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Assembly of PsaC subunit within stromal ridge of photosystem I core. Comparison of PsaC bound and solution structure

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Introduction

In photosystem I (PS I) charge separation occurs by electron transfer from P_{700} across the membrane via a chain of electron acceptors including chlorophylls (A_0) and quinones (A_1) to the iron-sulfur [4Fe-4S] clusters F_X, F_A and F_B. The PsaC subunit of PS I is a small (9.3 kDa) soluble protein which hosts the two iron-sulfur [4Fe-4S] centers F_A and F_B, which act as terminal electron acceptors. From the iron sulfur centers F_B the electron is donated to the soluble ferredoxin Fd, which docks to the stromal ridge structure made up of the PsaC together with PsaD and PsaE. The X-ray structure at atomic (2.5 Å) resolution of the PS I core complex of *Synechococcus elongatus* has just become available (Jordan 2001). This offers the unique opportunity to compare the structure of small soluble protein subunits (here PsaC, PsaD, PsaE) both, as integral part of the membrane bound functional PS I core complex and as unbound protein in solution. Solution structure information is available at different levels of detail for all three stromal subunits in unbound form, PsaC (Antonkine *et al* 2000 and 2001), PsaD (Jin 1999) and PsaE (Mayer 1999). In the case of PsaC a comparison is of particular interest because within PS I this subunit is tightly packed both, to the PsaA/PsaB stromal heterodimer surface and between the PsaD and PsaE subunits. Nevertheless, PsaC is also a readily soluble protein, which allows determination of its solution structure by NMR.

The tight packing and inner location of the PsaC subunit within the stromal ridge structure of the PS I core complex suggests a substantial network of interprotein binding contacts which are now accessible in the new PS I structure. From this we expect an interesting study case for a multiple interprotein contact network as well as for the step-wise assembly of the involved protein subunits. Last but not least, breakage of the interprotein contacts is likely to have a major influence on the solution structure of the unbound PsaC subunit. In fact, significant differences between bound and unbound PsaC structures are expected. This contrasts to the usual and often confirmed cases in which the structure of soluble small globular proteins turns out to be very similar between NMR solution structure and X-ray crystal structure. On the other hand, differences between bound and unbound structure will be significant for assembly of the PS I complex. It has been shown that PsaC alone can bind to the PsaA/PsaB heterodimer but the EPR properties of the reduced F_A and F_B centers do not correspond to those of the fully assembled complex (Li 1991), unless PsaD is added to the reconstitution mixture. Therefore, the detailed analysis of the PsaC contact network within the PS I complex and comparison with the solution structure can be expected

to yield valuable information on general aspects of protein assembly and protein-protein interaction.

This contribution provides a first account of inter-protein contacts and assembly with above stated goals in mind and outlines the need for a further more specific analysis. For space restrictions we will focus on the PsaC contacts to the PsaA/PsaB heterodimer and postpone closer analysis of additional PsaD and PsaE binding to a forthcoming publication.

Methods

The structural information used here for bound PsaC within the cyanobacterial PS I complex of *Synechococcus elongatus* is available (Jordan 2001) at Brookhaven data bank, PDB entry 1JB0. Solution structure information for unbound PsaC of *Synechococcus sp*. PCC 7002 has been obtained for the reduced (Antonkine 2000) as well as the oxidized state (Antonkine 2001). The NMR solution structure of oxidized unbound PsaC will be deposited to the Brookhaven data bank shortly.

