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Evidence for the existence of trimeric and monomeric Photosystem I complexes in thylakoid membranes from cyanobacteria

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Abstract

In cyanobacteria, solubilization of thylakoid membranes by detergents yields both monomeric and trimeric Photosystem I (PS I) complexes in variable amounts. We present evidence for the existence of both monomeric and trimeric PS I in cyanobacterial thylakoid membranes with the oligomeric state depending 'in vitro' on the ion concentration. At low salt concentrations (i.e. $\leq$ 10 mM $MgSO_4$) PS I is mainly extracted as a trimer from these membranes and at high salt concentrations (i.e. $\geq$ 150 mM $MgSO_4$) nearly exclusively as a monomer, irrespective of the type of salt used (i.e. mono- or bivalent ions) and the temperature (i.e. 4 °C or 20 °C). Once solubilized, the PS I trimer is stable over a wide range of ion concentrations (i.e. beyond 0.5 M). A model is presented which suggests a monomer-oligomer equilibrium of PS I, but also of PS II and the cyt. *b6/f*-complex in the cyanobacterial thylakoid membrane. The possible physiological role of this equilibrium in the regulation of state transitions is discussed.

Abbreviations: β-DM – dodecyl-β-D-maltoside; Chl – chlorophyll; cyt. *b6f* – cytochrome *b6f* complex; EM – electron microscopy; HPLC – high performance liquid chromatography; LDAO – N, N-dimethyl-N-dodecyl amine oxide; MES – 4-morpholino ethane sulfonic acid; PAGE – polyacrylamide gel electrophoresis; PBS – phycobilisome; PS – photosystem; SDS – sodium dodecyl sulfate; 2D – two dimensional; 3D – three dimensional

Introduction

The Photosystem I complex is a large membrane-bound enzyme which catalyses light-driven electron transfer between plastocyanin (or cytochrome *c*-553) and ferredoxin (see Golbeck and Bryant 1991 and Almog et al. 1992 for reviews). The isolated PS I complex of both cyanobacteria and higher plants contains at least 12 different subunits, 60–100 chlorophyll molecules as well as additional carotenoids, one phylloquinone and three iron sulphur clusters. Multimeric forms of PS I have been isolated from thermophilic cyanobacteria like *Synechococcus*, *Phormidium* and *Mastigocladus* (Boekema et al. 1987; Ford and Holzenburg 1988; Almog et al. 1991). Besides stable PS I monomers and trimers, rather unstable dimers have also been observed (Rögner et al. 1990a). Trimeric PS I has also been purified from a mesophilic cyanobacterium, *Synechocystis* PCC 6803 (Rögner et al. 1990b; Kruip et al. 1993), and from a phototrophic bacterium, *Prochlorothrix hollandica* (Van der Staay et al. 1993). Despite the advanced structural characterization of isolated monomers and trimers (Böttcher et al. 1992; Krauß et al. 1993; Kruip et al. 1993), little information is available about mono- or oligomeric state of PS I in cyanobacterial membranes (Hladik and Sofrova 1991). This information is also important for structural electron microscopy studies on 2D crystals of PS I (Böttcher et al. 1992) or for X-ray diffraction analysis of 3D crystals (Krauß et al. 1993). As

both studies up to now are mainly done with the highly purified trimer, the physiological relevance of this complex should be known.

Here we present an investigation on the content of monomeric and trimeric PS I complexes in the thylakoid membrane. We show that both forms can be extracted in variable amounts from this membrane and that the trimer to monomer ratio is dependent on the salt concentration present during the extraction procedure. In addition, we present evidence from electron microscopy that particles of similar size as trimers can be observed in the thylakoid membrane and introduce a model for a possible regulatory effect of PS I oligomerization. We extend this model to a hypothesis which could explain state transitions in cyanobacteria by an equilibrium of different oligomeric forms of the membrane complexes involved in photosynthesis.

