buffer volume ratios were 0.0, 0.14, 0.4, 0.6, and 0.8. The final glycerol concentration was 20% (v/v). Some 200 μL of the concentrated PSI particles was dialyzed against each of these buffers overnight at 4 °C. After dialysis the protein solutions were diluted to the desired concentration by the corresponding dialysis buffers and transferred to standard quartz spectrophotometer cells (Hellma, Mülheim, FRG). For D_2O volume fractions above 0.40, 400 μL was used in cuvettes with a path length of 2.0 mm, and for D_2O concentrations below 0.4 volume fraction, 200 μL was used in 1.0-mm cuvettes.

Two sets of experiments were performed: the first on Triton X-100 micelles in order to determine the buffer composition at which their zero-angle scattering fell to zero (matchpoint) and a second on the detergent-solubilized PSI particles.

All scattering curves were measured on the D11 instrument (Ibel, 1976) at the Institut Laue-Langevin, Grenoble, France. The detector is a 64 × 64 cm BF_3 multidetector. The sample-to-detector distance was 5.0 m for samples of Triton-solubilized PSI particles and 2.5 m for Triton solutions alone. The incident wavelength (λ) was 10 Å with $\Delta\lambda/\lambda = 8\%$. For matchpoint determination, scattering curves were measured from Triton X-100 solutions and the corresponding H_2O/D_2O/glycerol buffers as background. For the PSI complexes the background was the H_2O/D_2O/glycerol/Triton X-100 dialyzate. Neutron transmission measurements were carried out to determine the precise D_2O/H_2O content of the solutions and to verify that each sample and background had the same H_2O/D_2O content. The samples were thermostated at 16 °C. The beam cross section at the sample was 70 mm². Measuring times varied from about 10 min per scattering curve for samples with high D_2O content to 1 h for samples with low D_2O content. All scattering curves of samples were corrected for a nonuniform response of the different cells of the detector and were circularly averaged about the beam direction. The buffer scattering was subtracted. The scattering curves were normalized to the incoherent scattering of water, I_{inc}, by dividing by the scattering of a 1 mm thick sample of H_2O. These calculations were done according to standard programs (Ghosh, 1981). The resulting curves were plotted as ln $I(Q)$ versus Q^2 (Guinier plot) from which are obtained the radius of gyration and the intensity at zero angle, I_0.

For one sample of both Triton and the Triton-solubilized PSI complex, scattering curves were measured as a function of concentration to check for interference effects. For Triton, experiments were performed at 10, 5, and 2 mg/mL and for PSI–Triton complexes at protein concentrations of 2, 1.1, and 0.6 mg/mL. In neither case did the radius of gyration or the concentration-normalized intensity at zero angle vary with concentration. It was therefore concluded that interference effects were insignificant, and subsequent experiments were carried out at 10 mg/mL for Triton alone and at 2 mg/mL for the PSI complex.

Results

Purification of PSI Particles. During the development of the purification steps care was taken to retain the activity of the PSI complex.

According to the procedures described under Materials and Methods, solubilization of PSI-enriched membrane fractions and IEF of the extract were performed.

PSI particles were solubilized with 3% SB12 and focused in the Rotofor cell in the presence of 0.3% SB12. The main fraction focused between pH 4.6 and pH 5.2. These particles were inactive; i.e., a P_{700}^+ minus P_{700} difference spectrum could not be obtained. Similar results were obtained when 0.2% SDS was present in IEF after solubilization of the PSI particles with 2% SDS.

PSI particles were also solubilized with 1.5% (w/v) Triton X-100. The following observations were made: when 0.3–1% (w/v) Triton was present in the IEF cell, a slight inactivation of PSI occurs, indicated by a relatively high ratio of Chl/P_{700} of 250 and a concomitant shift of the chlorophyll band from 678 to 670 nm (see Figure 1). The main PSI fraction focused between pH 4.6 and pH 4.8. If the Triton X-100 concentration was reduced to 0.2%, a PSI particle with an IEP between pH 4.2 and pH 4.4, and a chlorophyll maximum at 678 nm was observed (see Figure 1). The ratio of Chl/P_{700} was 140.

ES12H and the ether form of this detergent (ET12H) and DM also kept the PSI complex redox titratable.

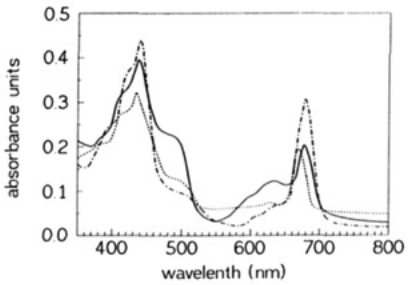

FIGURE 1: Spectra of the PSI-enriched thylakoid pellet (solid line) as well as of the main PSI fraction obtained by IEF in presence of 0.2% (w/v) Triton X-100 (dashed-dotted line) and of more than 0.3% (w/v) Triton X-100 (dotted line). A blue shift at higher Triton X-100 concentrations is seen. The spectrum of the PSI preparation obtained by gradient elution from Q-Sepharose is identical with the one obtained by IEF in the presence of 0.2% (w/v) Triton X-100.

