Protein−Protein Interactions between the Photosystem I Reaction Center Core and the PsaC Subunit

Hung-Cheng Chiou[†] and John Biggins*

Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island 02912

Received: February 19, 1998; In Final Form: August 18, 1998

The functional reconstitution of Photosystem I reaction center cores by wild-type and mutant PsaC subunits at high ionic strength indicated that hydrophobic interactions are dominant in stabilizing the reaction center. Deletion of the region E27 through C34 in the PsaC backbone resulted in a mutant subunit with impaired reconstitution, confirming that this segment is required for binding. Restoration of the deletion mutant protein to full length using a series of inserts of graded hydrophobicity, including residues with side chains of various sizes, established that this domain is involved in hydrophobic interactions with the reaction center core. The results indicate that a specific proline residue may be necessary to confer the requisite secondary structure in this domain of the PsaC subunit. The overall data provide additional indirect support for orientation of the PsaC subunit on the reaction center such that the F_B cluster is close to the core F_X redox center. We also report that an electrostatic interaction between residue D9 of PsaC and a complimentary basic residue in the core binding site is essential, and most likely ensures the correct orientation of the PsaC subunit on the core during assembly of the reaction center. We previously reported that residue R561 of the PsaB core subunit is also involved in an electrostatic interaction and contributes to the stability of Photosytem I [Rodday, S. M.; Schulz, R.; McIntosh, L.; Biggins, J. *Photosynth. Res.* **1994**, 42185]. We report here that this PsaB core residue is not paired with D9 of the PsaC subunit and postulate that it may be involved in a PsaB(A) intracore interaction.

Introduction

Charge separation in the PS I reaction center is promoted by fast electron transfer through several transient redox intermediates localized on the PS I heterodimer that is composed of two large subunits, PsaA and PsaB, hereafter referred to as the core. The terminal steps through two FeS centers, F_A and F_B, occur on the PsaC subunit that is bound to the stromal surface of the core and further stabilized by interaction with PsaD, a subunit which is also involved in docking ferredoxin.

We proposed a working model for the interaction of the PsaC subunit with the core[1] and suggested that the two identical interhelical loops [CDGPRGGTC] between helices Vlll and lX in PsaA and PsaB, containing the four cysteine ligands which coordinate the FeS center F_X, may be involved in PsaC binding. The model was tested by chemical modification[1] and site-directed mutagenesis experiments[2] which established the essential role of the R in the interhelical loop of the PsaB subunit (R561 in *Synechocystis* sp. PCC 6803). This finding implicated an electrostatic interaction between the loop R and an acidic side chain on PsaC, thereby prompting a search to identify such a complimentary residue on the small subunit.[3]

Charge reversal mutagenesis of all residues with negatively charged side chains in PsaC, in single changes to R, led to the construction of a series of mutant PsaC proteins that were then tested for binding to core preparations using a functional assay for PS I electron transfer.[3] The investigation revealed that residue D9 of PsaC was required for reconstitution and, indirectly, that the negative charge was most likely essential for binding. Mutants E27R and D32R also showed decreased levels of reconstitution, but the negative charges at positions 27 and 32 were not found to be necessary because the corresponding alanine mutants, E27A and D32A, were observed to behave similar to wild-type PsaC. The residues E27 and D32 are localized in an eight residue region of PsaC (E27−C34) which, when deleted, resulted in a mutant PsaC subunit that was also impaired in binding.[4] Thus, the results of our PsaC point mutants, E27R and D32R,[3] corroborate the observation obtained with the eight residue deletion strain.[4]

Here we extend investigations on the functional interaction of the PsaC subunit with the PS I reaction center core. The specific objectives were to obtain direct evidence that residue D9 of PsaC participates in an electrostatic interaction with a positively charged residue on the core[1−3] and to elucidate the structural role of the eight residue domain in PsaC (E27−C34) implicated in the binding mechanism.[4]

Experimental Section

Biological Preparations. PS I was isolated from the cyanobacterium *Synechocystis* sp. PCC 6803.[5] The concentration of P700 was determined by difference spectrophotometry using extinction coefficients of 64 000 M^{-1} cm^{-1} at 700 nm[6] and 6500 M^{-1} cm^{-1} at 820 nm.[7] PS I cores were prepared by dissociation of the low molecular mass subunits from PS I using urea as described.[1] The extent of electron transfer between the PS I core and the terminal redox centers on PsaC was selected as a stringent criterion for testing the interaction between the

* Corresponding author. Tel: 831-688-6508. Fax: 401-863-1201. E-mail: biggins@pacbell.net.

