Relationship between state transition and photosystem I structure under high light condition in cyanobacteria

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シアノバクテリアの強光順化における
ステート遷移と光化学系Ⅰ複合体の高次構造の関係

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修士論文

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Summary

In cyanobacteria, Photosystem I (PSI) exists mainly as trimer in thylakoid membrane, and this trimerization is mediated by the PsaL subunit of PSI. The role of PsaL and the function of trimeric PSI are unknown. I hypothesize that this trimerization of PSI may be important for the high light acclimation of cyanobacteria, based on the several different lines of evidence. Upon high light acclimation, cyanobacteria regulate the distribution of light energy absorbed by phycobilisome antenna either to PSII or to PSI by the process so-called state transition. In this report, I found that the *psaL* mutant was not able to perform state transition in high light-acclimated cells. Furthermore, PSI trimer was not formed under that condition in the *psaL* mutant. Apparently, there is a close relationship between state transition and PSI trimer formation in high light-acclimated cells. Three possible mechanisms for the relationship are discussed.

Introduction

All higher organisms on earth receive their energy directly or indirectly from oxygenic photosynthesis that is performed by plants, green algae and cyanobacteria. The oxygenic photosynthesis is a process that converts solar energy to chemical energy catalized by two large protein complexes, photosystem I (PSI) and photosystem II (PSII), which are located in the thylakoid membrane. Photosynthetic electron transfer leads to a proton gradient across thylakoid membranes, which is used to synthesize ATP by ATP synthetase. Electrons that are transported from PSII to PSI are eventually used for the reduction of $NADP^+$ to NADPH. Following the photosynthetic electron flow, NADPH and ATP that produced are used to fix CO_2 to carbohydrates.

Photosynthesis of cyanobacteria is almost identical to that of plants and algae. However, polypeptides composition and supramolecular organization in two photosystems are partially different. Most notable differences in the structure of photosynthetic apparatus between cyanobacteria and plants are found in PSI. PSI is a multi-subunit protein complex and consists of 15 core subunits (PsaA to PsaL, PsaN to PsaP) in plants and algae, whereas in cyanobacteria, PSI complex consists of 11 core subunits (PsaA to PsaF, PsaI to PsaM). Furthermore, isolated cyanobacterial PSI exists as trimer and monomer (Kruip et al., 1994), although trimeric PSI complexes have never been found in plants. I assume that it is very important to clarify the biological roles and significances of cyanobacterial PSI structure to understand the evolutionary diversity of the photosysthetic organisms.

For the study on PSI structure, we have now the X-ray crystal structure of PSI trimer at 2.5 Å resolution determined in cyanobacteria (Jordan et al., 2001) (Fig. 1). The PsaA and PsaB subunits form a heterodimeric core that harbors P-700, the primary electron donor, and a chain of electron acceptors A_0 , A_1 and F_X (Chitnis et al., 1993). All of the small membrane integral subunits, PsaF, PsaI, PsaJ, PsaK, PsaL and PsaM, are located peripherally to PsaA and PsaB. Among them, PsaL was first proposed to play a role in PSI trimarization by mutagenesis studies, in which no trimers can be detected in PsaL deletion mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 (Chitnis et al., 1993). Indeed, the structural information of X-ray crystallographic analysis demonstrated that PsaL are located close to the three-fold axis and far away from the lipid exposed to the surface of PSI trimer (Jordan et al., 2001) (Fig. 1). However, the cyanobacterium *Synechocystis* sp. PCC 6803 mutant that lacks PsaL did not significantly differ in the growth, photosynthetic activities and state transition compared with wild type, suggesting that PSI trimer is not essential under normal growth condition (Chitnis et al., 1993). This observation advocates a question of what the role of PsaL is, in turn, why cyanobacteria contain PSI trimer. Interestingly, a recent report provided the additional information on PsaL function. Under excess light (400 μmol $m^{-2}s^{-1}$) condition, the growth of the *psaL* mutant was reported to decrease, suggesting that PsaL subunit might be important in physiological responses under excess light condition (Wang et al., 2008). So far, molecular mechanisms of PsaL and PSI trimer for adaptation to excess light are still unclear.

Photosysthetic organisms can acclimate to changing light environment to optimize

the efficiency of photosynthesis or to avoid the photodamage. In the acclimation process, conformational modification of the photosynthetic apparatus is induced, which balances energy input and consumption. If energy supply exceeds, particularly under high light conditions, photosynthetic organisms change the distribution of light energy absorbed by antenna pigments to PSI and PSII in order to optimize the photosynthetic performance, or to avoid the damage from excess absorption of light energy.

Acclimation from low light to high light conditions can be classified into short-term and long-term processes. The long-term acclimation to high light is relatively slow because it involves changes in the composition, function, and structure of the photosynthetic apparatus as well as other photosynthesis-related components. Completion of the long-term processes may take hours or even days. Conversely, the short-term acclimation including ‘state transition’ occurs rather rapidly and is usually completed within several minutes. In the case of cyanobacteria, state transition regulates the distribution of light energy from the accessory light-harvesting system, phycobilisome, to the two photosynthetic reaction centers. State 1 is induced by illumination preferentially absorbed by chlorophyll *a* antenna of PSI and is characterized by a high efficiency of energy transfer from the phycobilisome to PSII (Fig. 2A). State 2 is induced by illumination predominantly absorbed by PSII and is characterized by increased efficiency of energy transfer to PSI (Allen et al., 1992) (Fig. 2B). State transition is induced not only by the change in light quality but also by the change in the light quantity. Under normal weak light condition, the light energy absorbed by phycobilisome is preferentially transferred to PSII. On the contrary, under

high light condition, the energy can be transferred not only to PSII but also to PSI (Campbell et al., 1998). In this case, photosynthesis may not be effective but there is an advantage to protect photosystems from photodamage. Thus, the state transition is important for cyanobacteria especially because the change of light intensity is faster than other environmental stresses.

Previously, our laboratory reported that PsaK2, a PSI subunit, is a factor of state transition under high light condition in the cyanobacterium *Synechocystis* sp. PCC 6803 (Fujimori et al., 2005). PsaK2 expresses only in high light-acclimated cells (Fujimori et al., 2005). Accordingly, it was suggested that the mechanism of state transition in high light acclimated cells is different from state transition in low light acclimated cells. Although PsaK2-dependent state transition is essential for the growth under continuous high light condition (Fujimori et al., 2005), we have no information on components of PSI, except for PsaK2, working for the cyanobacterial state transition in high light acclimated cells. In addition, it was reported that PSI trimer was stabilized by the high light-inducible polypeptides during the acclimatory process to high light (Wang et al., 2008). Moreover, PsaK2 is incorporated into PSI trimer only under high light condition (Fujimori et al., 2005). Taking these reports into account, I hypothesize that PSI trimer is related to state transition under high light condition.

In the process of response to high light, it is unknown whether PSI trimer is involved in energy transfer from phycobilisome to PSI. It is important to demonstrate the relationship between state transition and PSI trimer to understand the mechanism of physiological responses to avoid photodamage as well as to understand the significance

of PSI trimer. Here I present the investigation of state transition in high light-acclimated cells of the *psaL* mutant. Furthermore, I show that PSI trimer does not seem to be formed in high light-acclimated cells of the *psaL* mutant. I will discuss the relationship between state transition and PSI structure under high light condition in the cyanobacterium *Synechocystis* sp. PCC 6803.