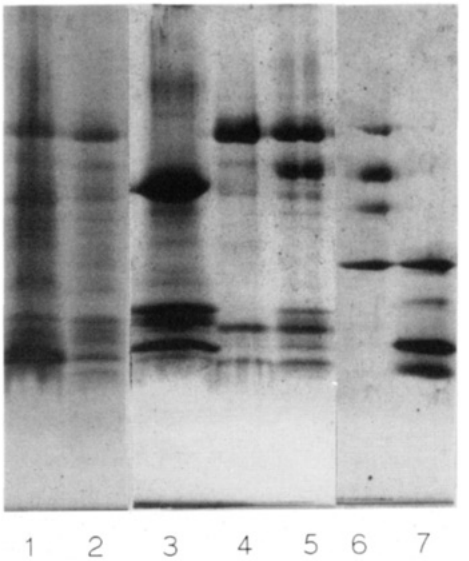

FIGURE 2: SDS–PAGE analysis of PSI particles at different stages of the purification: lane 1, PSI-enriched thylakoid pellets before isoelectric focusing; lane 2, greenish main fraction obtained by focusing in the presence of Triton X-100; lane 3, blue fraction obtained by washing of the Q-Sepharose column with buffer A with 4.8% (w/v) sodium cholate added; lane 4, PSI particles eluting from the Q-Sepharose column at 100 mM $MgSO_4$ after washing in the aforementioned way; lane 5, PSI particles eluting at 100 mM $MgSO_4$ after washing with buffer A with 0.06% (w/v) SDS added; lanes 6 and 7, marker proteins (molecular masses, from top to bottom: 92 500, 68 000, 45 000, 29 000, 21 000, 12 500, and 6500 Da).

SDS–PAGE analysis indicated that the samples obtained after solubilization and focusing with Triton X-100 were of higher purity than those obtained by use of ES12H, ET12H, and DM. This detergent was used therefore for IEF of PSI particles.

In SDS–PAGE, major polypeptide bands in the range of 110, 15, and 10 kDa, which are known from other PSI preparations, are seen (see Figure 2). Additional contaminating polypeptides are seen in the range of 20–40 kDa.

Anion-exchange chromatography was used as a further purification step of PSI material obtained by focusing in Triton X-100, as described under Materials and Methods. After the material was adsorbed to Q-Sepharose, the column was eluted with buffers containing different detergents. Three detergents were tried: the two ionic detergents SDS and sodium cholate and the zwitterion sulfobetaine (SB12).

The amount of SDS in elution buffer A was found to be critical for retaining the solubility of the PSI particles. If the concentration of SDS was higher than 0.05%–0.1%, either the elution of the particles in the $MgSO_4$ gradient was retarded,

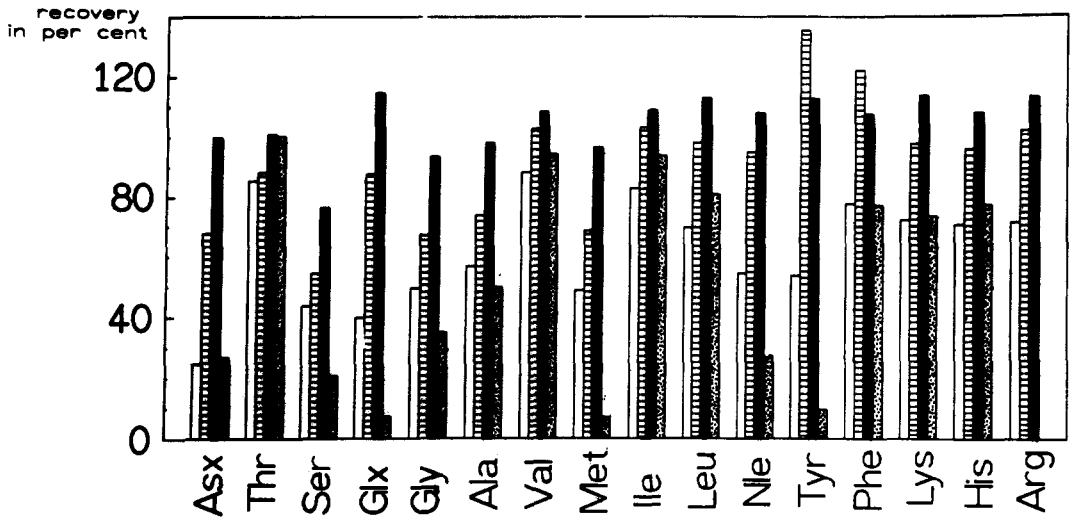

FIGURE 3: Recovery of amino acids of a standard amino acid mixture after treatment under hydrolysis conditions and quantitative analysis by chromatography as described under Material and Methods: bar 1 (empty), hydrolysis in the presence of HCl pro analysi and Tris; bar 2 (hatched), hydrolysis in the presence of HCl, Suprapur grade and Tris; bar 3 (solid), hydrolysis in the presence of HCl Suprapur; bar 4 (stippled), hydrolysis in the presence of HCl Suprapur and glycerol.