[†] Current address: Department of Plant Biology, Arizona State University, Tempe, AZ 85287-1601.

S1089-5647(98)01211-5 CCC: $15.00 © 1998 American Chemical Society
Published on Web 09/24/1998

TABLE l: Design of Oligonucleotides Used for Construction of the Mutant PsaC Subunits: Sequences of the Coding Strand

PsaC mutant	oligonucleotide sequence	amino acid sequence
RR	5′-TTAACCGTGTTCCTTGGCGCGGCC	NRVPWDGC
QQ	5′-TTAACCAGGTTCCTTGGCAGGGCC	NQVPWQGL
SN	5′-TTAACAGCAACAGCAACGATAACC	NSNSNDNL
AV	5′-TTAACGCAGTTGCAGGTGCAGGTC	NAVAGAGL
PV	5′-TTAACGCAGTTCCAGGTGCAGGTC	NAVPGAGL

PS I core and wild type and mutant PsaC subunits. Accordingly, functional reconstitution of the cores using PsaC in vitro was performed at 4 °C by modifications of procedures developed by Golbeck and co-workers[8−10] as described[1] except that PsaD was included. The PS I dissociation and the extent of reconstitution of the cores was followed by flash kinetic spectrophotometry as outlined below.

Mutagenesis and Production of Wild-Type and Mutant PsaC Apoproteins. *psa*C from *Synechococcus* sp. PCC 7002[11] was kindly provided by Dr. J. H. Golbeck. The PsaC E27−C34 deletion clone (PsaC-ΔL) was engineered by PCR and a unique *Afl* ll restriction site was created at the new junction L26K35. Eight amino acid segments (Table 1) were then introduced at the *Afl* ll site by linearization of the PsaC-ΔL plasmid followed by ligation of double stranded DNA inserts. The DNA inserts were prepared by annealing complementary 5′-phosphorylated 24-mer deoxyoligonucleotides obtained from Operon Technologies, Alameda, CA, and used without further purification. The coding strand sequences used for the inserts are shown in Table 1. The mutants were selected from *Escherischia coli* JM 109 transformants and miniprep scale plasmid DNA was used for dideoxy-DNA sequencing to confirm the insertion and correct sequence. Cloned plasmid DNA of confirmed sequence was then established in the expression host *E. coli* BL21(DE3) pLysS for protein production. Large scale growth of the host cells, protein overproduction, and purification were conducted as described previously.[3] The purity of the wild type and mutant PsaC apoproteins was verified by SDS-PAGE.

Spectroscopy. Laser flash transient absorption spectrophotometry was performed at either 700 or 820 nm to monitor the recombination kinetics between $P700^+$ and reduced acceptors following charge separation using standard protocols[6] and optical apparatus and signal processing methods as described.[12]

Results

Electrostatic Interactions. To investigate whether the residue D9 of PsaC is unequivocally involved in an electrostatic interaction with a positively charged domain in its binding site on the PS I core heterodimer,[3] we performed functional reconstitutions of PS I cores with the mutant PsaC D9R in the presence of phosphate. It is known that arginyl phosphate and diarginyl phosphate interactions are prevalent in the active sites of many enzymes, and the structure of these complexes may be predicted based upon the crystallographic study of the model compound dimethylguanidinium phosphate.[13] Therefore, we reasoned that the postulated electrostatic repulsion between PsaC D9R and a positively charged residue in the wild-type core binding site[3] might be "rescued" by the formation of such phosphate complexes. Examples of primary data showing the results of such reconstitutions are presented in Figure 1. Recombination reactions between $P700^+$ and reduced PS I acceptors for wild-type PS I (trace 1), PS I cores devoid of PsaC (trace 2), and cores reconstituted with PsaC (trace 3) are shown as controls in Figure 1A. The optical signals indicate that the