Results

In this study, I used two mutant strains to analyze the relationship between PSI structure and high light acclimation. One is the *psaL* mutant, which lacks the ability to form PSI trimer. Another is the *psaK2* mutant, which lacks the ability to perform state transition under high light. Growth of both strains is not so much different from that of wild type. After 24 hours-culture under low light conditions, the number of cells of the *psaL* mutant and the *psaK2* mutant were 2.6-2.8 x 10^7 cells ml^{-1} (Table 1, left column), the value similar to 2.7 x 10^7 cells ml^{-1} observed in wild type. The situation was the same under high light conditions, and the number of cells of wild type, the *psaL* mutant and the *psaK2* mutant were around 5.0 x 10^7 cells ml^{-1}. The content of photosynthetic pigments usually decreased upon the sift to high light condition, and this high light acclimation was observed in wild type cells, as well as in the *psaL* mutant and the *psaK2* mutant. Both chlorophyll content and phycocyanin content decreased under high light condition in all three strains (Table 1, center and right columns). The results indicate that the *psaL* mutant and the *psaK2* mutant could acclimate to high light similarly in the case of wild type.

When cyanobacterial cells were excited in liquid nitrogen at 77 K with blue light (400-600 nm) that could be absorbed by phycobilin and chlorophyll *a*, the fluorescence from the cells adapted to either state 1 or state 2 shows emission peaks at 663, 685, 695 and 725 nm. The fluorescence around 663 nm arises from phycobilisome, while the fluorescence around 685 nm and 695 nm arises from PSII. In the case of 685 nm peak,

there is some contribution from the terminal emitters of phycobilisome. The 725 nm peak exclusively arises from PSI. To investigate the role of PsaL in state transition under high light condition, I examined the energy transfer from phycobilisome to two photosystems by monitoring 77 K emission spectra of chlorophyll fluorescence from cells either in state 1 or in state 2.

In low light-acclimated cells of wild type, the *psaL* mutant and the *psaK2* mutant, relative intensity of PSI fluorescence was much greater in cells adapted to state 2 than that adapted to state 1 (Fig. 3A, B, C and Table 2). This increase of PSI fluorescence in state 2 reflects the enhanced energy transfer from phycobilisome to PSI. These results indicate that the *psaL* mutant and the *psaK2* mutant can perform state transition under low light condition as previously reported (Chitnis et al., 1993, Fujimori et al., 2005).

In high light-acclimated cells of wild type, relative intensity of PSI fluorescence was much greater in cells adapted to state 2 than to state 1 (Fig. 4A and Table 2). In high light-acclimated cells of the *psaL* mutant and the *psaK2* mutant, however, relative intensity of PSI fluorescence in state 2 was close to that in state 1 (Fig. 4B and C). In high light-acclimated cells of the *psaL* mutant and the *psaK2* mutant, the ratio of state 1 to state 2 was 0.97 ± 0.05 and 0.84 ± 0.03, respectively (Table 2). These results indicate that the high light-acclimated cells of the *psaL* mutant and the *psaK2* mutant are not able to perform state transition. In addition, the *psaL* mutant was more incompetent in state transition than the *psaK2* mutant. Apparently, PsaL was necessary for the normal function of state transition under high light condition.

If the chlorophyll fluorescence emission spectra were compared between the low

light-acclimated cells and high light-acclimated cells, the relative intensity of PSI fluorescence (at around 725 nm) was greater in the low light-acclimated cells than in the high light-acclimated cells (compare Fig.3 and Fig.4). This reflects another acclimatory response of cyanobacteria, i.e. the regulation of photosystem stoichiometry (Hihara et al., 1998). Upon acclimation to high light, relative PSI content decreased in wild type as well as in the two mutant strains. It is concluded that the regulation of photosystem stoichiometry is not impaired by the lack of PsaL or PsaK2. It is concluded therefore that the perception of environmental light condition itself was not impaired in these mutants.

To examine the changes of the PSI structure, I isolated chlorophyll proteins complexes by sucrose density gradient after solubilization of thylakoid membranes by detergent. In the case of wild type, three distinct bands were resolved after ultracentrifugation, the upper orange band, the middle green band, and the lower green band (Fig. 5). The gradients were fractionated into 16 parts from top to bottom of the gradients and chlorophyll and P-700 contents of the each fraction were quantified. In the case of wild type, the two green bands were clearly observed both in low light-acclimated cells and high light-acclimated cells (Fig. 5A and B). Since the two bands contain P-700, the reaction center chlorophyll of PSI, it is concluded that PSI complex is present in both bands. From the results of earlier works (Sun et al., 1998), it could be assumed that the middle band contains monomer PSI while the lower band contains trimer PSI. The lower value of P700/Chl in the upper band (Fig. 7A) suggests that this band contains not only PSI complexes but also PSII complexes.

In the low light-acclimated cells of the *psaL* mutant, only one peak was observed in the same fractions that correspond to the PSI monomer fractions of wild type (Fig. 6A and C). The absolute value of the peak was higher than PSI monomer or PSI trimer peak of wild type (Compare Fig.5C and Fig.6C), suggesting that PSI trimer could not be formed in the *psaL* mutant as reported earlier (Chitnis et al., 1993). The ratio of P-700/Chl in the monomer fraction of the *psaL* mutant was higher than those of wild type (Compare Fig. 7A and B). The result shows that the relative contribution of PSII to the value of P-700/Chl ratio became smaller in the *psaL* mutant than in the case of wild type due to higher concentration of PSI in this fraction.

In the high light-acclimated cells of the *psaL* mutant, the middle green band expanded and the lower green band of PSI trimer was obscure (Fig. 6B). Examination of the chlorophyll distribution shows that the broad peak appeared and the absolute value of the peak was lower than that under low light condition (Fig. 6D). These results indicate that PSI exists in the different structure from that of wild type. Nonetheless, PSI trimer does not seem to be formed in high light-acclimated cells of the *psaL* mutant.

Table 3 shows the relative content of PSI trimer in wild type and the *psaL* mutant cells acclimated to low light or high light conditions. The relative content of PSI trimer was estimated as percentage of (total trimer fractions)/(total monomer + trimer fractions) determined by the content of either P-700 or chlorophyll. Since momomer fraction contains PSII chlorophyll as explained above, trimer content should be underestimated in the calculation based on chlorophyll content. About 70% of the PSI was present in the form of trimer in wild type, regardless of the growth light conditions.

In the case of the low-light grown *psaL* mutant, trimer formation was apparently inhibited. On the other hand, trimer/monomer ratio could not be evaluated by the results of Table 3 in the case of the high-light grown *psaL* mutant, since the band pattern of the sucrose density gradient is not comparative with that of wild type.

Discussion

In the course of state transition of cyanobacteria, light energy absorbed by phycobilisome is transferred to PSI in state 2. In spite of the vast accumulation of physiological data, the molecular mechanism of interaction between phycobilisome and PSI is poorly understood. Although the PSI complexes in cyanobacteria are organized in thylakoid membrane preferentially as trimer (Tsiotis et al., 1995, Karapetyan et al., 1999), physiological significances of cyanobacterial trimeric PSI are largely unknown. In this report, I found that the mutant lacking PsaL, a subunit of PSI, was not able to perform state transition in high light-acclimated cells (Fig. 4). Therefore, I concluded that PsaL seems to be necessary for the induction of state transition only in high light-acclimated cells. Furthermore, PSI of the *psaL* mutant exists in the different structure from that of the wild type and PSI trimer does not seem to be formed in high light-acclimated cells of the *psaL* mutant. Taken together, I assume that the structure of PSI trimer is somehow involved in state transition in high light-acclimated cells.