or they did not elute at all. At a concentration of 0.05% SDS, a Chl/P_{700} ratio of 40 was found.

If 0.6% SB12 was used in the elution buffer, the purity was not as good as that with SDS, as seen from the polypeptide pattern. When 4.8% sodium cholate was used in the elution buffer, the PSI particles eluted at 100 mM $MgSO_4$, and the purity of PSI was superior to that of other preparations. Figure 2, lane 4, shows three main bands of 110, 15, and 10 kDa. Bruce and Malkin (1988) report the disappearance of a 110-kDa band by solubilization at elevated temperature. We never observed the individual 65-kDa band when heating the PSI particles before application to the gel. In addition to the three main bands, some contamination of other bands is seen.

The P_{700} concentrations for SDS-washed and sodium cholate washed PSI particles were determined by the redoxtitration method as described under Materials and Methods. For cholate-washed particles, the Chl/P_{700} ratio has a mean value of 60 with a standard deviation of about 20. For two samples, which had been used for SANS before, the Chl/P_{700} ratio was determined by redoxtitration and by kinetic measurement of the initial absorption change at 820 nm, using $\Delta\epsilon$ of 6500 M^{-1} cm^{-1}. Similar values for the ratio of 64 and of 63 were obtained, respectively.

Henceforth, we used the following scheme for purification of PSI particles. The PSI-enriched pellets were treated with Triton X-100, at a detergent to chlorophyll weight ratio of 30 and placed in the IEF cell. The concentration of Triton X-100 was adjusted to 0.2%. The main band focused at a pH of 4.3. For the final purification step, the anion-exchange chromatography column was equilibrated with 0.03% Triton X-100 in buffer A and loaded with the dialyzed PSI material. The adsorbed protein on the column was washed with 4.8% sodium cholate in buffer A and subsequently eluted with a linear $MgSO_4$ gradient (0–250 mM). Highly active and pure PSI eluted at 100 mM $MgSO_4$.

The yields of protein after focusing and after DEAE chromatography were approximately 50 and 25%, respectively, of the amount of protein of the PSI-enriched membranes used at the beginning.

Upon storage at 4 °C for 4 weeks, the PSI particles showed a slow decrease in the initial rise of the absorption change at 820 nm (not shown), indicating a high stability.

Electron micrographs of negatively stained PSI particles showed particles of ellipsoidal shape. The long and the short axes of 20 particles were measured, and mean values of 14.8 and 9.1 nm, respectively, were obtained.

Quantitative Amino Acid Analysis. The calculation of molecular mass from SANS measurements requires knowledge of the protein concentration. The error in the calculated mass thus is proportional to the error of the concentration determination. The Lowry assay as well as other staining assays is known to yield results that deviate from the true concentration in a manner depending upon the nature of the protein. We therefore determined the amino acid composition quantitatively in the protein hydrolysate. In preliminary experiments we found that certain conditions during the hydrolysis of the protein cause a low recovery of the amino acids in the hydrolysate. Thus in some experiments with PSI material we found only 60% of the amount of norleucine that was originally added to the protein samples as an internal standard. In order to find the reasons for this poor recovery, control experiments were made with an amino acid standard mixture dissolved in various buffers. These solutions were treated identically with the protein solutions as described under Materials and Methods and were hydrolyzed at 110 °C for 24 h. The recovery [100 × (quantity found after hydrolysis)/(quantity originally added)] was calculated.

Figure 3, bar 1, shows the result for amino acids dissolved in 50 mM Tris-HCl, pH 8, 3 mM NaN_3, and p.a. grade hydrochloric acid. The recovery is typically around 50%. This value increased to some 90% when Suprapur-grade in place of p.a.-grade HCl was used (see Figure 3, bar 2). When Tris was absent, the recovery was approximately 100% for each amino acid (see Figure 3, bar 3). The mechanisms causing these losses are unknown.

When a solution of norleucine was subjected to hydrolysis conditions in the presence and absence of Tris, we found that Tris caused a peak eluting after norleucine. The area of this peak correlated strongly with the quantity of Tris buffer in the sample. Consequently, there are no nonsystematic losses of material during the various steps of the hydrolysis and analysis procedure.