Materials and methods

Cells of a glucose-tolerant strain from the mesophilic cyanobacterium *Synechocystis* PCC 6803 were grown in BG 11 medium essentially as in (Rögner et al. 1990b). Isolation of the thylakoid membranes was done as in (Rögner et al. 1990b; Kruip et al. 1993). 3 ml membrane suspension (1 mg Chl/ml) was incubated with stirring at 4°C for 20 min in buffer A (20 mM MES, pH 6.5, 500 mM mannitol, 10 mM $CaCl_2$) supplemented with different ions at concentrations as indicated. Membrane proteins were then solubilized for an additional 20 min by incubation in the presence of 1% dodecyl-β-D-maltoside (β-DM). Remaining membrane fragments were pelleted by centrifugation (12 000 × *g*, Eppendorf centrifuge) and the extract (500 μl) was loaded directly on a Mono Q column (Pharmacia, Sweden) equilibrated with buffer A (supplemented by 0.03% β-DM). Elution from the column was done with a linear gradient of $MgSO_4$ and the elution profile was recorded at 280 nm and at 435 nm as in (Rögner et al. 1990b). The area at 435 nm of the monomeric and the trimeric PS I peak, respectively, was the basis for quantification. The Chl content of the membranes was determined according to (Arnon 1949). Electron microscopy of the membranes was performed as in (Kruip et al. 1993). Prior to staining with uranyl acetate, these membranes were washed with 2 M NaBr to remove surface-exposed proteins, essentially the CF_1-part to the ATPase. NaBr was then removed through multiple washing steps with a buffer containing < 10 mM $MgSO_4$ (from now on referred to as 'low salt buffer').

Results

Figure 1 shows the elution profile of extracts from the thylakoid membrane, which had been pretreated with two different salt concentrations during the extraction procedure (see 'Materials and methods'), from an anion exchange column. The attribution of the individual elution peaks to components of the photosynthetic electron transport chain, namely monomeric PS I, cyt. *b6/f*-complex, PS II and trimeric PS I, has already been done by extensive structural and functional characterization (Rögner et al., 1990b; Bald et al., 1992; Kruip et al., 1993). According to this analysis, at high salt concentrations ($\geq$ 150 mM $MgSO_4$) only about (15±10)% trimeric PS I can be found, while at low ion concentrations (<10 mM $MgSO_4$) the trimeric form of PS I is dominant ((75 ± 10)%). As under both conditions 80 to 90% of the total Chl were routinely extracted from the thylakoid membrane, the different salt concentrations apparently have no influence on the quantity of extracted PS I. Figure 2 shows the amount of extracted PS I trimer relative to total extracted PS I (i.e. as monomer and trimer) in the presence of different $MgSO_4$ concentrations during the extraction procedure. These results suggest that a 1:1 ratio of monomers and trimers can be expected at a $MgSO_4$ concentration of (70 ± 20) mM $MgSO_4$, where the range of error is mainly due to variations within different thylakoid membrane preparations. The same 'salt effect' was also observed with other salts such as $MgCl_2$, $CaCl_2$, NaCl and Na_2SO_4 (tested from 10 mM to 200 mM) although at slightly different concentrations depending on the type of salt (data not shown). It should be mentioned, however, that the differences between various salts are too small to be characteristic for the respective salt and are within the range of error mentioned above, i.e. ±30%. Irrespective of bivalent ions (i.e. Mg^{2+} or SO_4^{2-}) or monovalent ions (i.e. Na^+ or Cl^-) maximal monomerization of PS I is routinely reached at salt concentrations of about 150–200 mM.

When the extraction was done at 20 °C instead of 4 °C, no substantial difference could be observed. In addition, all experiments were repeated with membranes of the closely related cyanobacterium *Synechocystis* PCC 6714, yielding identical results.

Once extracted, the PS I trimer is remarkably stable against dissociation into monomers: Even in the