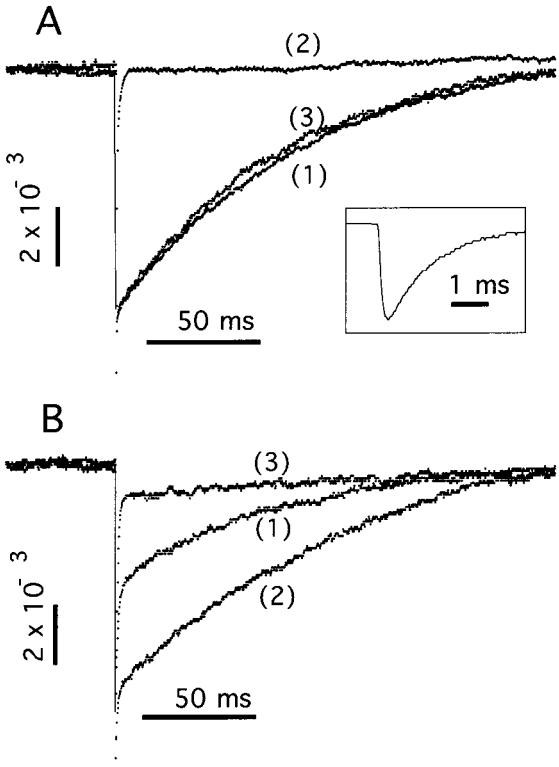

Figure 1. Flash-induced absorption transients measured at 700 nm showing recombination reactions between $P700^+$ and reduced acceptors in PS I. (A) Controls for intact PS I (trace 1), PS I cores devoid of PsaC (trace 2), and cores reconstituted with PsaC (trace 3). The insert shows the kinetic trace of the PS I core on a faster time scale. (B) PS I cores reconstituted with the PsaC mutant D9R at various concentrations of P_i. No addition (trace 1), 10 μM to 1 mM range (trace 2) and 100 mM (trace 3). The samples contained 13 μg of chlorophyll/mL, 5 mM Na ascorbate, 100 μM TMPD, and PsaC protein (molar ratio PsaC/P700 = 40).

ca. 1 ms recombination reaction between $P700^+$ and F_X^- in the core preparation (Figure 1A, trace 2 and insert) is replaced by a slower kinetic phase identical to that for intact PS I when reconstituted using PsaC (trace 3) indicating restoration of complete forward electron transfer to the terminal centers, F_A and F_B. Figure 1B shows corresponding data for the reconstitution of cores using the mutant PsaC D9R. As was shown previously,[3] only ca. 55% restoration of electron transfer was observed (trace 1) which we suggested was most likely due to impaired interaction of the mutant subunit with the core. However, inclusion of low concentrations (10 μM to mM range) of P_i in the reconstitution protocol resulted in restoration of wild-type levels of electron transfer (trace 2). Higher concentrations of P_i were found to inhibit the reconstitution (trace 3) and the full set of data is shown in Figure 2 for both wild-type and mutant PsaC subunits. The slope of the inhibition of reconstitution using the D9R mutant PsaC is similar to that observed for the wild-type PsaC. We suggest that at low concentrations the P_i interacts with the R at position 9 on the mutant PsaC and another basic residue in the core binding site, most likely another R, providing favorable ion pairing between the PS I subunits. If the basic residue on the core is indeed an R, then four hydrogen bonds could be involved between the two guanidinium groups and a single P_i group in this interaction.[13] The inhibition of reconstitution by both wild type and mutant PsaC subunits at higher concentrations of P_i most likely occurs by electrostatic repulsion between a core arginyl phosphate and residue D9 in the case of wild-type PsaC, and D9-arginyl phosphate in the case of the mutant D9R. Figure 3 shows that a similar rescue