In a further experiment with Suprapur-grade HCl and Tris buffer, we found that the presence of glycerol during hydrolysis reduces the recovery again to some 25% (see Figure 3, bar 4). This showed the necessity of removing glycerol before hydrolysis.

In an independent experiment we tested whether glycerol

Table I: Amino Acid Composition (mol %) of PSI Particles

	barley[a]	spinach[b]	tobacco[c]	oat[c]	beet[c]	phormidium[c]	*S. elongatus*[d]
Asx	7.8	7.7		7.6	8.4	8.8	7.4
Thr	6.1	5.6	4.2	4.5	5.2	5.9	7.0
Ser	6.1	6.5	4.4	3.5	5.5	5.9	3.9
Glx	6.8	6.9	6.6	6.6	7.8	6.5	6.9
Gly	10.6	12.6	10.1	9.9	11.0	10.0	9.2
Ala	10.5	9.8	9.0	9.2	9.8	10.0	10.3
Cys	1.1				0.3	0.9	"0.9"
Val	4.5	4.7	5.6	4.8	6.3	6.2	7.8
Met	1.4	3.3			1.4	1.9	1.8
Ile	8.6	5.4	6.2	6.6	6.5	6.2	6.7
Leu	14.6	11.3	10.6	10.6	11.4	11.2	11.9
Tyr	2.6	3.0	1.8	2.2	2.7	3.1	3.5
Phe	7.2	6.1	6.8	6.2	6.8	6.2	6.8
Lys	1.6	3.0	2.5	2.7	2.9	3.4	3.8
His	3.4	5.2	3.4	3.6	5.9	4.0	2.7
Arg	3.5	4.0	2.4	3.3	3.6	3.4	5.1
Pro	3.6	4.9	4.9	3.6	4.2	4.7	"4.8"

[a] Taken from Vierling and Alberte (1983). [b] Taken from Setif et al. (1980). [c] Taken from Thornber (1979). [d] This study.

might interfere with the performance of the chromatographic separation and the monitoring of the fluorescence assay. The standard amino acid mixture was chromatographed with and without glycerol (20% v/v) present in the elution buffers. The peak areas for the amino acids were found to be the same as before. Hence, the decrease of the recovery is due to the presence of glycerol during the hydrolysis procedure. As the presence of sugars during hydrolysis of proteins is known to interfere with the quantitative amino acid analysis, we think that the effect of glycerol may be of a similar nature.

To remove glycerol before the hydrolysis, we extracted the pelleted protein with a mixture of ether and ethanol as described under Materials and Methods. As a further benefit the extraction also removes lipids, pigments, and Tris, the latter causing a reduction in the recovery. On the other hand, the extraction mixture dissolves a small fraction, approximately 10%, of the protein as well, as can be shown by the Coomassie assay. A corresponding correction has to be applied to the result.

Two samples (typically 100 μL) of each of the PSI protein solutions in 0, 14, 40, 60, and 80% (v/v) D_2O, which had been used for the SANS measurement, were extracted, hydrolyzed, and analyzed as described under Materials and Methods for 24, 48, and 96 h. The quantities of individual amino acids did not change with the length of time of hydrolysis. The recovery of norleucine, which was added as an internal standard prior to the hydrolysis procedure, was 91% with a standard deviation of 5% of the 10 samples analyzed. It is therefore assumed that a loss of 10% of all amino acids occurred by an unknown process. The amounts of all amino acids therefore were corrected for the loss of norleucine. Table I presents the amino acid composition found by us with previously reported data from other species. As there is no fluorescence for Pro and a low one for Cys, we assumed a content of 0.9 and 4.8 mol % for Cys and for Pro, respectively, by comparison with data from other PSI preparations given in Table I. In order to include the contribution of these amino acids, we corrected the sum of the concentrations of the amino acids by a factor of 1.09. The total PSI weight can be estimated by including the chlorophylls. Taking the weight ratio of protein to chlorophyll as 4.66, a further correction of the weight by a factor of 1.21 was applied. The resulting concentrations correlate well with the values obtained with the Bio-Rad assay, the former being 4% higher than the latter.

Small-Angle Neutron Diffraction. (A) SANS of Triton X-100 Solutions. The scattering-length density of Triton X-100 can be calculated from its average molecular formula and published partial molar volume. This leads to a scattering-length density of 0.573×10^{-4} cm Å^{-3} in H_2O and 0.676×10^{-14} cm Å^{-3} in D_2O, the difference being due to the single exchangeable proton. From this we calculate that Triton X-100 would be contrast matched in a solution containing 16.5% (v/v) D_2O/H_2O in good agreement with an experimental value of 17% (w/v) given by Wise and Karlin (1979). In our experiments, however, the buffer contained 20% (v/v) glycerol. The scattering-length density of a H_2O/20% (v/v) glycerol solution and a D_2O/20% (v/v) glycerol solution can be calculated to be -0.327×10^{-14} cm Å^{-3} in H_2O and 5.249×10^{-14} cm Å^{-3} in D_2O, giving a matchpoint of 13.1% (v/v) D_2O.