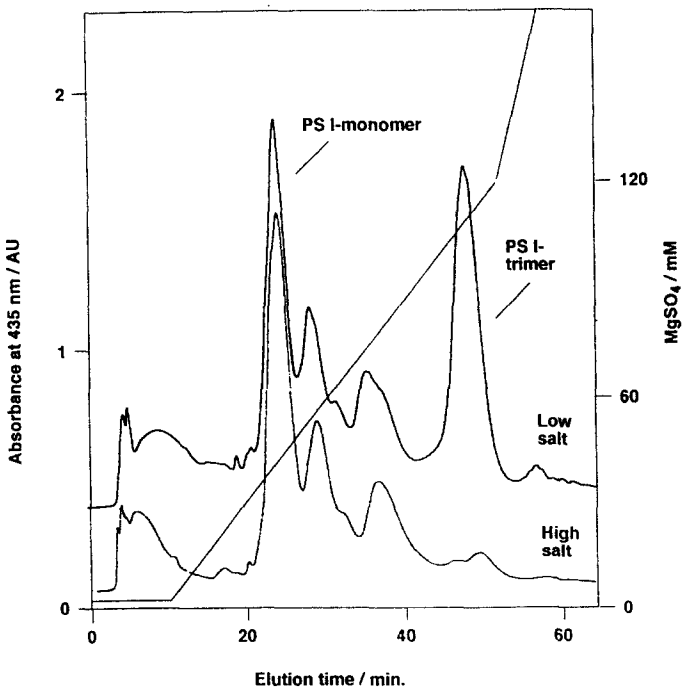

Fig. 1. Ratio of monomeric to trimeric PS I complexes extracted from thylakoid membranes of *Synechocystis* PCC 6803 in the presence of 5 mM ('low salt) and 150 mM $MgSO_4$ ('high salt'). The amount of monomeric and trimeric PS I was determined by HPLC analysis of membrane extracts (1% β-DM) on a Mono Q anion exchange column (Pharmacia, Sweden). In order to investigate the salt dependence, membranes were incubated for 20 min. at 4 °C in buffer with the respective salt concentration prior to extraction.

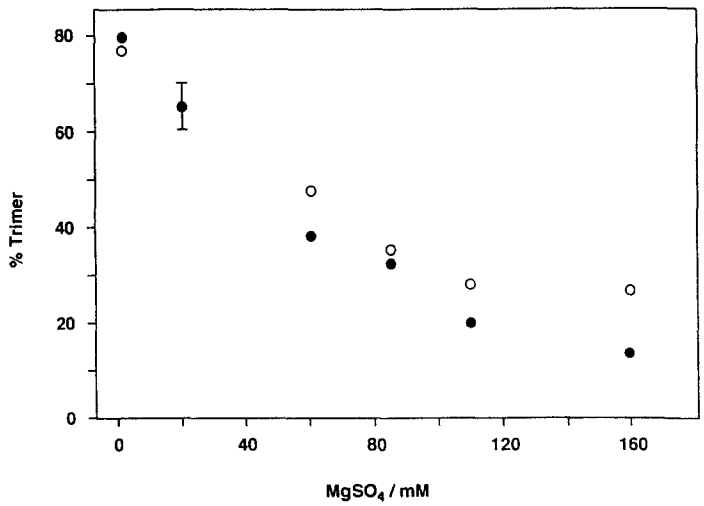

Fig. 2. Trimeric PS I extracted from thylakoid membranes (as percentage of total PS I, extracted as monomer or trimer) in dependence of the $MgSO_4$ concentration present during the extraction procedure. Different symbols (i.e. open and closed circles) represent extractions from different thylakoid membrane preparations. The value at 20 mM $MgSO_4$ represents an average value from preparations done under standard conditions. The ratio of monomer to trimer has been determined from elution profiles of analytical anion exchange column runs (see Fig. 1) by integration of the respective peak areas.

presence of 0.5 M $MgSO_4$ or 0.5 M sodium phosphate no dissociation could be observed after several hours of incubation.

Uranyl acetate-stained membrane fragments were examined by electron microscopy at low ion concentrations (i.e. $\leq$10 mM $MgSO_4$). These membrane frag-

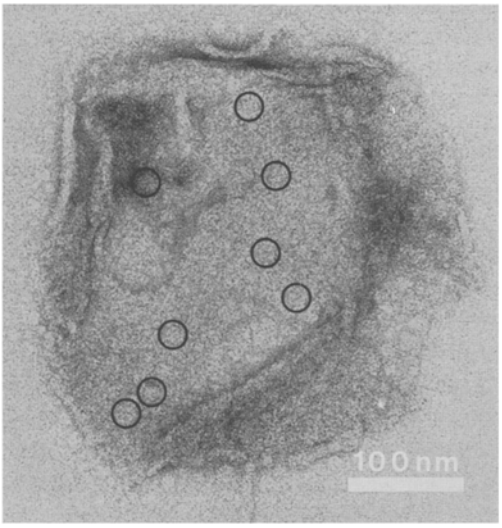

Fig. 3. Negatively stained (1% uranyl acetate) membrane of *Synechocystis* PCC 6803, washed with 2 M NaBr. Projections with the expected size of native PS I are encircled.