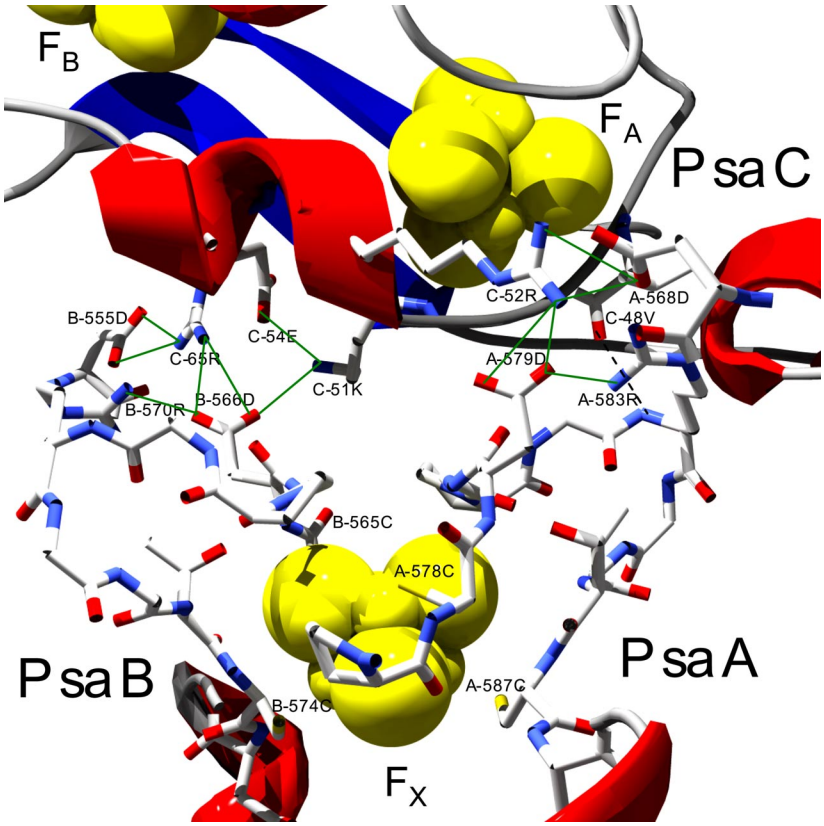


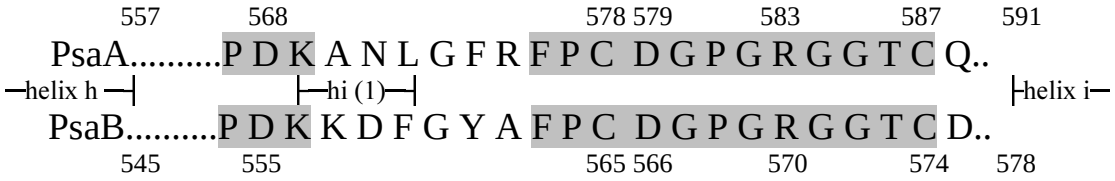
Fig. 1. Central contact area between PsaC subunit and the PsaA/PsaB heterodimer surface. All contacts are listed in Table 1 and concern the loops between helices h and i (which also contain the F_X binding cysteines). Ionic contacts are indicated by solid lines. One H-bond is shown by a broken line. Note the perfect C_2-symmetry of the PsaA and PsaB contributions to the contact network.

Results and Discussion

Analysis of the A/B – C contacts. The central contact area of PsaC bound to the stromal surface of the PsaA/PsaB heterodimer complex is shown in Fig.1. The all amino acids involved are part of the stromal loop connecting the helices h and i of either PsaA or PsaB. The relevant sequence parts are reproduced at the top of Table 1. The ionic and H-bond contacts (with a distance cut off at 3.1 Å) between the respective residue atoms are listed in Table 1 and organized according to progressive numbers in this fully conserved sequence parts of PsaA and PsaB (shaded sequence area). At both sides of the contact network in Fig.1 note the corresponding contact system A/B-C-A/B with the same Asp – Arg – Asp binding motif. The respective PsaC Arg residues are: C-52R for the PsaA contact and C-65R for the PsaB contact. Note that these argenines belong to very different structural elements of PsaC, the former to the central pair of equally charged residues C-51/52 at the start of the one turn α-helical connection

from the F_A to F_B cysteine binding motif, while the latter sits in the center of the final β-sheet (β4a) connecting to the PsaC C-terminus. Although the network of contacts in Fig.1 looks quite different for the PsaA(right)- versus PsaB(left)-side corresponding to the different amino acids and structural elements involved from the PsaC side, we stress the perfect local C_2-symmetry of the whole A/B heterodimer complex and correspondingly its stromal surface. A rotation of the PS I complex around this local C_2-symmetry axis does not only overlay perfectly all binding residues and atoms of the PsaA and PsaB stromal surface but also both C_ζ-atoms of C-52R and C-65R. In fact, all contacts on the right and left in Fig.1 can be formed equally well when PsaC binds in the C_2 rotated form.

Table 1: Inter-(or intra-)protein contacts (ionic and H-bond) between PsaA/PsaB heterodimer and PsaC subunit. The arrangement of the participating PsaA and PsaB amino acids reflects the perfect C_2-symmetry in this part of the AB stromal surface. This is also obvious from the sequence comparison (above table) for part of the stromal loop between the transmembrane helices h and i of PsaA and PsaB. Framed areas are fully conserved and contain all (numbered) contact partners to PsaC and the cysteines of the F_X binding motifs which are also listed in the Table.