PsaL is a small intrinsic membrane protein and forms most of the contacts between monomers to form a trimer (Muhlenhoff et al., 1993, Karapetyan et al., 1999, Chitnis et al., 1993). In the structure of 2.5 Å resolution (Jordan et al., 2001) (Fig. 1), PsaL are located in the center and inside of PSI trimer. Moreover, it was concluded that PsaL and then PsaK were the last subunits of PSI to be assembled (Duhring et al., 2008). PsaK1 was present in the PSI of the *psaL* mutant (Chitnis et al., 1993). Practically, PsaL have no effects in assembly of all the other subunits. In addition, PsaL was not essential in

the growth, photosynthetic activities and state transition under normal growth condition (Chitnis et al., 1993). This is consistent with my study that the *psaL* mutant could induce the state transition under low light condition as similar to the wild type (Fig. 3). Moreover, under high light (200 μmol m^{-2} s^{-1}) condition, PsaL was not essential in their growth (Table 1). These studies indicate that PsaL itself have no important functions except for the trimerizarion of PSI. It could not be assumed that PsaL is involved directly in the regulation of energy transfer from phycobilisome to PSI. This is supported by the fact that the *psaL* mutant can perform state transition in low light-acclimated cells.

PSI trimer does not seem to be formed in high light-acclimated cells of the *psaL* mutant (Fig. 6). Furthermore, PSI exists not simply as monomer but as some different structure such as dimer or as monomer containing many carotenoids in high light-acclimated cells of the *psaL* mutant (Fig. 6). By high light stress, PSI monomers might assemble incorrectly and form a kind of dimer. Alternatively, content of carotenoids increase in high light-acclimated cells and more carotenoids might bind with the surface of PSI monomer in the *psaL* mutant. The modified PSI structure might be the cause of the defect in state transition in the *psaL* mutant, which in turn, the normal PSI structure for PSI trimer organization appeared to be important for state transition under high light condition.

As mechanism of the involvement of PSI trimer in state transition, I hypothesize three possibilities (Fig. 8). First, in the PSI trimer, all subunits inside are protected from the external stress and proteolysis. Indeed, PsaL was protected from proteolysis in

trimers but not in monomers of PSI (Chitnis et al., 1993). PSI trimer could protect PsaI, PsaM and PsaK located between PSI monomers. The main function of these small subunits is the stabilization of the antenna system and the quaternary structure of PSI. The protection of these subunits would guarantee the efficient function of PSI and of state transition.

Secondly, in the form of trimer, PsaK2 subunit can contact with other subunits that cannot contact in the form of monomer. PsaK2 is a factor of state transition under high light condition (Fujimori et al., 2005). In a previous report, upon high light acclimation of the cyanobacterium *Synechocystis* sp. PCC 6803, the expression of all PSI genes was simultaneously suppressed with only one exception of the *psaK2* gene (Hihara et al., 2001). The genome of the cyanobacterium *Synechocystis* sp. PCC 6803 contains two unlinked *psaK* genes, *psaK1* and *psaK2*. PsaK was located on the outer edge of PSI monomer and between PSI monomers when PSI formed trimer (Jordan et al., 2001). PsaK is not in contact with any of the small membrane intrinsic proteins. PsaA is the only subunit in immediate neighborhood of PsaK in the form of monomer. Two models of PsaK2 position under high light condition are suggested. The former model is that PsaK2 replace PsaK1 (Duhring et al., 2007). The latter model is that PsaK2 binds to the opposite position of PsaK1 where PsaG located in the case of higher plant (Fujimori et al., 2005). In either event, PsaK2 in PSI trimer can be in contact with both PsaA and PsaB. Under high light condition, this contact might be necessary for the normal function of state transition.

Thirdly, PsaK2 is stabilized or retained in the trimer much more than in the case of

monomer. PsaK2 is expressed only in high light-acclimated cells (Fujimori et al., 2005). It was revealed that PsaK2 is not essential for trimerization of PSI and PsaK2 can be assembled in PSI monomer (Fujimori et al., 2005, Duhring et al., 2007). However, PsaK2 might be more stable in PSI trimer than in PSI monomer under high light condition. If the binding of PsaK2 to PSI monomer is weaker than that to PSI trimer, the formation of trimer increased the ratio of PSI that bind PsaK2, leading to the normal function of state transition in high light-acclimated cells. PSI complexes almost exist as PSI trimer and the trimer structure might be important for the normal function of state transition on these reasons above. Considering the evolution of plant, cyanobacterial trimeric PSI might involve with phycobilisome because these are peculiar to cyanobacteria.

Interestingly, no differences in state transition between wild type and the *psaL* mutant were observed under low light-acclimated cells (Fig. 3). This supported the model that at least two kinds of mechanisms for state transition might exist, such as light quality and light quantity or low light- and high light-dependent state transition (Fujimori et al., 2005). Under low light or biased light (PSII or PSI light) condition, RpaC-dependent state transition might enable the effective distribution of energy between PSII and PSI in order to perform efficient photosynthesis (Emlyn-Jones et al., 1999). Under high light condition, PsaK2-dependent state transition might allow cells to be protected against the photodamage (Fujimori et al., 2005). In this report, I add PSI trimer to factors of state transition under high light condition.

In my study, I concluded that there is a close relationship between state transition

and PSI trimer under high light-acclimated cells. The relationship would be a key to understand the mechanism of physiological responses to avoid photodamage under high light.

Materials and Methods

Growth of Cultures and Strains

Wild type and mutant strains of the cyanobacterium *Synechocystis* sp. PCC 6803 were grown in BG-11 medium (Rippka et al., 1979) supplied with 10 mM TES, pH 8.0. Cells were grown at 30^{o}C in 50-ml glass tubes and bubbled with air under continuous illumination provided by fluorescent lamps. Photon flux density at 20 and 200 μmol $m^{-2}s^{-1}$ was regarded as low light and high light, respectively. The *psaL* mutant was constructed by insertion of the kanamycin-resistant cassette. The *psaK2* mutant was constructed by insertion of the kanamycin-resistant cassette into *AgeI* site in the *psaK2* gene. Mutants were usually maintained with kanamycin at the final concentration of 20 μg ml^{-1}.

Fluorescence Emission Spectra

Low temperature fluorescence emission spectra were determined at 77 K using a custom-made apparatus as described by Sonoike and Terashima (Sonoike et al., 1994). Cells acclimated to low or high light condition for 24 h were collected and concentrated by centrifugation. Cells were adjusted to a concentration of 0.5 μg chlorophyll ml^{-1} with BG-11. State 1 conditions were achieved by illumination of cells with blue light in the presence of 10 μM DCMU for 2 min and subsequent freezing in liquid nitrogen under the same light condition. State 2 conditions were achieved by dark incubation of cells for 10 min at room temperature and subsequent freezing in liquid nitrogen in the dark.