ments have not been exposed to detergent before electron microscopy. As surface-exposed parts of membrane proteins such as the CF_1 part of the ATPase obscure a clear view of the PS I complex, they were removed by a NaBr treatment prior to exposing them to low salt concentration followed by staining (see 'Materials and methods'). Also, direct detection of PS I trimers in the membrane by electron microscopy is complicated by the fact that the lipid bilayer prevents substantial negative stain penetration, resulting in grainy and low contrast images. Despite the low contrast, Figure 3 shows circular shaped projections with a diameter of 14–18 nm, in agreement with the diameter of trimeric PS I complexes (Boekema et al. 1987; Rögner et al. 1990a; Kruip et al. 1993).

Discussion

a. Evidence that the PS I trimer is not an artifact

Despite extensive characterization of isolated monomeric and trimeric PS I complexes the existence of mono- and/or trimeric PS I complexes in the thylakoid membrane in vivo is still a matter of debate (Ford and Holzenburg 1988; Hladik and Sofrova 1991; Hefti et al. 1992). Concerning function, up to now no substantial differences in recombination half time (Kruip et al. 1993), steady-state polarized light spectroscopy (Van der Lee et al. 1993) or time resolved fluorescence pattern (S. Turconi, J. Kruip, G. Schweitzer, M. Rögner and A.R. Holzwarth, unpublished) could be found between isolated monomeric and trimeric PS I complexes. On the other hand, monomeric and trimeric PS I complexes isolated from various mesophilic and thermophilic cyanobacteria have been structurally characterized by PAGE under native conditions (Ford and Holzenburg 1988; Rögner et al. 1990), HPLC gel filtration (Rögner et al. 1990ab), electron microscopy (Boekema et al. 1987; Ford and Holzenburg 1988; Rögner et al. 1990a; Kruip et al. 1993) and crystal analysis (Witt et al. 1987; Ford et al. 1987; Almog et al. 1991; Böttcher et al. 1992; Krauß et al. 1993). However, as no particles with the approximate size of PS I trimers could be detected on electron micrographs of negatively stained thylakoid membranes, some authors assigned the trimeric form to an artifact caused by in vitro detergent treatment (Ford and Holzenburg 1988).

Results of this report show that, at a constant detergent concentration, the ratio of PS I monomer to trimer extracted from the thylakoid membrane can be shifted considerably by adjusting the ionic strength of the medium. Apparently, this equilibrium can be shifted in the absence of detergent only due to surface charge effects contradicting the detergent-induced aggregation hypothesis (Ford and Holzenburg 1988). It seems plausible that the extracted monomer/trimer equilibrium reflects their ratio in the thylakoid membrane prior to extraction which is supported by the fact that under low salt conditions EM of the thylakoid membranes indeed shows large projections with the expected diameter of membrane embedded trimers. Although, at this resolution, it is not possible to be certain that these projections represent trimeric PS I, this results is different from a report where electron microscopy was performed on similar stained membranes yielding no evidence for particles of trimeric size (Hefti et al. 1992). This difference may be due to monomerization of membrane-embedded PS I, as these membranes are treated by the ionic detergent LDAO prior to analysis by EM, while in our case membranes had not been exposed to any detergent before.