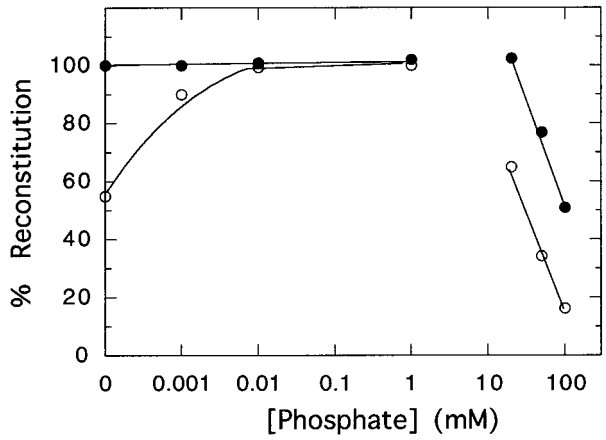

Figure 2. Reconstitution of PS I cores by wild-type PsaC and PsaC mutant D9R at various concentrations of P_i. Experimental conditions were as in Figure 1. (●) Wild-type PsaC; (○) mutant D9R.

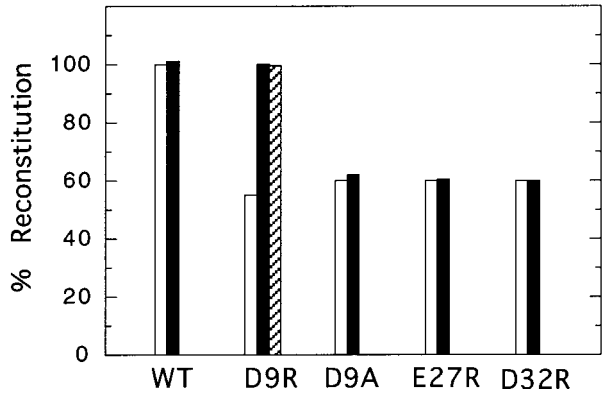

Figure 3. The effect of divalent anions on the in vitro reconstitution of PS I cores using wild-type PsaC and the PsaC mutants D9R, D9A, E27R, and D32R. Experimental conditions were as in Figure 1. Open bar, no addition of P_i; solid bar, plus 10 μM to 1 mM P_i; and hatched bar, plus 10 μM to 1 mM molybdate.

of reconstitution of PS I cores by PsaC D9R can also be achieved using low concentrations of molybdate as well as P_i. This indicates that the anions must be forming ion pairs between two positively charged domains on the core and the PsaC subunit. We conclude that the negatively charged residue D9 of wild-type PsaC interacts with the same positively charged domain on the core.

We had shown previously that, although the mutant PsaC subunits E27R and D32R were impaired in binding to the core, the corresponding alanine mutants, E27A and D32A, behaved similar to wild type.[3] This suggested that the negative charges at positions 27 and 32 were not required for the PsaC−core interaction. The data shown in Figure 3 confirms the earlier observations because the unfavorable reconstitutions of cores using the PsaC mutants E27R and D32R could not be rescued using P_i. As expected, the alanine mutant D9A also showed no enhancement of reconstitution in the presence of P_i. The data establish that the specific residue at PsaC position 9 is involved in an electrostatic interaction between the small subunit and the PS I core.

In a test of our working model of the binding site for PsaC on the core heterodimer, we reported[2] that the core mutant R561E (PsaB) in the cyanobacterium *Synechocystis* sp. PCC 6803 was unstable and that in vitro reconstitution studies using wild-type PsaC confirmed that the interaction between PsaC and the mutant core was impaired as a result of the residue change. Recently, DNA sequence analysis of a revertant of

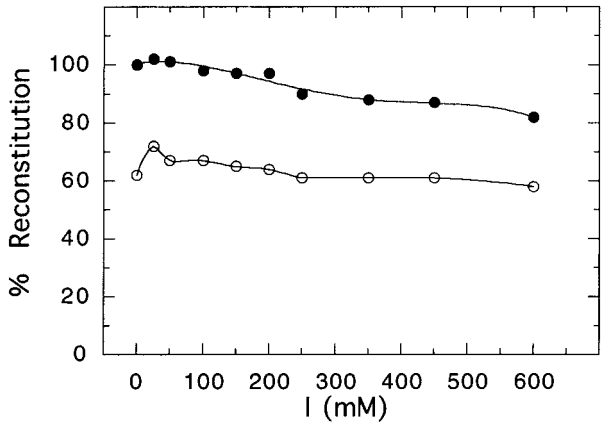

Figure 4. Effect of ionic strength [NaCl] on the in vitro reconstitution of PS I cores using wild-type PsaC and the PsaC mutant D9R. Experimental conditions were as in Figure 1. (●) Wild-type PsaC; (○) mutant D9R.