Isolation of Thylakoid Membrane and Resolution of PSI monomers and trimers

Cyanobacterial cells of wild type and the mutant strains were harvested and suspended in 0.4 M sucrose, 10 mM NaCl, 1 mM $CaCl_2$, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine, and 50 mM MOPS, pH 7.0 (Chitnis et al., 1993). Cells were then broken with a bead-beater (Model 1107900, Biospec Products). Unbroken cells and debris were removed by low speed centrifugation at 4,700 x g. Thylakoid membranes were pelleted by centrifugation at 140,000 x g for 45 min and resuspended in 50 mM MOPS, pH 7.0 . Thylakoid membranes were solubilized with β-D-dodecyl-maltoside for 15 min on ice. The ratio of chlorophyll to β-D-dodecyl-maltoside was 1:15. The solubilized thylakoid membranes were centrifuged at 10,000 x g for 3 min, and the resulting supernatant was layered on a 10, 20, 30 % step gradient of sucrose in 10 mM MOPS, pH 7.0 , and 0.05 % β-D-dodecyl-maltoside (Sun et al., 1998). The total chlorophyll of thylakoid membranes on each gradient was 0.06 mg chlorophyll. The quantity of layered thylakoid membranes, 10 %, 20 % and 30 % sucrose solution was 200 μl, 500 μl, 800 μl and 800 μl, respectively. Three distinct bands were resolved after ultracentrifugation at 140,000 x g for 2 h with an angle rotor (70T, Hitachi, Yokohama, Japan). The upper orange band contained carotenoid, the middle green band contained PSII complexes and monomers of PSI complexes, and the lower green band contained trimers of PSI complexes. The gradients were fractioned into 16 aliquots from top to bottom of the gradients.

Determination of P-700

The photooxidisable concentration of P-700 was measured with a spectrophotometer (Model 356, Hitachi, Japan) at 700 nm in a double-beam mode (Terashima et al., 1994). Actinic light at a saturating PPFD of 200 μmol m^{-2} s^{-1} (400-700 nm) was provided by a tungsten lamp passing through two layers of blue filters (CS 4-96, Corning Glass, Corning, N.Y., USA) and a 7-cm layer of water. The photomultiplier was protected with a cut-off filter (R-69, Toshiba) and an interference filter (Toshiba, transmittance peak at 701 nm). The reaction mixture contained, 50 mM Tris-KOH, pH 7.5, 10 μM DCMU, 10 mM ascorbate, 80 μM N,N,N',N'-tetramethyl-*p*-phenyl-enediamine, and 10 μM methyl viologen.

Absorption Spectra

Absorption spectra of whole cells of wild type and mutants were measured using a spectrophotometer (Model 356, Hitachi, Japan) with a cuvette placed just in front of photomultiplier. Concentration of chlorophyll and phycocyanin were calculated by the equations of Arnon et al. (1974). Optical density at 730 nm (OD_{730}) of whole cells was determined using a spectrophotometer (GeneSpec III, Hitachi, Japan). The wild type cells gave 1.1 x 10^{8} cells ml^{-1} at 1 OD_{730}, when the OD was determined by the same spectrophotometer (Ozaki et al., 2007). The number of cells was converted by this equation. Chlorophyll concentration was determined after extraction to 100 % methanol as reported earlier (Grimme et al., 1972) using the same spectrophotometer.

Figures

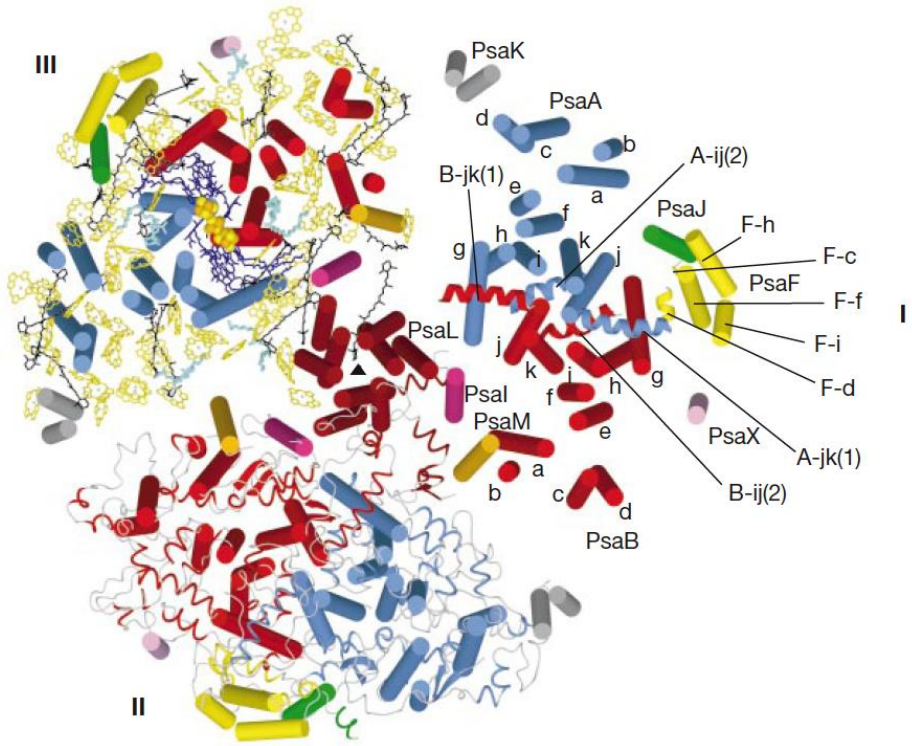

Fig.1. Photosystem I structure in cyanobacteria.

In cyanobacteria, photosystem I (PSI) exists as monomer or trimer. Structual model of PSI trimer at 2.5 Å resolution determined by X-ray crystal analysis (Jordan et al., 2001) is shown. PsaL is localized on the trimer-forming domain. PsaK is localized on the outside edge of PSI trimer.