On the other hand, in agreement with our results, a substantially higher content of PS I trimer at low salt (5 mM $MgCl_2$) in contrast to high salt (150 mM $MgCl_2$) is evident from SDS-PAGE of PS I-enriched thylakoid membranes under mildly denaturing conditions, although this fact is not commented (Hefti et al. 1992). It should be pointed out, however, that a comparison with our results is complicated by the fact that these authors use three different detergents (LDAO,

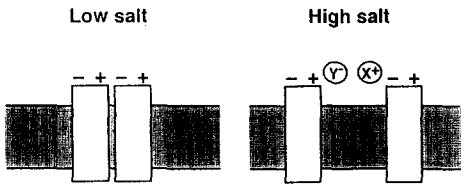

Fig. 4. Proposed model for the arrangement of PS I complexes in the thylakoid membrane under low salt (i.e. $\leq$10 mM $mgSO_4$) or high salt (i.e. $\geq$ mM $MgSO_4$) condition.

β-DM and SDS) in the course of their PS I purification and analysis by PAGE while in our case all steps from extraction to analysis by HPLC are done in the non-ionic and mild detergent β-DM. Further possible impact of the PS I preparation procedure on the analysis by EM and PAGE are discussed elsewhere (Rögner et al. 1990a).

Further strong evidence for 'native' PS I trimers suggested by this report comes from crosslinking experiments performed on cyanobacterial thylakoid membranes (Hladik et al. 1990) and from CD spectra of isolated trimers showing an additional highly ordered carotenoid absent in monomers (Hladik and Sofrova 1991). Also, in averaged projections of trimers recorded by electron microscopy and in the 3D reconstruction at 6 Å resolution from X-ray diffraction, a 'connection domain' is visible in the middle of the trimer (Krauß 1993; Kruip et al. 1993). This domain is more exposed in solubilized PS I monomers and therefore more likely to be attacked by detergent. As the PsaL content of PS I monomers is reduced by about 50%, a role for this subunit in trimer formation was postulated (Kruip et al. 1993). This is strongly supported by site-directed mutants of *Synechocystis* 6803 with deleted PsaL, from which, even under low salt conditions (acc. to Bald et al. 1992), no PS I trimer could be extracted (Chitnis et al. 1993ab). Yet, while all these results strongly suggest the existence of trimers in vivo, the physiological role of the oligomeric state is still unresolved, especially as the PsaL deficient strain and the wild type showed nearly identical growth behaviour (Chitnis et al. 1993b).

b. Model for PS I monomer/trimer equilibrium

The fact that isolated PS I trimers are stable up to very high ionic strength (i.e. 0.5 M $MgSO_4$) and that membrane-embedded PS I shows reversible monomerization at much lower ionic strength (i.e. 50% value at about 70 mM $MgSO_4$) leads us to propose a monomer/trimer equilibrium model shown in Fig. 4. Within the thylakoid membrane the association/dissociation of PS I complexes apparently is controlled by two physical forces: (1) Electrostatic interaction, and (2) Hydrophobic interaction. The hydrophobic tails of thylakoid membrane lipids will act to shield the membrane embedded parts of the monomers from irreversible aggregation. This environment allows the electrostatic surface charge attraction between monomers to become the dominant factor in controlling the state of aggregation. At low salt concentrations the surface charges on the monomers are able to attract each other resulting in (reversible) trimer formation. By the addition of salt the electrostatic surface charges are screened. As a result, charges between monomers are prevented from attracting each other, leading to isolated monomers within the membrane due to intercalating lipid molecules. As the oligomeric state of the extracted and purified PS I complex is dependent on the salt concentration present at the time of detergent solubilization, the detergent 'freezes' the ratio of monomers to trimers existing at the moment of extraction within the membrane. The ratio remains constant, as monomers do not aggregate in the presence of detergent (Rögner et al. 1990a) and trimers do not dissociate into monomers due to the lack of lipids which are replaced by the detergent around the trimer. This way, upon extraction 'reversible trimers' are transformed into 'irreversible trimers'. Apparently, in the native membrane lipids reduce the hydrophobic interaction to such a level that electrostatic interaction becomes the dominant factor controlling oligomerization. Possible stages of such a dynamic process have recently been outlined in a 'molecular recognition hypothesis' (Allen 1992).

