The Molar Mass of an Active Photosystem I Complex from the Cyanobacterium *Synechococcus* PCC 7002

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The molar mass of photosystem I (PS I) from the cyanobacterium *Synechococcus* PCC 7002 was determined by sedimentation equilibrium analysis in the analytical ultracentrifuge. For that purpose, the "trimeric" form of PS I was isolated and studied in three different nonionic detergents. After determining the partial specific volume of the protein/pigment complex, from the ultracentrifuge data, as (0.788 ± 0.010) ml g^{-1}, M_r was obtained as $830{,}000 \pm 60{,}000$.

Photosystem I (PS I) is a multiprotein complex, which mediates light-driven electron transfer from plastocyanin or cytochrome c_{553} to ferredoxin. The PS I complex is located in the thylakoid membrane of higher plants, green algae and cyanobacteria (Goldbeck and Bryant, 1991). Active "monomeric" and "trimeric" forms of the cyanobacterial PS I have been isolated by using nonionic detergents for the solubilization of the thylakoid membrane (Rögner *et al.*, 1990a, b; Tsiotis *et al.*, 1993). The isolated "monomeric" PS I contains two protein subunits of about 83 kDa, to which 60–100 chlorophyll *a* (Chl *a*) molecules are bound. In addition, the PS I complex contains a series of other subunits with molar masses below 20 kDa (Goldbeck and Bryant, 1991). The molar mass of "monomeric" and "trimeric" PS I was estimated by electron microscopy and measurements of the apparent Stokes radius by HPLC gel filtration and was found to be around 250–300 kDa for the "monomeric" and 700–750 kDa for the "trimeric" form (Rögner *et al.*, 1990a, b; Tsiotis *et al.*, 1993).

It is well-known that the methods used for the molar mass determination of the cyanobacterial PS I complex are based on assumptions which may not be fulfilled (Boonstra *et al.*, 1993; Tanford and Reynolds, 1976). We have, therefore, performed a molar mass determination by a rigorous thermodynamic method, sedimentation equilibrium analysis in the analytical ultracentrifuge. The most critical parameter in the analysis, the partial specific volume $\bar{v}$ of the protein/pigment complex, was determined by a recently described adaptation of a method of Edelstein and Schachman (1967) to intrinsic membrane proteins in detergent solutions (Schubert *et al.*, 1994). In the procedure, which for the first time allows a reliable experimental $\bar{v}$-determination of intrinsic membrane proteins, data obtained in three different detergents were combined, to yield simultaneously both M and $\bar{v}$ of the complex. The system studied was the active trimeric form of PS I from a phycobilisome-less mutant of the cyanobacterium *Synechococcus* PCC 7002 (Tsiotis *et al.*, 1993).

Trimeric PS I was isolated as described by Tsiotis *et al.* (1993). The final purification step was HPLC gel filtration on a TSK 4000 SW column (Toso Haas) equilibrated with 20 mM MES, 10 mM $MgCl_2$, 10 mM $CaCl_2$, 0.05% lauryl-β-D-maltoside ($C_{12}M$). Detergent exchange against 0.2% nonaethyleneglycol lauryl ether ($C_{12}E_9$; Sigma) or 0.1% of the hydrogenated form of Triton X-100 (TX; Aldrich) was performed by an additional gel filtration step using the same column. The peak position during elution of the protein was virtually unaffected by the detergent exchange, which indi-

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cates that the protein complex was stable in the detergents used. For the ultracentrifuge experiments, the samples were brought to the required concentrations of D_2O and $D_2{}^{18}O$ (Edelstein and Schachman, 1967; Schubert *et al.*, 1994). In addition, appropriate buffer stock solutions were added to restore the detergent and salt concentrations given above plus 50 mM NaCl. Final protein concentration varied between 2.8 and 8.0 µg/ml.

Sedimentation equilibrium experiments on the purified PS I were performed in a Beckman Optima XL-A analytical ultracentrifuge, using 6-channel centerpieces. Rotor speed was 8000 to 10000 rpm, and rotor temperature 6 °C. The concentration distributions were recorded at 422 nm. The samples contained either $C_{12}M$, $C_{12}E_9$ or TX. The parameter to be determined was the effective molar mass of the protein/pigment complex, $M_{eff} = M(1-\bar{\nu}\varrho_o)$, at that solvent density ϱ_o which equals the density ϱ_d of the corresponding detergent (Schubert and Schuck, 1991; Schubert *et al.*, 1994; Tanford and Reynolds, 1976). In the case of $C_{12}M$, extrapolation of the M_{eff}-values measured at different values of ϱ_o to the density ϱ_d had to be performed (Schubert *et al.*, 1994). With the latter two detergents, ϱ_o could be adjusted to ϱ_d, thus avoiding the need for density variation series (Schubert and Schuck, 1991; Schubert *et al.*, 1994; Tanford and Reynolds, 1976).