The scattering curves for the Triton X-100 solutions were plotted as Guinier plots (not shown) from which were obtained the radii of gyration and the intensity at zero angle. The variation of $\sqrt{I_0}$ with D_2O content of the solvent yielded a matchpoint of 12.6% in good agreement with the value calculated above. The radii of gyration clearly do not vary greatly with contrast, having values varying between 30 and 32 Å. A more quantitative interpretation of these data is precluded by the slightly curved nature of the plots arising probably from slight aggregation and the polydispersity of the detergent. The micelle molecular weight and hence aggregation number may be calculated from the intensity at zero angle. This is done most precisely from a sample in H_2O where any error in the knowledge of the partial specific volume is minimized (Jacrot & Zaccai, 1981). For Triton X-100, using a calculated scattering-length density in H_2O of 0.573×10^{-14} cm Å^{-3}, we find a micelle molecular weight of 57.3×10^3 corresponding to an aggregation number of 89. This value is very close to that found by Wise et al. (1979) although the R_g is significantly larger, indicating a much more elongated micelle. Such differences could be due to the presence of glycerol in our experiments and/or the fact that our measurements were made at 16 °C rather than at 8 °C as for Wise et al. (1979).

(B) SANS of PSI Particles. The aim of these measurements was to determine in situ the molecular mass of the protein moiety by measuring its forward scattering [$I(0)$] at the matchpoint of the detergent. In principle, this can be done by a single measurement in the H_2O/D_2O mixture at which the Triton X-100 is contrast matched. We preferred, however, to interpolate these data from a contrast variation series in order to minimize the errors due to inexact determination of this match point.

Scattering curves for PSI/Triton X-100 micelles obtained at x = 0.0, 0.14, 0.4, 0.6, and 0.8 mole fraction of D_2O are

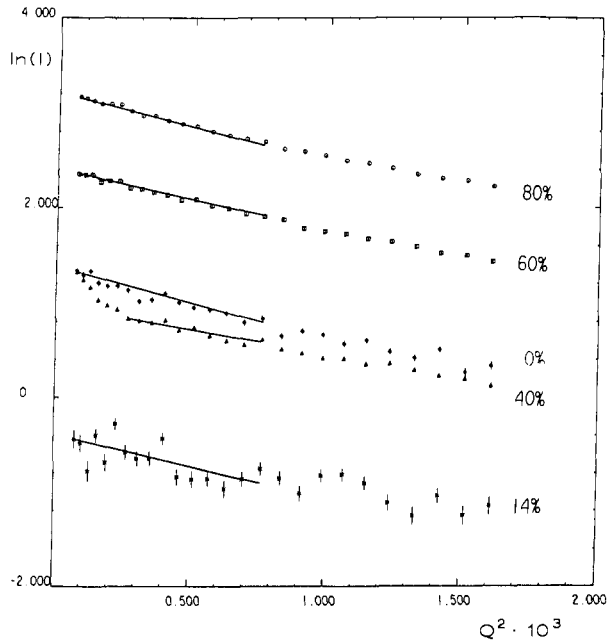

FIGURE 4: Guinier plots [ln I vs $Q^2 \times 10^3$, where $Q = 4\pi(\sin\theta/\lambda)$ is given in units of $Å^{-1}$ and 2θ is the scattering angle] of PSI–Triton X-100 complexes as a function of the D_2O content of the buffer (shown alongside each curve). The curves are all normalized to unit concentration, sample thickness, and neutron transmission and are relative to the scattering of a 1 mm thick sample of H_2O. The sample in 40% (v/v) D_2O shows significant aggregation, and the first six points were, therefore, not included in the fitting of the least-squares straight line.

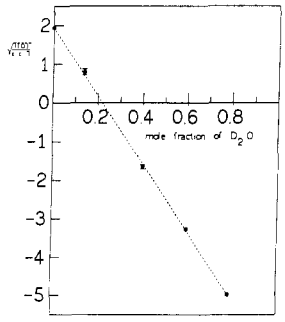

FIGURE 5: Plot of $\sqrt{I_0}$ normalized to sample concentration, thickness, and transmission versus D_2O mole fraction. This plot yields the matchpoint of the PSI–Triton X-100 complexes and hence the protein to detergent stoichiometry.

shown in Figure 4 from which were obtained $I(0)$ and R_g. Figure 5 shows $\sqrt{I_0}$ (normalized to unit concentration, thickness, and neutron transmission) as a function of x. This plot is linear as expected for a monodisperse solution. From this plot we find for $x = 0.126$ (where the Triton scattering is matched) $I_0/c = 0.038$.