As the capability to form stable 'native' trimers apparently is lost upon deletion of the PsaL subunit (Chitnis et al. 1993ab), charges responsible for the salt-induced dissociation of PS I observed in this study most probably are located on this subunit and/or stroma-exposed PsaD and -E, which are located close to the contact area (Kruip et al. 1993). A loss of PsaL in the course of solubilization would also explain the failing to reconstitute trimeric PS I out of purified monomers (Rögner et al. 1990a). Nevertheless, at this stage also the contribution of lipids with charged head groups cannot be excluded from the surface charge effects. Such lipids have been reported to be closely associated with some membrane proteins, for example PS II (Sprague et al. 1985) and the LHC II complex (Nußberger et al. 1993).

Interestingly, the salt concentration at which trimers are converted into monomers within the isolated thylakoid membranes falls into the physiological ion concentration range found in cyanobacterial cells, with K^+ and Cl^- being the dominating ions (Richardson et al. 1984). Thus, it may be possible that the monomer to trimer transition is a regulatory switch, especially as indications for changes of the K^+ concentration in combination with changes in the photosynthetic activity have been shown for *Synechocystis* 6714 (Reed et al. 1985). More likely, the shift of monomers to trimers by salts observed 'in vitro' could mimic other factors 'in vivo': Reversible phosphorylation or acidification of the thylakoid lumen could lead to a change in the overall electric charge of PS I which in turn induces a monomer to trimer transition. In this context a membrane protein of about 15 kDa with unknown function, showing light dependent phosphorylation (Allen et al. 1985) deserves special interest as a possible candidate for trimerization and might be identical with PsaL.

c. Correlation with 'state transitions' (hypothesis)

There is increasing evidence for the existence of oligomeric structures of all three integral proteins which are involved in the photosynthetic non-cyclic electron transport chain. This prompted us to extend our model to a hypothesis which may be able to explain state transitions in cyanobacterial membranes by reversible oligomerization of PS I, PS II and the cyt. *b6/f* complex.

Evidence for the existence of functional PS II dimers in the cyanobacterial thylakoid membrane is given by both structural (Mörschel and Schatz 1987; Rögner et al. 1987; Dekker et al. 1988) and functional investigations: Isolated PS II dimers consist of two monomers in highly ordered, antiparallel arrangement (Boekema et al. 1994) and show a high fluorescence increase ($F_{max}/F_o \geq 7$) in fluorescence induction measurements with pronounced cooperativity (G.H. Schatz, M. Rögner, J.P. Dekker and A.R. Holzwarth, unpublished). Also, recently a highly active dimeric cyt. *b6/f* complex has been isolated from thylakoid membranes of spinach under high salt conditions, similar to this report. Under low salt conditions, this complex was isolated as monomer with impaired electron transport (B. Cramer et al. 1992). This may indicate a general significance of the salt effect (or a concomitant effect) reported in this paper beyond the PS I complex.

We therefore modify and extend a model proposed as the 'mobile antenna model' (for review see Allen 1992) by correlating reversible monomer/oligomer transitions of photosynthetic membrane proteins with state transitions in cyanobacteria. According to our model, illustrated in Fig. 5, in state 1 phycobilisomes are coupled to dimeric PS II, enabling linear electron transport via a dimeric cytochrome *b6f* complex (cyt. *b6f*) to monomeric PS I. In state 2 phycobilisomes are bound to trimeric PS I, while PS II is dissociated into a monomer, unable to bind phycobilisomes. This state, according to our model, supports mainly cyclic electron transport between PS I and monomeric cyt. *b6f*. Basis of our model is the experience about the oligomeric structure of the three photosynthetic membrane proteins under defined conditions: Under conditions favouring monomeric PS I complexes (i.e. conditions simulated by high salt) both PS II and cyt. *b6/f* complex will be dimeric, while under conditions simulated by low salt trimeric PS I complexes will be combined with monomeric PS II and cyt. *b6/f* complexes. Our model is further based on the assumption that di- or trimeric complexes are better docking sites than monomeric complexes for phycobilisomes to form a highly oriented PS–PBS complex. Such a well-ordered super-complex is the structural basis for efficient excitation energy transfer from the PBS to the respective photosynthetic reaction center. Regarding the fact that the mass of a PBS is greater than that of monomeric PS I and PS II core complexes by a factor of about 10, the assumption of a larger stabilizing 'base plate' in form of oligomeric PS I and PS II complexes, respectively, seems reasonable. This assumption is supported by the observation that PBS are bound by PS II dimers arranged in rows that favor PS II- PS II energy transfer (Dekker et al. 1988; Vernotte et al. 1990) and that the PS II complexes are randomly distributed upon transition in state 2, decreasing PS II-PS II interaction (Olive et al. 1986). Additionally there is increasing evidence for the existence of a functional PBS-PS I complex in cyanobacteria (Mullineaux and Holzwarth 1991; Mullineaux 1992). Whether or not the 'wandering' of PBS as a result of state 1-state 2 transition is accompanied by a phosphorylation similar to higher plants is presently unknown (Allen et al. 1985; Allen 1992).