this strain to wild-type phenotype revealed the intragenic change R561E to E561K (Dr. Lee McIntosh, personal communication) confirming the requirement for a residue with a positively charged side chain at position 561 in PsaB. Therefore, we investigated the possibility of showing a specific interaction between the residues R561 of PsaB and D9 of PsaC by conducting an in vitro reconstitution of the core mutant R561E with the PsaC mutant D9R. A positive result of this experiment would have been observation of a level of reconstitution close to that normally seen in controls between wild-type PsaC and wild-type cores (100%). However, the result of this potential compensatory experiment was negative, the reconstitution of core R561E by PsaC D9R being ca. 55% and identical to that using wild-type PsaC as observed previously.[2] We conclude that candidate residue D9 of PsaC does not interact with R561 of the F_X intercysteinyl loop of PsaB.

Hydrophobic Interactions. We suggested previously that the electrostatic interaction between D9 of PsaC and a basic residue in the core binding site be used to establish the correct orientation of the small subunit on the core and then hydrophobic interactions between residues on the contact surface between the two subunits stabilize the complex. To examine the latter possibility we studied the effect of ionic strength on the in vitro reconstitution of the PS I core using both wild type PsaC and mutant D9R subunits (Figure 4). The level of reconstitution in both cases was only slightly affected by increasing the NaCl concentration to 600 mM confirming that the binding of PsaC to the core was, in fact, primarily stabilized by hydrophobic interactions. The small but reproducible increase in reconstitution of the PS I core by PsaC D9R at low ionic strength is probably due to the shielding effect by Cl^- on the basic residue(s) on the core and the arginine of PsaC D9R. The low concentration of salt most likely has the effect of reducing the level of unfavorable electrostatic repulsion between the two interacting domains.

Role of the PsaC Strand 3−4 β-sheet. Although the negative charges on PsaC at positions 27 and 32 were shown to be unnecessary for PsaC binding,[3] the region in the protein secondary structure is strongly implicated in the binding mechanism. Naver et al.[4] reported that deletion of the eight residues E27 through C34 (mutant PsaC-ΔL), which is most likely part of a small region of β-sheet between strands 3 and 4 in the PsaC secondary structure,[14] resulted in a polypeptide that folded correctly as judged by the fact that the two FeS centers could be successfully inserted. However, the PsaC-ΔL mutant subunit promoted very low levels of reconstitution of

TABLE 2: Effect of Various Substitutions in the Strand 3-4 β-Sheet Domain of PsaC on the Reconstitution of PS I Cores[a]

PsaC mutant	amino acid sequence	hydrophobicity[b] (kJ mol^{-1})	% reconstitution[c]
AV	NAVAGAGL	6.14	75
PV	NAVPGAGL	4.85	93
D32A[d]	EMVPW*A*GC	3.85	96
E27A[d]	*A*MVPWDGC	3.45	95
WT	EMVPWDGC	−0.15	100
QQ	NQVPWQGL	−2.12	76
D32R[d]	EMVPW*R*GC	−4.65	60
E27R[d]	*R*MVPWDGC	−5.05	65
RR	NRVPWRGL	−11.32	66
SN	NSNSNDNL	−13.8	64
ΔL	deletion		66

[a] Inserts cloned into the mutant subunit PsaC-ΔL. [b] Calculated using data from ref 15. These reconstitutions were conducted in the absence of PsaD. [c] The reconstitution of PS I cores was determined as in Figure 1. The error limits were determined to be ±1%. [d] Data from ref 4. The residue change from the wild-type sequence is underscored in each case.