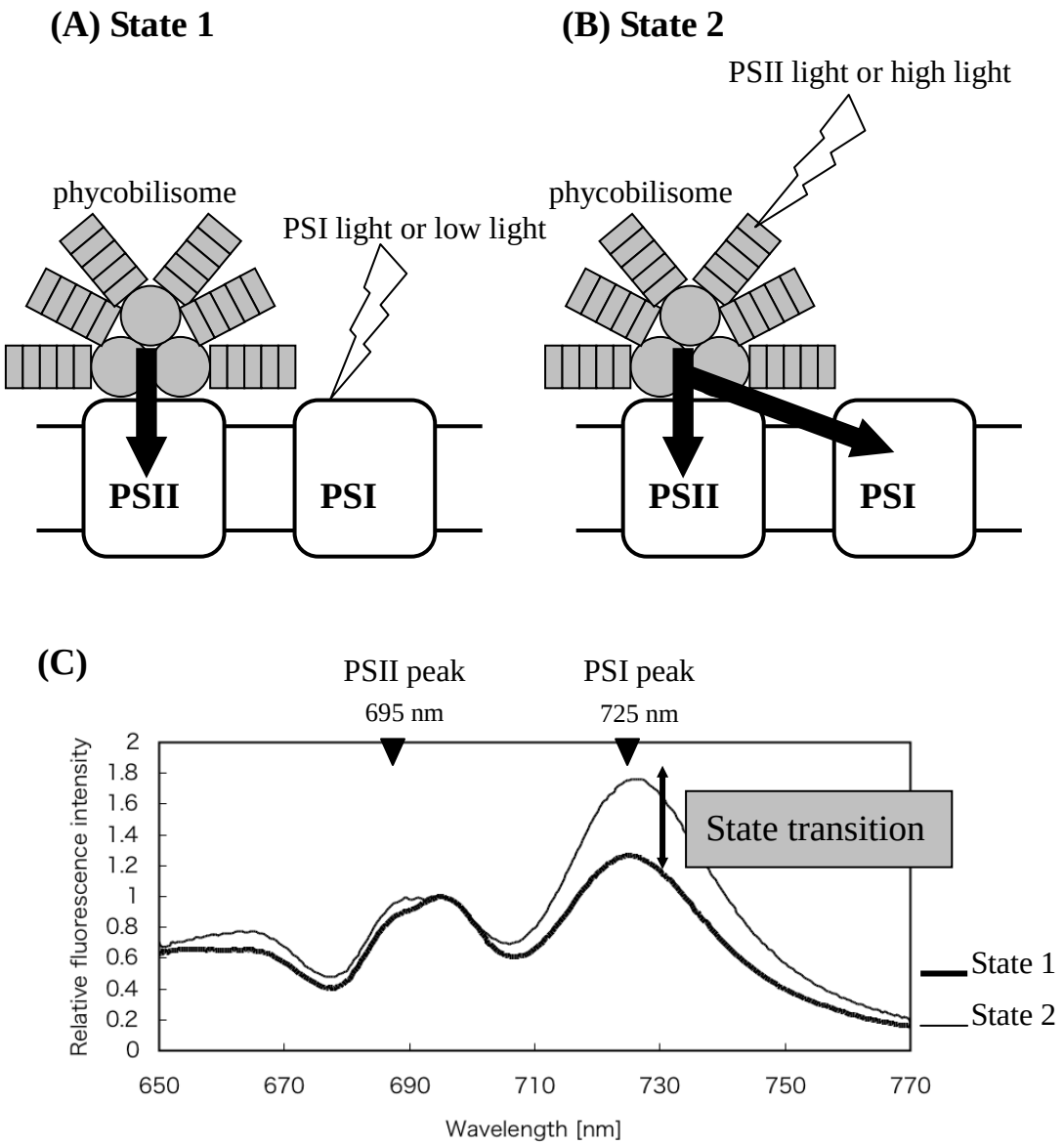

Fig.2. State transition in cyanobacteria and analysis in state transition estimated by chlorophyll fluorescence emission spectra determined at 77 K.

State transition is the regulation of energy distribution between photosystem II (PSII) and photosystem I (PSI). When PSI is preferentially excited, the energy absorbed by phycobilisome is transferred mainly to PSII (State 1, A). On the contrary, when PSII is predominantly excited, the energy is delivered primarily to PSI (State 2, B). The spectra were measured with the cells in either state 1 or state 2 induced by the respective treatments (C). The spectra were normalized to the intensity of the fluorescence peak at 695 nm (PSII peak), and state transition was estimated by the ratio of fluorescence peak at 725 nm (PSI peak) in state 1 and that in state 2.

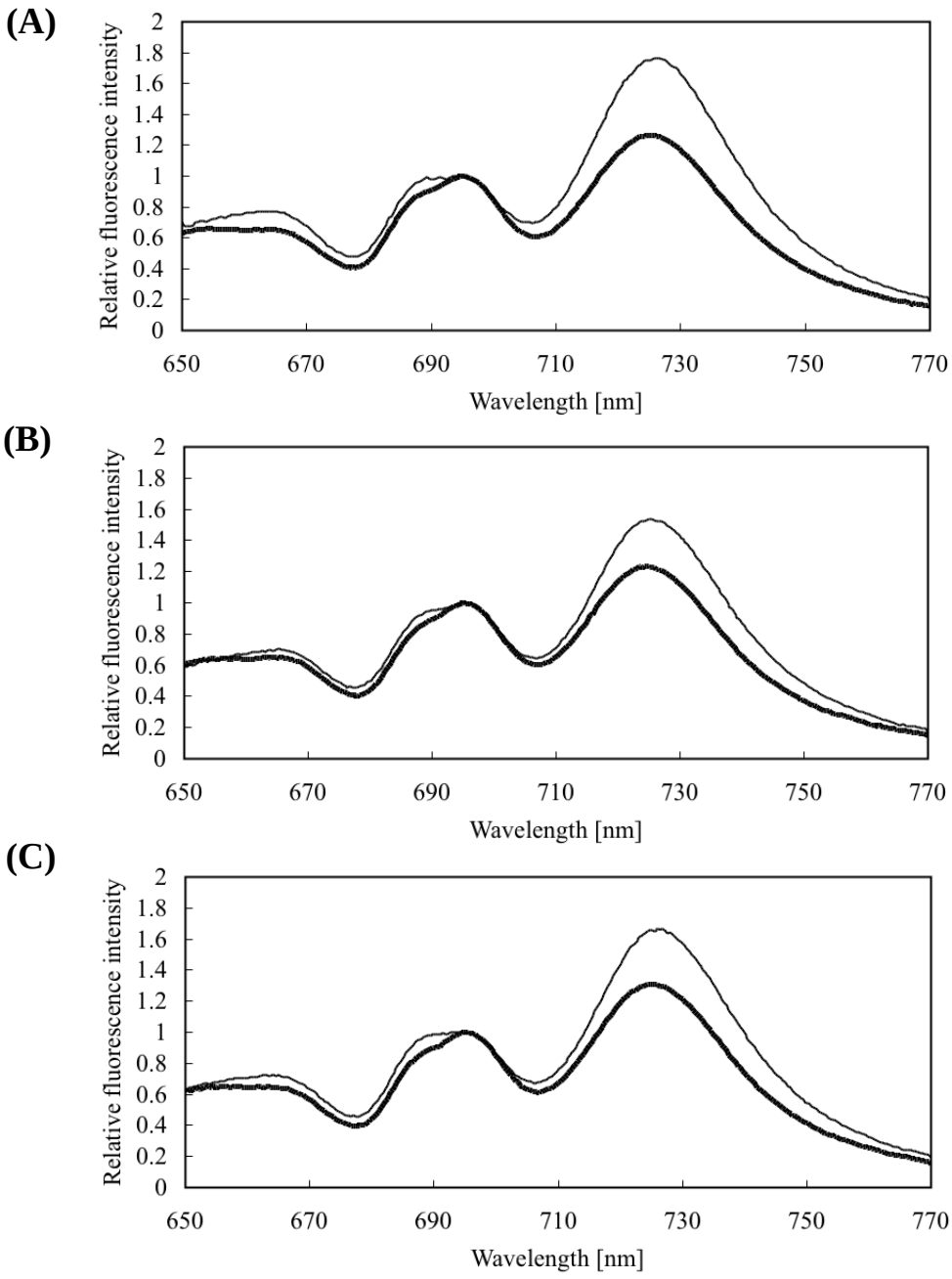

Fig.3. Chlorophyll fluorescence emission spectra of low light-acclimated cells determined at 77 K.