In our model, the cyt. *b6f* complex could play a special role in the regulation between linear and cyclic electron transport: While the dimeric form supports linear electron transfer between PS II and PS I, the monomeric form would be active in cyclic electron flow. This is in accordance with a highly active dimeric and a less active monomeric form of cyt. *b6f* com-

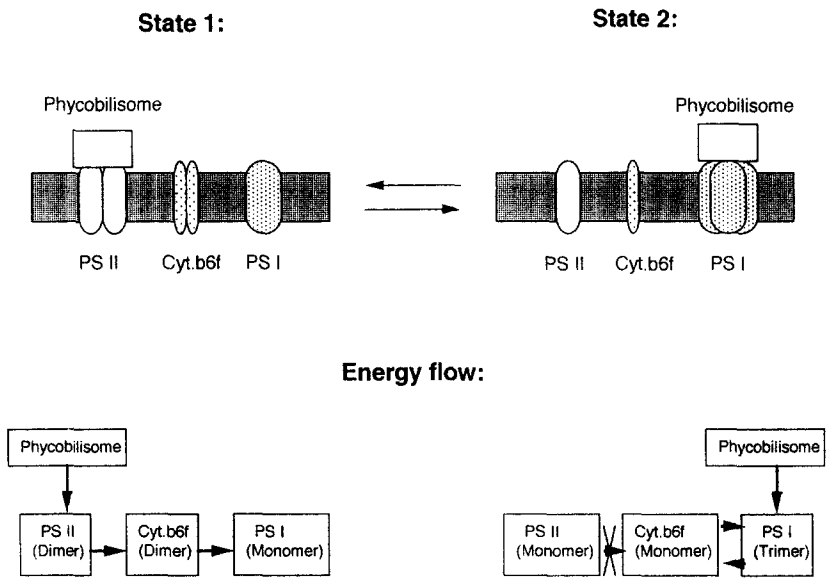

Fig. 5. Proposed model of reversible oligomeric forms of PS I, PS II and cyt. *b6/f*-complex for state transitions in cyanobacteria.

plex recently isolated (Cramer et al. 1992) and with a much slower turnover of cyclic electron flow carriers under normal circumstances (Fork and Herbert 1993). Unfortunately, up to now no in vitro test system for cyclic electron transport of isolated *b6/f*-complexes is available.

The salt-induced effect which led us to this hypothesis may be regarded as a model mechanism by which dissociation/association of the membrane proteins involved in state transitions can be induced in vitro in the dark; in vivo these processes should be triggered by light leading to changes in surface charge density (i.e. phosphorylation or other posttranslational modifications of proteins) which are not necessarily combined with changes in salt concentrations. In any case, however, fluidity of the lipid phase seems to be important for these oligo-/monomerisation processes: Preliminary experiments with thylakoid membranes of the thermophilic cyanobacterium *Synechococcus elongatus* showed that monomerisation of PS I by high salt treatment could only be achieved at $\geqslant 35$ °C reflecting the much higher phase transition temperature of lipids from thermophiles in comparison to the mesophilic *Synechocystis* investigated in this study (I. Claes, D. Bald, J. Kruip and M. Rögner, unpublished results).

Originating from the structural point of view, this hypothesis presents a new concept for the regulation of cyanobacterial photosynthesis. To prove it, the exact conditions for the isolation of dimeric PS II and cyt. *b6/f* complexes from cyanobacterial thylakoid membranes have to be verified, followed by functional studies on isolated membranes under exactly these conditions. While these experiments are now in progress, this hypothesis should stimulate further discussions as well as the design of new experiments.

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