PS I cores (52%) and $NADP^+$ photoreduction, and they concluded that the eight-residue domain of PsaC interacts with the PS I−A/B heterodimer (core). Here we extend this line of investigation in an attempt to establish the mechanism of the interaction between this domain of PsaC and the core.

The fact that the PsaC point mutants E27R and D32R in this region interact poorly with the core[3] could be because introduction of the bulky hydrophilic arginine side chains resulted in either (a) a steric interference in PsaC binding, (b) a disruption of the β-sheet secondary structure, the architecture of which may be specifically required for interaction with the binding site, or (c) a general decrease in hydrophobicity in the domain which may weaken the overall protein−protein interaction.

To investigate these possibilities we constructed the same PsaC-ΔL mutant from the cyanobacterium *Synechococcus* sp. PCC 7002 and confirmed that the deletion mutant was impaired in the in vitro reconstitution of PS I cores (Table 2) as judged from flash kinetic analysis of recombination reactions as outlined in Figure 1. We then engineered several mutant subunits by cloning eight residue inserts into the PsaC-ΔL strain using designs of varying hydrophobicity and side chain length and tested them in the core reconstitution assay (Table 2). It can be seen that all the PsaC mutants with hydrophilic inserts were impaired in binding, and the RR and SN mutants reconstituted at levels even lower than the deletion strain itself. The similarity between the low levels of reconstitution of the RR and SN mutants suggests that it is the reduced hydrophobicity of the domain rather than the steric impedance caused by the large side chains that leads to reduced levels of PsaC binding. Mutant QQ, which has residues with intermediate length side chains but overall sequence similar to that of wild-type PsaC, was only marginally better than the deletion strain. On the other hand, mutant AV, which has an insert of hydrophobic residues with small side chains and no resemblance to wild-type sequence, although still impaired in binding, reconstituted at a level much higher than the mutants with hydrophilic inserts (compare particularly mutant SN). More significantly, inclusion of a proline residue in the AV sequence at a position identical to that found in wild-type PsaC resulted in a mutant (PV) that reconstituted at a level 93% of wild type. Overall, the data show that there is a good correlation between hydrophobicity of the domain and PsaC binding, and the proline residue may be necessary to ensure the correct folding of this region of the PsaC backbone.

Discussion

Electrostatic Interactions. The results presented above considerably extend our previous reports on the binding of the PsaC subunit to the core heterodimer of the PS I reaction center.[1−3] We have now established that a specific electrostatic interaction between residue D9 on PsaC and a basic residue(s) on the core is required for subunit binding. We reported earlier[2] that the core residue R561 of the PsaB subunit is also involved in PsaC binding through an electrostatic interaction. However, the potential in vitro compensatory experiment matching the PsaC mutant D9R with the core mutant R561E with the possibility of observing wild-type levels of reconstitution was negative. This result is unequivocal and we conclude that D9 of PsaC is not paired with R561 of PsaB. Clearly, additional mutagenesis experiments are now required to establish the residue pairing with D9, and the R in the homologous intercysteinyl loop of PsaA (R579 in *Synechocystis* sp. PCC 6803) is a prime candidate. An alternative possibility for the role of residue R561 in PS I structure may be that it is involved in an essential PsaB intrasubunit interaction affecting the PsaC binding site itself rather than in a PsaB−PsaC intersubunit ion pair. We conclude that the electrostatics involving D9 of PsaC, R561 of PsaB, and their ion pairs are weak secondary interactions most likely serving to position the small PsaC subunit in its correct orientation on the core during assembly. This contrasts with other related interactions such as those between PS I/plastocyanin and cytochrome c_{552},[16] cytochrome *c*/cytochrome oxidase,[17] and ferredoxin/ferredoxin−$NADP^+$ reductase[18] where electrostatic forces are strongly implicated because the protein−protein interactions were significantly affected by relatively low ionic strengths ([NaCl] ≥ 50 mM).