Calculation of the Molecular Mass. The molecular mass M_r of a protein complex is related to the normalized neutron scattering intensity at zero angle by the following formula (Jacrot & Zaccai, 1981):

$$\frac{I(0)}{I_{inc}(0)} = f\frac{4\pi T_S}{1 - T_W}CN_A t \times 10^{-3}\left(\frac{1}{M_r}\sum b - \rho_s V\right)^2 M_r \qquad (1)$$

f is a wavelength correction factor that is 1.0 for $\lambda = 10$ Å. T_S and T_W are the transmissions of the sample (=0.502) and of water (=0.46), respectively. C is the protein concentration of the sample (in mg/mL). N_A and t are Avogadro's number and the sample cell thickness in cm, respectively. $\sum b$ is the sum of the scattering lengths of all amino acids, ρ_s is the scattering-length density of the surrounding buffer, and V is the volume of the protein complex. $(1/M_r)(\sum b - \rho_s V)$ is the excess scattering length per molecular weight unit of the protein complex over the surrounding solution. It is calculated by forming the product of the excess scattering-length density of the protein complex $\sum b/V - \rho_s$ and of $\bar{V}/N_A$. Volumes and scattering lengths of the amino acids in D_2O and H_2O were taken from Jacrot (1976) and Jacrot and Zaccai (1981). The scattering-length density of a chlorophyll molecule was approximated as the sum of the corresponding fractions of scattering length and volume for ethylchlorophyllide *a* and phytol. From crystal data of Chow et al. (1975), we calculated a volume of 803.8 $Å^3$ for the former. The scattering length was calculated from the sum formula $C_{37}H_{37}O_7N_4$ and the atomic scattering lengths published by Bacon (1975). The scattering length of phytol was obtained analogously as above; the volume was calculated from its known density and molecular weight (*Merck Index*, 9th ed.) to be 579.5 $Å^3$.

With the known amino acid composition and the known chlorophyll/protein weight ratio, the scattering-length density of the PSI complex as a function of the D_2O content x can be calculated. The result is

$$\frac{\sum b}{V}(\text{PSI complex})\ (10^{-12}\ \text{cm/Å}^3) = 0.016\,249 + x0.009\,606 \qquad (2)$$

The scattering-length density of the surrounding buffer solution ρ can be approximated by a $D_2O/H_2O/20\%$ glycerol (v/v) solution and was calculated from the known scattering lengths and volumes of water, D_2O (Jacrot, 1976), and glycerol:

$$\rho(D_2O/H_2O/20\%\ \text{glycerol})\ (10^{-12}\ \text{cm/Å}^3) = -0.003\,27 + x0.069\,70 \qquad (3)$$

where $0 \le x \le 0.8$. With the known amino acid composition and the known chlorophyll/protein weight ratio, $\bar{V}/N_A$ was calculated and found to be 1.319 $Å^3$ per mole fraction unit.

The excess scattering length per mass unit at the matchpoint found for Triton X-100, $x = 0.126$, follows from (2) and (3):

$$0.015\,76\ (10^{-12}\ \text{cm/mass unit})$$

With I_0 and C determined as given above, the molecular mass can be calculated to be 217 kDa.

We recalculated the molecular mass after making several error assumptions in order to illustrate their influence on the end result. If there is only 75% deuteration of the exchangeable hydrogens of the protein instead of 100%, we get 228 000 Da. If 10% of the protein was lost during the extraction procedure, the molecular mass would be 195 000 Da. If 10% of the sample was aggregated to trimers or bigger oligomers, the molecular weight would be 241 000. We also tested the influence of the amino acid composition of those amino acids with the largest contribution to the scattering length. If Asx, Glx, and Thr would be present at concentrations lower by 2% while Ala, Ile, and Phe would be present at concentrations higher by 2% as compared to the values found by amino acid analysis, the result would be 231 000 Da. The molecular mass thus could be in error by up to ±30 kDa from the value given above.

Figure 5 also contains information on the bound detergent from the point at which the complete protein–Triton complex is contrast matched. This is found to be for a D_2O mole fraction of 0.225 corresponding to a scattering length density of 0.0124×10^{-14} cm/$Å^3$. From the scattering densities of protein and detergent and their corresponding partial specific

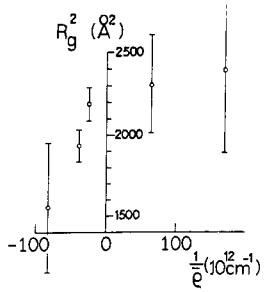

FIGURE 6: Radii of gyration of PSI–Triton X-100 mixed micelles represented as a Stuhrmann plot (R_g^2 versus $1/\bar{\rho}$).

volumes, we find that the complex consists of 44% protein and 56% Triton X-100. The mass of the Triton bound per PSI monomer therefore is approximately 276 000, which corresponds to about 430 molecules of Triton bound per PSI monomer. Errors in the matchpoint determination lead to an uncertainty of about 10% in these values.