PsaA $\overset{[Å]}{\longleftrightarrow}$ PsaC	Contact type	PsaB $\overset{[Å]}{\longleftrightarrow}$ PsaC
A-568D (Asp $O_{\delta 2}$) $\overset{2.9}{\longleftrightarrow}$ C-52R (Arg $N_{\eta 1}$)	inter	B-555D (Asp $O_{\delta 1}$) $\overset{3.0}{\longleftrightarrow}$ B-555D (Asp $O_{\delta 2}$) $\overset{3.1}{\longleftrightarrow}$ C-65R (Arg $N_{\eta 1}$)
A-578C (Cys)	F_X	B-565C (Cys)
A-579D (Asp $O_{\delta 1}$) $\overset{2.7}{\longleftrightarrow}$ C-52R (Arg $N_{\eta 2}$)	inter	B-566D (Asp $O_{\delta 2}$) $\overset{2.6}{\longleftrightarrow}$ C-65R (Arg $N_{\eta 2}$)
A-579D (Asp $O_{\delta 2}$) $\overset{3.0}{\longleftrightarrow}$ C-52R (Arg N_{ε})	inter	B-566D (Asp $O_{\delta 1}$) $\overset{2.7}{\longleftrightarrow}$ C-51K (Lys N_ζ)
A-579D (Asp $O_{\delta 1}$) ┐ 3.0 A-583R (Arg $N_{\eta 2}$) ┘	intra A or B	┌B-566D (Asp $O_{\delta 2}$) ┐ 2.7 └B-570R (Arg $N_{\eta 2}$) ┘
A-583R (Arg N) $\overset{2.8}{\Longleftrightarrow}$ C-48V (Val O)	intra C H-bond A⇐⇒C	┌C-51K (Lys N_ζ) ←┐ 3.0 └C-54E (Glu $O_{\varepsilon 1}$)←┘
A-587C (Cys)	F_X	B-574C (Cys)
	H-bonds B⇐⇒C	B-678Q (Gln $O_{\varepsilon 1}$) $\overset{3.1}{\Longleftrightarrow}$ C-80Y (Tyr OH) B-702K (Lys N_ζ) $\overset{3.1}{\Longleftrightarrow}$ C-73T (Thr $O_{\gamma 1}$) B-703P (Pro O) $\overset{2.7}{\Longleftrightarrow}$ C-80Y (Tyr OH)

This leaves us with the important questions: How can PsaC distinguish between the two perfectly equivalent binding options (F_A- F_B connecting vector pointing to the upper left as in our figures or to the right)? Do symmetry breaking features exist which favor one of the two PsaC binding options? The only obvious symmetry breaking contact concerns the C-terminus (C-73T and C-80Y) which has specific H-bond contacts to PsaB only, as listed at the bottom of Table 1. However, these contacts involve major conformational changes when PsaC binds from the solution structure and is therefore unlikely to be the main decisive element. As expected for the tightly bound structure of the fully assembled PS I complex, an overlay of both C_2-symmetric positions of PsaC results in a large number of clashes between side chains in the alternative binding option to that of Fig.1. PsaC binding to the A/B heterodimer alone, in the absence of PsaD, results in weaker binding, which is consistent with the previous EPR data (Li 1991). Nevertheless, the symmetry breaking aspects mentioned above may introduce sufficient imbalance between the two PsaC binding options in a kind of dynamic equilibrium that favors the correct choice which can then be stabilized and locked into tight binding by the presence of PsaD and PsaE during PS I assembly. We add a final point concerning the network of contacts between PsaC and the stromal surface of the A/B heterodimer. The ionic contacts of the main Arg residues on PsaC and A-579D and B-566D (connected also intramolecularly to A-583R and B-570R, respectively) will be strengthened by the presence of hydrophobic patches nearby. Indeed, the headgroups of C-52R and C-65R have indeed hydrophobic neighbors as seen from the sequences above Table 1.

Details of additional binding of PsaD and PsaE will be described in the forthcoming publication. We mention here only a few key points. More important is PsaD binding. This alone establishes the tightly bound state of PsaC (Li 1991). Indeed, multiple contacts are added both to PsaA/PsaB and to PsaC upon binding of PsaD. The N-terminal part (D-1 to about D-95) clearly stabilizes the specific PsaC C-terminus binding area to PsaB (near the quinone binding site of the PsaB-side). In the middle part (D-95 to D-109) PsaD forms a multiple contact “clamp” around the stromal central area of PsaC with the exception of a short loose loop including D-104K and D-107K, presumably involved in Fd docking (Jordan 2001). The next part (D-112 to D-122) is strongly involved in stabilizing the β-sheet (β4a) formation of PsaC which is not found in the solution structure of unbound PsaC. Although PsaE has considerably less contacts than PsaD and therefore may not add much PsaC stabilization, it has noteworthy contacts to PsaA near the quinone binding site of the PsaA-side. These few remarks clearly indicate significant functional relevance of many of these contacts.

Owing to the space restrictions we have to postpone a detailed description and evaluation of the differences between the PsaC solution and bound structure. The same holds for a revisit of the orientation dependent EPR studies of the reduced F_A and F_B clusters in single crystals of PS I. With the X-ray structure at atomic resolution, these data can be used to assign the g-tensors to the cluster axes which turn out to be different for F_X and F_A, F_B on the other hand. For further details see a forthcoming publication.

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