Cells of wild type (A), the *psaL* mutant (B) and the *psaK2* mutant (C) were acclimated to low light (20 μmol $m^{-2}s^{-1}$) and the emission spectra were measured under state 1 (solid line) or state 2 (thin line). Cells were either illuminated with blue light in the presence of 10 μM DCMU (state 1) or incubated in the dark for more than 10 min (state 2). The spectra were normalized at 695 nm (i.e. at PSII peak). Spectra are the average of those determined with three independent cultures.

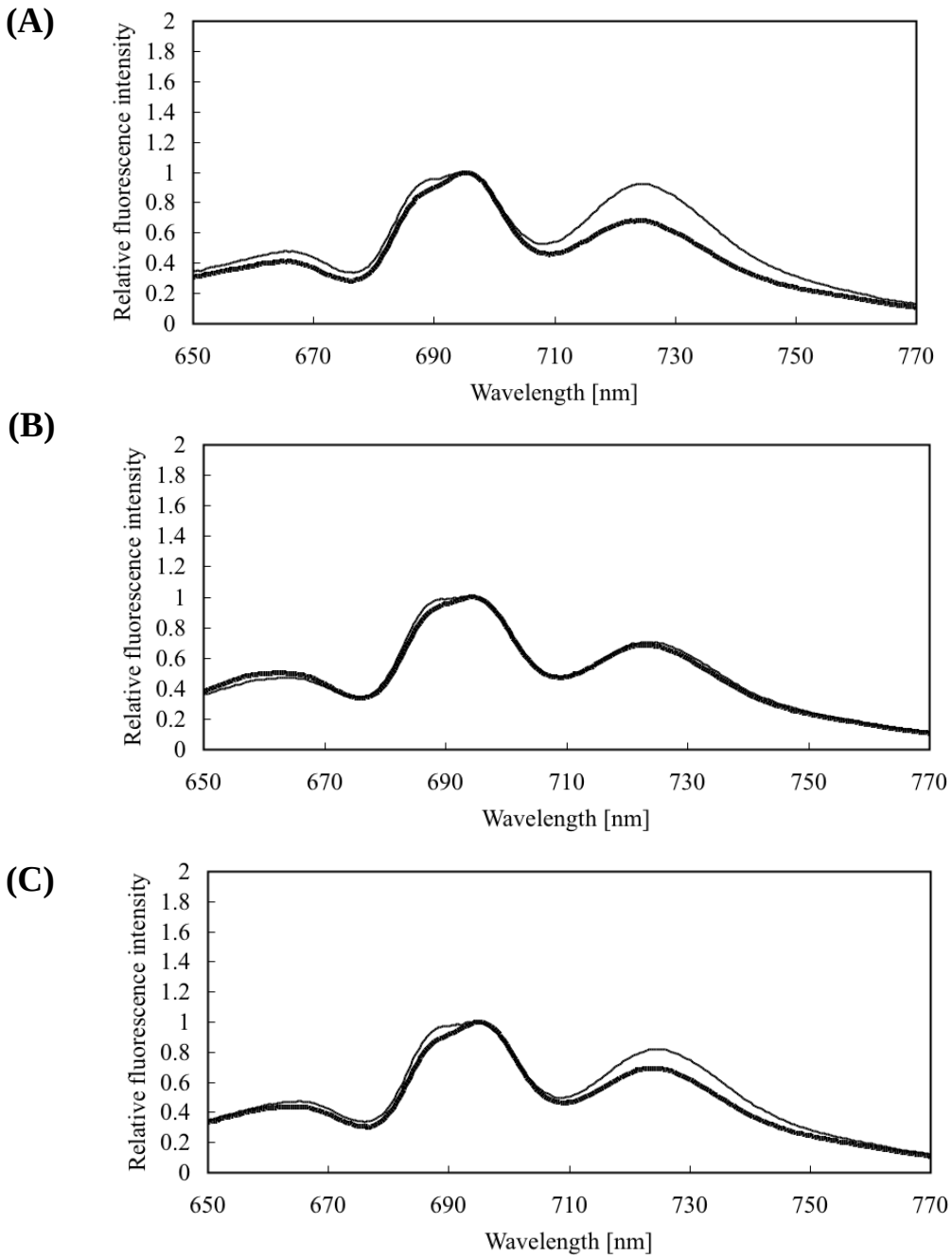

Fig.4. Chlorophyll fluorescence emission spectra of high light-acclimated cells determined at 77 K.

Cells of wild type (A), the *psaL* mutant (B) and the *psaK2* mutant (C) were acclimated to high light (200 μmol $m^{-2}s^{-1}$) and the emission spectra were measured under state 1 (solid line) or state 2 (thin line). Cells were either illuminated with blue light in the presence of 10 μM DCMU (state 1) or incubated in the dark for more than 10 min (state 2). The spectra were normalized at 695 nm (i.e. at PSII peak). Spectra are the average of those determined with three independent cultures.