In most of the experiments, the absorbance-versus-radius distributions obtained could be fitted by a single exponential, which indicates homogeniety of particle mass. However, with a few samples which had been stored frozen, the presence of an additional component of much lower molar mass was apparent from the fits. This component, with a typical Chl *a*-spectrum, obviously represented a degradation product. Data obtained with the latter samples were not considered in the evaluations following.

In the sedimentation equilibrium experiments, the effective molar mass of PS I in solutions of $C_{12}E_9$ und TX, at the density of the respective detergents ($\varrho(C_{12}E_9) = 1.059$ g ml^{-1} (Schubert *et al.*, 1994), ϱ(TX) = 1.085 g ml^{-1}*) was found to be 140,000 ± 2,000 ($C_{12}E_9$; n = 6) and 122,000 ± 3,000 (TX; n = 5). The data obtained in solutions of $C_{12}M$, at different solvent densities ϱ_o, are shown in Fig. 1; M_{eff} extrapolated to the density of the detergent ($\varrho_d = 1.229$ g ml^{-1} (Schubert *et al.*, 1994)) is 40,000 ± 5,000. The M_{eff}-values at the respective detergent densities are plotted, as a function of ϱ_o, in Fig. 2. The two figures yield approximate values

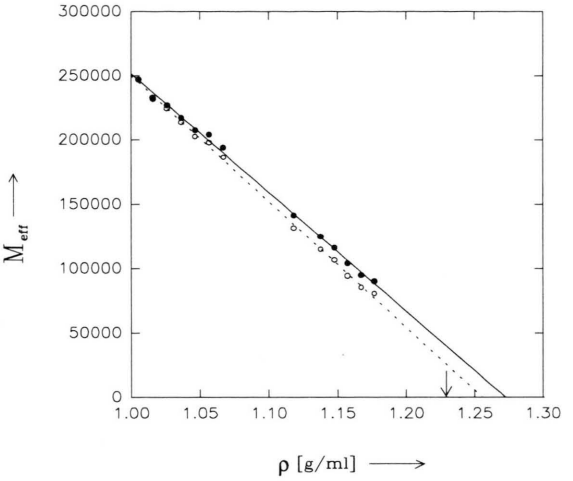

Fig. 1. PS I in solutions of $C_{12}M$: Dependency of M_{eff} on solvent density. (●) Uncorrected data; (○) data corrected for H-D exchange. The arrow indicates the buoyant density of $C_{12}M$.

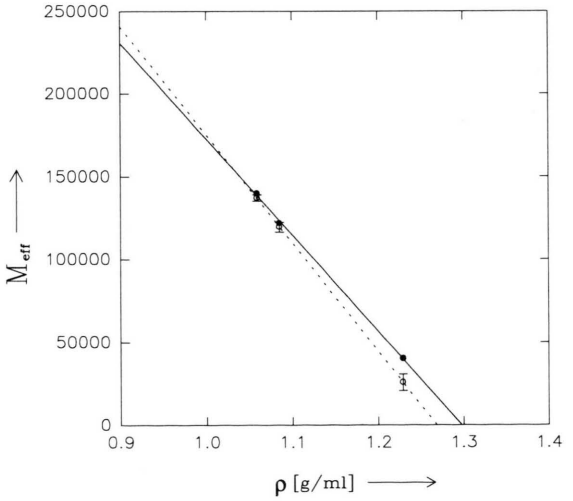

Fig. 2. Dependency of the effective molar mass of PS I on solvent density, under the conditions of density matching. (●) Uncorrected data; (○) data corrected for H-D exchange.

* The density of the hydrogenated form of TX, used in this study, was found to be distinctly lower than that of the unsubstituted form ($\varrho = 1.115$ g ml^{-1}).

(uncorrected for H-D exchange) for the molar mass of the protein/pigment and of the protein/pigment/$C_{12}M$ complex and thus also for the relative amount of PS I-bound $C_{12}M$. Applying these data, corrections for H-D exchange in the protein and in the protein-bound $C_{12}M$ were calculated, assuming $\Delta k_{p,max} = 0.008$ (where $(1 + \Delta k_{p,max})$ is the ratio of the protein's molar mass in 100% D_2O and H_2O) (Schubert *et al.*, 1994). The corrected data are included in the figures. Those in Fig. 2 directly yield the final results for the protein/pigment complex: $\bar{v} = (0.788 \pm 0.010)$ ml g^{-1} and $M_r = 830{,}000 \pm 60{,}000$. Assuming $\Delta k_{p,max} = 0.0155$ (Edelstein and Schachman, 1967), the corresponding figures would be $\bar{v} = 0.797$ ml/g and $M_r = 860{,}000$. As discussed earlier (Schubert *et al.*, 1994), we consider the former figures as the more probable ones. – It follows that, in solutions of $C_{12}M$, the exact amount of detergent bound to the protein/pigment complex is (0.48 ± 0.06) g per g of complex.

The figures obtained for the molar mass of the protein/pigment complex are surprisingly close to those estimated from electron microscopy and from HPLC gel filtration, two techniques which have to rely on quite disputable assumptions.

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