Hydrophobic Interactions. We also observed that the in vitro reconstitution of PS I cores by PsaC is not significantly attenuated if conducted in the presence of high concentrations of salt. This result establishes that hydrophobic interactions are dominant in stabilizing PsaC in the core binding site. We confirm the results of Naver et al.[4] that the eight residue segment E27−C34 in PsaC is required for binding the subunit to the core. This domain was assumed by Naver et al.[4] to be an extra loop in PsaC not found in other bacterial ferredoxins. Comparison of the amino acid sequence of PsaC with those of other dicluster bacterial ferredoxins, some structures of which have been resolved via X-ray crystallography,[14] indicates that the domains containing the cysteine residues for ligation of the FeS clusters are similar and, therefore, the fold of the PsaC α-chain may be similar. If so the span E27−C34 of PsaC is most likely an extension of a small region of antiparallel β-sheet formed between the main chain strands 3 and 4 localized external to the α-helix involved in ligation of the F_B cluster. Our observations reported above indicate that if substitutions in this region include more hydrophilic residues then the PsaC constructs were further impaired in binding. The fact that PsaC mutants containing inserts with residues unrelated to wild-type sequence but with more hydrophobic side chains showed enhanced reconstitution over the deletion strain is consistent with this region being involved in PsaC binding through hydrophobic interactions. Furthermore, the results point to the requirement for a specific proline residue in the antiparallel β-sheet between strands three and four in the subunit backbone, the architecture of which may be necessary for efficient interaction between PsaC and the reaction center core.

Following interpretation of our previous results on the reconstitution of PS I cores using the PsaC point mutants D9R, E27R, and D32R,[3] we claimed that the data were consistent

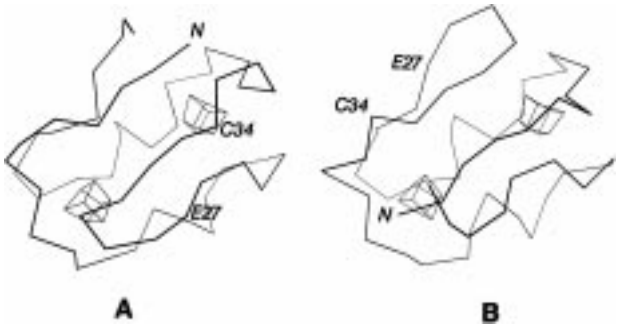

Figure 5. Models showing the two possible orientations of PsaC in the PS I core binding site.[19] The models show the tertiary structure of the ferredoxin of *Peptostreptococcus asaccharolyticus* (formerly *Peptococcus aerogenes*, PDB code 1FDX) as a simulator of the main chain of PsaC as used previously.[3,19] (A) Orientation of the subunit with the N-terminal FeS cluster (analogous to F_B) proximal to the core cluster, F_X. (B) Orientation of the subunit with the C-terminal FeS cluster (analogous to F_A) proximal to the core cluster, F_X. In both cases the domain of the F_X cluster lies immediately below the PsaC subunit as shown. The numbered residues are the predicted positions in the PsaC main chain based upon sequence alignment. The strand 3−4 β-sheet containing the residues E27−C34 is conserved in all ferredoxins for which the 3D structure is known.[14] Therefore, we suggest that the 8 residues present in PsaC not represented in this structure are C-terminal to this domain as in *Bacillus thermoproteolyticus* (PDB code 1FXR).[14]

with an orientation of PsaC in its binding site such that the F_B cluster of the subunit is close to the core redox center F_X in agreement with the conclusion of Naver et al.[4] This structural arrangement is modeled in Figure 5A after the proposal of Kamlowski et al.[19] based on their EPR spectroscopy of PS I crystals. Our finding that the region E27−C34 must contain residues with side chains capable of hydrophobic interactions, together with the demonstration that such interactions are primary forces stabilizing this region of PS I structure (Figure 4), lends additional support for this conclusion. The model in Figure 5B represents the alternative orientation of PsaC whereby the cluster F_A is proximal to F_X.[19] It is unlikely that the E27−C34 hydrophobic domain, shown above to be required for functional interaction between PsaC and the core, would be localized on the stromal surface of the PsaC subunit as shown in Figure 5B. We point out, however, that there is increasing biophysical evidence favoring the alternative arrangement whereby the cluster F_A of PsaC is proximal to F_X.[20−24]

Acknowledgment. We thank Dr. J. H. Golbeck for supplying the *psa*C clone from *Synechococcus* sp. PCC 7002. This work was supported by the National Science Foundation (MCB-9404744).