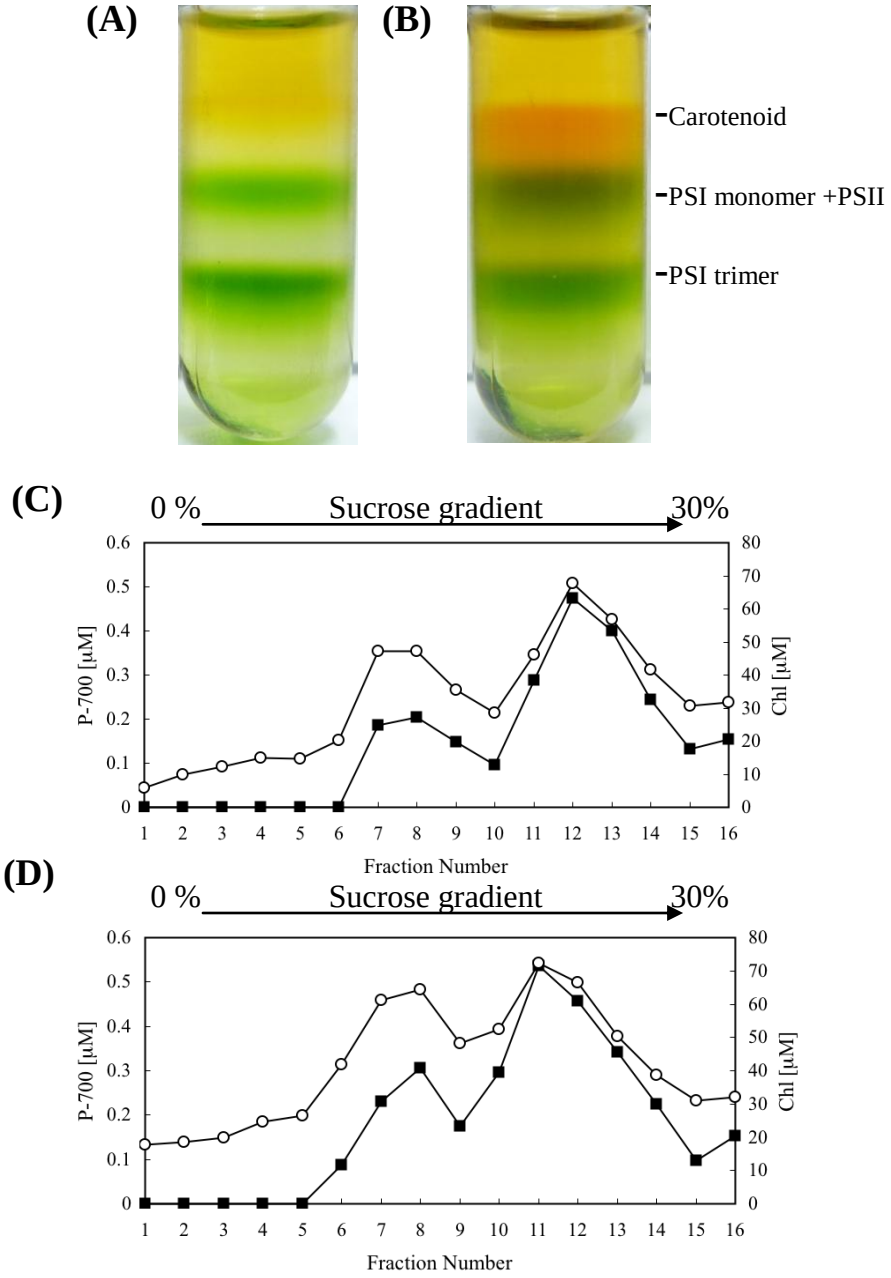

Fig.5. Resolution of monomeric and trimeric forms of photosystem I from wild type by sucrose gradient ultracentrifugation and the determination of P-700 and chlorophyll content.

Thylakoid membranes of wild type acclimated to low light (20 μmol $m^{-2}s^{-1}$) (A, C) or high light (200 μmol $m^{-2}s^{-1}$) (B, D) were solubilized by β-dodecyl-maltoside (the ratio of chlorophyll to β-dodecyl-maltoside was 1:15).Thylakoid membranes containing 0.06 mg chlorophyll were loaded on each sucrose gradient ultracentrifugation. The gradients were fractionated into 16 parts and P-700 content (solid square) and chlorophyll content (open circle) of each fraction were determined.

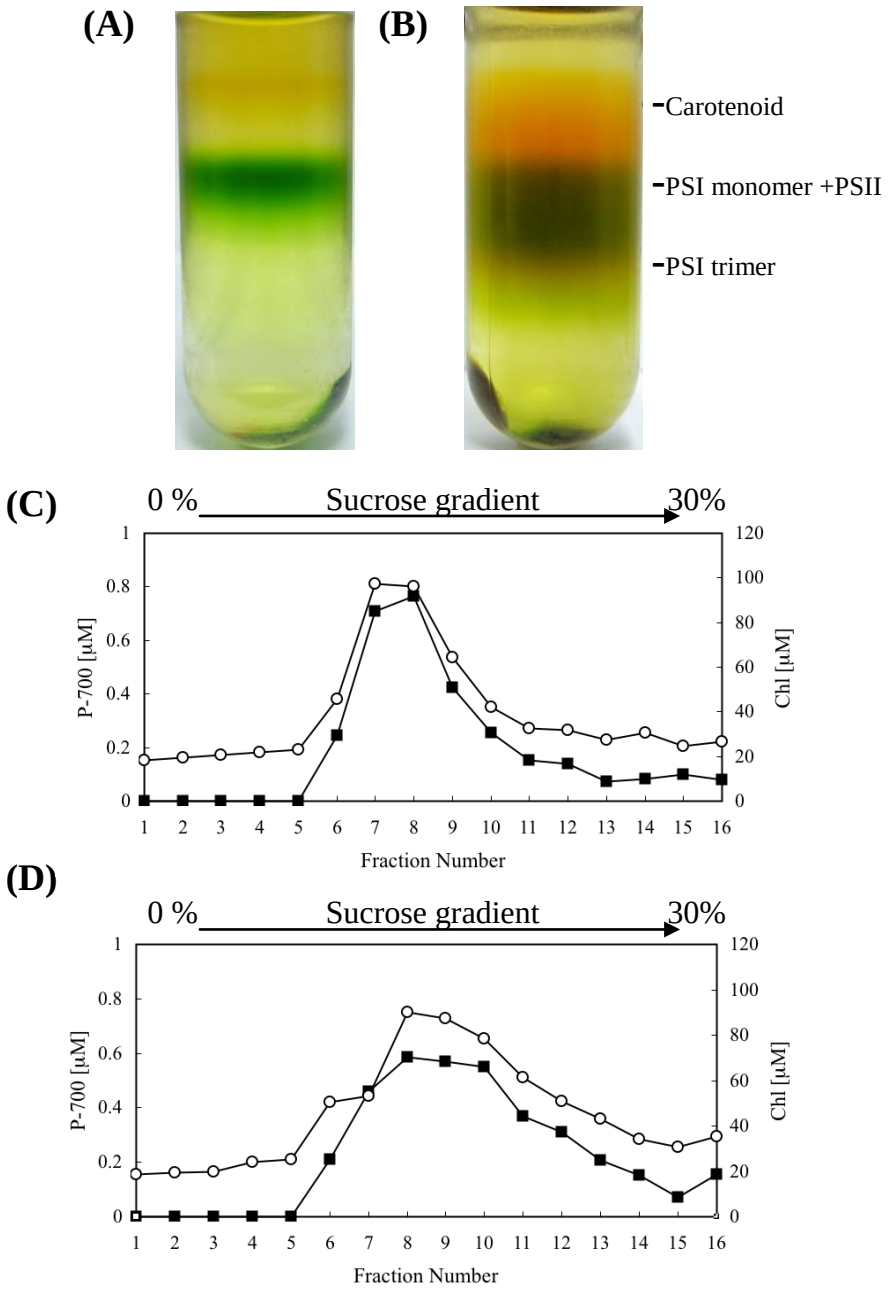

Fig.6. Resolution of monomeric and trimeric forms of photosystem I from the *psaL* mutant by sucrose gradient ultracentrifugation and the determination of P-700 and chlorophyll content.

Thylakoid membranes of the *psaL* mutant acclimated to low light (20 μmol $m^{-2}s^{-1}$) (A, C) or high light (200 μmol $m^{-2}s^{-1}$) (B, D) were solubilized by β-dodecyl-maltoside (the ratio of chlorophyll to β-dodecyl-maltoside was 1:15). Thylakoid membranes containing 0.06 mg chlorophyll were loaded on each sucrose gradient ultracentrifugation. The gradients were fractionated into 16 parts and P-700 content (solid square) and chlorophyll content (open circle) of each fraction were determined.