The radii of gyration obtained as a function of contrast are displayed as a Stuhrmann plot (R_g^2 vs inverse contrast) in Figure 6. The radius of gyration of ~48 Å at low detergent contrast is indicative of the size of the protein alone but must be interpreted with caution as inhomogeneities in the Triton scattering density could influence this value. However, it is consistent with an ellipsoidal cylinder of dimensions of 148 Å (length) × 60 Å × 91 Å (cross section) from electron microscopy as described by Ford et al. (1987) for PSI preparations. The error bars in this plot are, however, such that no firm conclusions may be drawn concerning the relative dispositions of protein and detergent. A globular protein surrounded by a detergent belt having a common center of mass would show a straight line of negative slope whereas asymmetrically disposed detergent would produce a parabolic curve. The situation is complicated by the presence of large amounts of chlorophyll, which we expect to be situated within the protein and which have a contrast behavior similar to detergent. At low solvent-scattering density the complex would therefore have a somewhat "Swiss cheese" appearance. Interpretation of the contrast variation is further complicated by the presence of 20% glycerol, which, if preferentially excluded from certain areas (e.g., a hydration shell), can substantially alter the radius of gyration at high D_2O contents. This reservation does not concern conclusions based on the data at low D_2O concentrations (i.e., molecular weight determination), although it could lead to an overestimation of the bound detergent.

Discussion

A purification scheme for PSI has been developed. Separation of PSI and PSII is achieved in two steps, by an extraction procedure using the zwitterionic detergent SB12, according to Schatz and Witt (1984), and by preparative isoelectric focusing in the presence of Triton X-100. We consider the new method advantageous, as it does not need the lengthy use of a density gradient ultracentrifugation that is used in the majority of previously published methods. In the presence of Triton X-100 at a concentration of 0.2%, the PSI particles remained redox titratable and focused at pH 4.3. In the presence of higher Triton concentrations or of ionic detergents like SB12 or SDS, the isoelectric point shifted to 4.7, and there were indications of inactivation of PSI. Satoh and Butler (1978) reported a similar isoelectric point for their F_1 band in a preparative isoelectric column. The PSI particles so prepared have a reduced carotenoid content, are free of most phycobilisomes, but are contaminated with several polypeptides with apparent molecular masses between 20 and 25 kDa. We thus continue the purification by anion-exchange chromatography. When the column was eluted with a sodium cholate containing buffer, the contaminating polypeptides were found in the eluate. Concomitantly, the ratio of Chl/P_{700} decreases. Our method is similar to one reported by Boekema et al. (1987), who use the extraction step, a density gradient fractionation, and anion-exchange chromatography, but without the preparative focusing and the washing step.

As we observed a stable charge separation reaction, we suppose that one of the two bands of 15- and 10-kDa apparent molecular mass represents the iron–sulfur-rich F_A–F_B binding polypeptide (Hoj et al., 1987; Wynn & Malkin, 1988; Golbeck et al., 1988). We observed a slow decay of the P_{700}^+ signal in the presence of ascorbate, TMPD, and benzylviologen, which seems to be a general feature of all highly purified PSI particles (Ratajczak et al., 1988). As the polypeptide that was reported to be involved in plastocyanin docking can be stripped off under conditions similar to the ones used by us (Wynn & Malkin, 1988), we suppose that it is missing in our preparation.

We calculated the molecular mass from the zero-angle neutron scattering intensity. Other methods were excluded as they either require radioactive detergents or dialyzable detergents. Triton X-100, which we used for solubilization of PSI, is known to dialyze exceedingly slowly (Robinson & Tanford, 1975).

In order to calculate the molecular mass of the PSI particle, a precise determination of the protein concentration was necessary. Chemical protein assays, as, e.g., the Lowry method or the Coomassie method, are generally considered to be imprecise for an absolute concentration determination. We therefore used the quantitative determination of amino acids after hydrolysis of the protein. Before this method could be applied, it turned out to be necessary to elaborate hydrolysis conditions which minimize the loss of amino acids. Use of the purest hydrochloric acid was found to be essential. Furthermore, the presence of glycerol and to a minor extent of Tris was found to decrease the recovery of the amino acids. The samples therefore were extracted with ether/ethanol, which removed glycerol, Tris, and pigments from the protein. After hydrolysis of the samples, norleucine, which was added as an internal standard, was recovered typically to 95%. The loss of protein during the extraction procedure was shown to be smaller than 10% of the amount used. When protein concentrations determined by quantitative amino acid analysis, the Coomassie assay, and the Lowry assay were compared, the first two values agreed within the percent range, while the last one was larger by up to 30%. Control experiments showed that chlorophyll gives a strong reaction in the Lowry assay, while Coomassie does not (unpublished).