References and Notes

(1) Rodday, S. M.; Jun; S−S.; Biggins, J. *Photosynth. Res.* **1993**, *36*, 1.
(2) Rodday, S. M.; Schulz, R.; McIntosh, L.; Biggins, J. *Photosynth. Res*. **1994**, *42*, 185.
(3) Rodday, S. M.; Do, L. T.; Chynwat, V.; Frank, H. A.; Biggins, J. *Biochemistry* **1996**, *35*, 11832.
(4) Naver, H.; Scott, P. M.; Golbeck, J. H.; Moller, B. L.; Scheller, H. V. *J. Biol. Chem.* **1996**, *271*, 8996.
(5) Smart, L. B.; Anderson, S. L.; Mcintosh, L. *EMBO J.* **1991**, *10*, 3289.
(6) Hiyama, T.; Ke, B. *Biochim. Biophys. Acta* **1972**, *267*, 160.
(7) Mathis, P.; Sétif, P. *Isr. J. Chem.* **1981**, *21*, 316.
(8) Parrett, K. G.; Mehari, T.; Warren, P. G.; Golbeck, J. H. *Biochim. Biophys. Acta* **1989**, *973*, 324.
(9) Parrett, K. G.; Mehari, T.; Golbeck, J. H. *Biochim. Biophys. Acta* **1990**, *1015*, 341.
(10) Li, N., Zhao, J.; Warren, P. V.; Warden, J. T.; Bryant, D. A.; Golbeck, J. H. *Biochemistry* **1991**, *30*, 7863.
(11) Zhao, J.; Warren, P. V.; Li, N.; Bryant, D. A.; Golbeck, J. H. *FEBS Lett.* **1990**, *276*, 175.
(12) Biggins, J. *Biochemistry* **1990**, *29*, 7259.
(13) Cotton, F. A.; Hazen Jr. E. E.; Day, V. W.; Larsen, S.; Norman, J. G.; Wong, S. T. K.; Johnson, K. H. *J. Am. Chem. Soc.* **1973**, *95*, 2367.
(14) Moulis, J.-M.; Sieker, L. C.; Wilson, K. S.; Dauter, Z. *Prot. Sci.* **1996**, *5*, 1765.
(15) Eisenberg, D.; Weiss, R. M.; Terwilleger, C. Y.; Wilcox, W. *Faraday Symp. Chem. Soc.* **1982**, *17*, 109.
(16) Hervas, M.; De La Rose, M. A.; Tollin, G. *Eur. J. Biochem.* **1992**, *203*, 115.
(17) Hazzard, J. T.; Rong, S.-Y.; Tollin, G. *Biochemistry* **1991**, *30*, 213.
(18) Walker, M. C.; Pueyo, J. J.; Navarro, J. A.; Gomez-Moreno, C.; Tollin, G. *Arch. Biochem. Biophys.* **1991**, *287*, 351.
(19) Kamlowski, A.; van der Est, A.; Fromme, P,; Krauss, N.; Schubert, W.-D.; Klukas, O.; Stehlick, D. *Biochim. Biophys. Acta* **1997**, *1319*, 199.
(20) Fujii, T.; Yojayama, E.; Inoue, K.; Sakurai, H. *Biochim. Biophys. Acta* **1990**, *1015*, 41.
(21) He, W.; Malkin, R. *Photosynth. Res.* **1994**, *41*, 381.
(22) Jung, Y.-S.; Yu, L.; Golbeck, J. H. *Photosynth. Res*. **1995**, *46*, 249.
(23) Díaz-Quintana, A.; Liebl, W.; Bottin, H.; Sétif, P. *Biochemistry* **1998**, *37*, 3429.
(24) Vassiliev, I. R.; Jung, Y.-S.; Yang, F.; Golbeck, J. H. *Biophys. J.* **1998**, *74*, 2029.