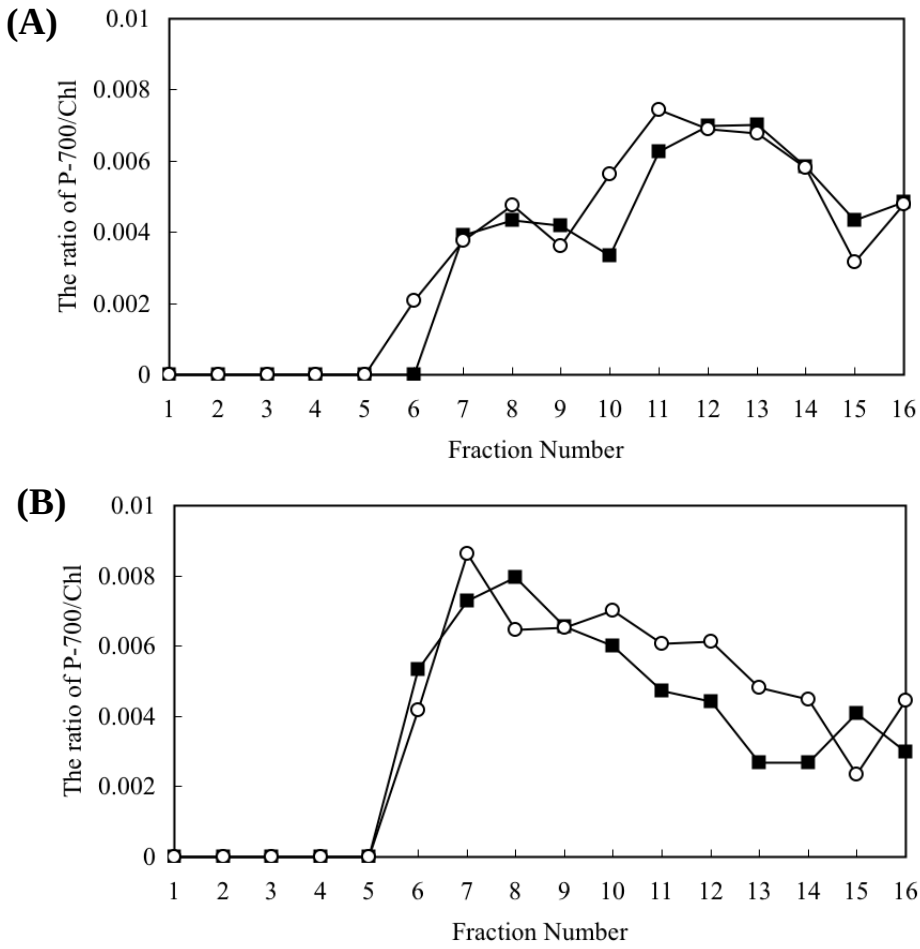

Fig.7. The ratio of P-700 content and chlorophyll content in wild type and the *psaL* mutant cells acclimated to low light or high light conditions

The ratio of P-700 content and chlorophyll content (P-700/Chl) in wild type (A) and the *psaL* mutant (B) cells acclimated to low light (solid square) or high light (open circle) conditions was calculated based on the values of Figures 5 and 6.

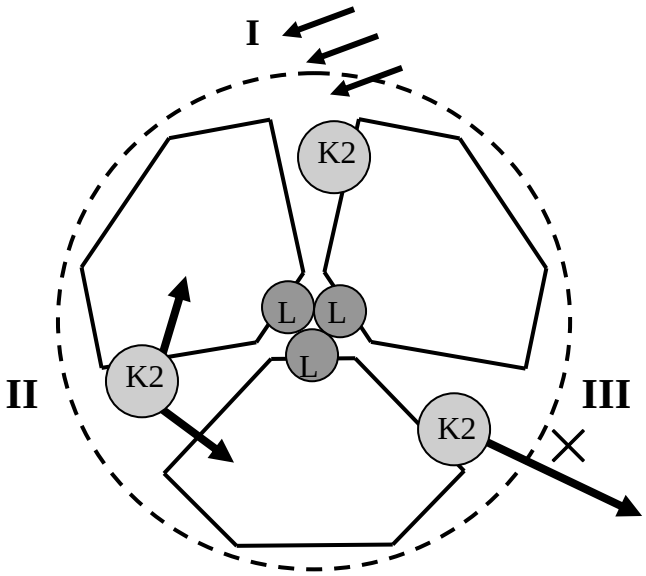

Fig. 8. Model for the function of photosystem I trimer.

This model shows the three possibilities of PSI trimer function. (I) First, in the PSI trimer, all subunits inside are protected from the external stress. (II) Second, PsaK2 subunit can contact with other subunits that cannot contact in the form of monomer. (III) Third, PsaK2 is stabilized or retained in the trimer much more than in the case of monomer.

Tables

Table 1. The number of cells, chlorophyll concentration and phycocyanin concentration of the cell cultures grown for 24 hours.

Wild type, the *psaL* mutant and the *psaK2* mutant were grown under low light (20 μmol $m^{-2}s^{-1}$) or high light (200 μmol $m^{-2}s^{-1}$) condition for 24 hours. At the start of the culture, OD_{730} was adjusted at 0.05. The number of cells was calculated assuming that the wild type cells gave 1.1 x 10^8 cells ml^{-1} at 1 OD_{730}. The concentration of chlorophyll and phycocyanin determined by measuring absorption of these mutants at 678 nm and 620 nm. The values were normalized by OD_{730}.

	Cells [10^7cells ml^{-1}]	Chlorophyll *a* [μg ml^{-1}]	Phycocyanin [μg ml^{-1}]
Low light condition			
Wild type	2.7 ± 0.06	64.6	536.2
Δ psaL	2.6 ± 0.19	64.1	537.7
Δ psaK2	2.8 ± 0.14	68.5	553.2
High light condition			
Wild type	4.9 ± 1.02	33.4	252.4
Δ psaL	5.4 ± 0.65	23.6	169.5
Δ psaK2	5.0 ± 0.43	34.4	247.9

Table 2. The relative photosystem stoichiometry calculated from the chlorophyll fluorescence emission spectra.

The ratio of F_{725}/F_{695} (fluorescence intensity at 725 nm / fluorescence intensity at 695 nm) was determined for low or high light-acclimated cells in either state 1 or state 2. Values are the average ± S.D. of those determined with three independent cultures. The experimental conditions were the same with those of figures 3 and 4.

	State1	State2	State1/State2
	725nm/695nm	725nm/695nm	
Low light condition			
Wild type	1.26 ± 0.04	1.75 ± 0.05	0.72 ± 0.005
Δ psaL	1.23 ± 0.06	1.54 ± 0.08	0.80 ± 0.01
Δ psaK2	1.31 ± 0.03	1.65 ± 0.07	0.79 ± 0.02
High light condition			
Wild type	0.68 ± 0.02	0.92 ± 0.05	0.73 ± 0.01
Δ psaL	0.68 ± 0.06	0.70 ± 0.05	0.97 ± 0.05
Δ psaK2	0.69 ± 0.03	0.82 ± 0.04	0.84 ± 0.03

Table 3. The relative content of photosystem I trimer in wild type and the *psaL* mutant cells acclimated to low light or high light conditions.

Thylakoid membranes were isolated from wild type and the *psaL* mutant cells acclimated to low light or high light were isolated, and solubilized, and fractionated by sucrose gradient ultracentrifugation as in Figures 5 and 6. In the case of wild type, fractions number 7-9 and 11-14 were regarded as PSI monomers and trimers, respectively. In the case of the *psaL* mutant, fractions number 7-9 and 10-13 were regared as PSI monomers and trimers, respectively. The PSI trimer content was estimated as percentage of P-700 content or chlorophyll content, i.e. trimer / (trimer + monomer) x 100.

	PSI trimer contents [%]	
	The determination of P-700 contents	The determination of Chlorophyll contents
Wild type		
Low light	72.3 %	62.1 %
High light	69.6 %	58.2 %
Δ psaL		
Low light	19.2 %	32.2 %
High light	(47.1 %)	(50.3 %)

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