The zero-angle neutron scattering intensity was determined by Guinier extrapolation. Together with the known protein concentration and the Chl to protein weight ratio, the molecular mass of the PSI particle without bound Triton X-100 was calculated to be 217 000 Da. Error considerations in the determination of the protein concentration indicated that the true mass may deviate by up to 30 000 Da from this value.

The P_{700} content was determined by redox titration and by the kinetic method, and both methods gave identical values. The Chl/P_{700} ratio thus was found to be 60. The protein to chlorophyll weight ratio was found to be 4.66. Assuming one P_{700} per particle and all P_{700} to be redox titratable, a molecular mass of 305 000 Da can be calculated. The difference of the latter value and the molecular mass calculated with the SANS data indicates that some 30% of all P_{700} centers are unti-

tratable. This could be due to partial inactivation of the complexes during the purification process. The true Chl/P_{700} ratio thus would be 40.

Fish et al. (1985) calculated molecular masses of 83 200 and 82 500 Da for the psaA and psaB gene products, respectively. According to Fish and Bogorad (1986), both polypeptides are present in a 1:1 stoichiometry, resulting in a molecular mass of some 165 000 Da for the heterodimer. According to several studies (Lundell et al., 1985; Bruce & Malkin, 1988; Nechustai & Nelson, 1981), the smallest unit of the PSI complex contains one copy of the heterodimer as well as one copy of the 20- and the 10-kDa polypeptide. Due to the presence of some 40 chlorophyll molecules, the smallest possible molecular mass thus should be approximately 230 000 Da. As the psaA and psaB gene products may be processed before the assembly of the PSI complex and as the apparent molecular masses for the 20- and 10-kDa polypeptide may be overestimated, this value may be an overestimation of the true molecular weight. This value is within the error estimation of our zero-angle neutron scattering calculation. We thus conclude that the PSI particles represent a smallest PSI particle containing one copy of each of the polypeptides. Our results also indicate that this particle posesses one P_{700} which is capable of transferring electrons to the F_A–F_B iron–sulfur acceptor complex.

Boekema et al. (1987) have isolated a PSI particle from *Synechococcus* sp. and estimated the molecular mass to be approximately 600 000 Da by gel filtration. Using electron microscopic imaging techniques, they find a trimeric arrangement. In the average structure, the monomers appear as elliptic cylinders with semiaxes of 13.6 and 6.8 nm and a height of 6 nm. Using the formula for the radius of gyration of an elliptic cylinder (Burchard, 1983) a value can be calculated, which is in accord with the R_G value of 4 nm found by SANS at the Triton X-100 matchpoint. Ford and Holzenburg (1988) find 14.8 and 9.1 nm for the semiaxes of their PSI particles, which they assume to be monomers. Our molecular weight determination corroberates this assumption as we find particles in electron micrographs of similar size as the monomers reported in the two above-mentioned studies.

Ford (1987) found apparent molecular masses of 105 000, 150 000, and 450 000 Da in SDS–PAGE. Only the latter two particles contain the small molecular weight proteins as well. Ford assumes that the 450-kDa particle is a trimer and the 150-kDa particle is a monomer. The low apparent molecular mass of the monomer (150 000 Da) thus suggests that its electrophoretic mobility is higher than would correspond to its true molecular weight. Moreover our results show that these monomers can be purified in a functionally active form.

Other molecular weight calculations, based on the combination of chlorophyll, P_{700}, and quantitative amino acid determination by Vierling and Alberte (1983) and by Lundell et al. (1985), result in molecular masses of 350 000 and 460 000 Da, respectively. This may also be due to a loss of the redox titratability of P_{700} during the purification process.

The Chl/P_{700} ratios for PSI particles found in the literature show a surprising variation (between 10 and 120). We found ratios of 40 for SDS-treated particles and of 60 for sodium cholate treated particles by redox titration. These high values may be partly due to inactivation of P_{700} and partly due to a tendency of the PSI complex itself toward loss of its antenna chlorophylls.

Our results as well as work by Bruce and Malkin (1988) disfavor models (Lundell et al., 1985) in which more than two of the 65-kDa polypeptides are aggregated to form the active charge separating the PSI reaction center complex. A heterodimer of two 65-kDa polypeptides binding P700, A0, A1, and Fe–X, which follows from our mass determination, may indicate a general construction principle of all reaction centers of purple bacteria, green sulfur bacteria (Nitschke et al, 1987), cyanobacteria, algae, and green plants: the primary donor and the early electron acceptors are held in a defined spacial position with respect to each other in a heterodimer of two homologous polypeptides.

Acknowledgments

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