Identification of Precise Electrostatic Recognition Sites between Cytochrome c₆ and the Photosystem I Subunit PsaF Using Mass Spectrometry*

Received for publication, August 3, 2006, and in revised form, September 19, 2006. Published, JBC Papers in Press, September 19, 2006, DOI 10.1074/jbc.M607384200

Frederik Sommer§1, Friedel Drepper†1, Wolfgang Haehnel§, and Michael Hippler†1

From the 1Institute of Plant Science, Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104 and 2Biochemie der Pflanzen, Biologie II, Universität Freiburg, Schänzlestrasse 1, 79104 Freiburg, Germany

The reduction of the photo-oxidized special chlorophyll pair P700 of photosystem I (PSI) in the photosynthetic electron transport chain of eukaryotic organisms is facilitated by the soluble copper-containing protein plastocyanin (pc). In the absence of copper, pc is functionally replaced by the lumenal soluble electron carrier plastocyanin (pc) across the membrane to the stromal soluble electron acceptor ferredoxin. Under copper deficiency, pc can be functionally replaced by the heme-containing protein cytochrome c₆ (cyt c₆) in the green alga Chlamydomonas reinhardtii. Binding and electron transfer between both donors and PSI follows a two-step mechanism that depends on electrostatic and hydrophobic recognition between the partners. Although the electrostatic and hydrophobic recognition sites on pc and PSI are well known, the precise electrostatic recognition site on cyt c₆ is unknown. To specify the interaction sites on a molecular level, we cross-linked cyt c₆ and PSI using a zero-length cross-linker and obtained a cross-linked complex competent in fast and efficient electron transfer. As shown previously, cyt c₆ cross-links specifically with the PsaF subunit of PSI. Mass spectrometric analysis of tryptic peptides from the cross-linked product revealed specific interaction sites between residues Lys70 of PsaF and Glu69 of cyt c₆ and between Lys23 of PsaF and Glu69/Glu70 of cyt c₆. Using these new data, we present a molecular model of the intermolecular electron transfer complex between eukaryotic cyt c₆ and PSI.

In photosynthetic electron transfer, photosystem I (PSI) functions as an integral light-driven plastocyanin:ferredoxin oxidoreductase. It is a thylakoid-embedded multiprotein complex that consists of ~14 subunits in Chlamydomonas reinhardtii (1). Using light energy, PSI transports electrons from the luminal soluble electron carrier plastocyanin (pc) across the membrane to the stromal soluble electron acceptor ferredoxin. Under copper deficiency, pc can be functionally replaced in green algae and in cyanobacteria by cytochrome c₆ (cyt c₆) (2–5).

Electron transfer between pc or cyt c₆ and the eukaryotic PSI can be described by a multistep model of donor binding, PSI-donor complex formation, electron transfer, and donor unbinding (6, 7). In eukaryotic organisms, binding of pc or cyt c₆ to the PSI is mainly driven by two different forces, electrostatic attraction and hydrophobic contact. Long range electrostatic attractions between basic patches of PsaF and acidic regions of pc and cyt c₆ (8–12) as well as the hydrophobic contact between the electron transfer site of the donors and PSI, including PsaA-Trp651 and PsaB-Trp627 in C. reinhardtii (9, 13), are required for stable electron transfer complex formation and efficient electron transfer. Cross-linking studies, knock-out, and reverse genetics experiments have established the crucial function of the positively charged eukaryotic N-terminal domain of PsaF for the binding of both donors (10, 12, 14–16). Hereby, Lys23 of PsaF from C. reinhardtii was found to be the key residue for the electrostatic interaction between PSI and both donors (12). The positively charged N-terminal domain is missing in prokaryotic organisms, and correspondingly, knock-out experiments and functional electron transfer measurements have demonstrated that, in the cyanobacterium Synechocystis sp. PCC6803, efficient binding and electron transfer between PSI and pc or cyt c₆ are independent of PsaF (10, 17, 18). Introduction of the basic PsaF domain from C. reinhardtii into cyanobacterial PsaF allowed efficient binding and fast electron transfer between the algal donors and the chimeric cyanobacterial PSI, proving that the positively charged N-terminal domain of PsaF is essential for electrostatic attraction of the donors and formation for the electron transfer complex competent in fast electron transfer (15).

As outlined, in cyanobacteria, electrostatic interactions between the electron donors and the oxidizing side of PSI are independent of PsaF or any other peripheral PSI subunit. However, site-directed mutagenesis of pc or cyt c₆ from Synechocystis sp. PCC6803 and Anabaena PCC 7119 reveal the importance of electrostatic attractions between the reaction partners. Analysis of binding and electron transfer between the altered donors and the cyanobacterial PSI revealed that a positively charged amino acid located at the “northern face” of either pc or cyt c₆ is crucial for the interaction with the reaction center (19–21). Interestingly, an equivalent positively charged exposed amino acid is present in cyt c₆ from C. reinhardtii (22) but absent from pc of C. reinhardtii (23) and other eukaryotic pc structures. It is tempting to speculate that this positively charged amino acid expressed by pc or cyt c₆ in cyanobacteria is important for electrostatic recognition of a negatively charged amino acid present in the core subunits PsaA or PsaB, thereby helping the formation of an electron transfer complex.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: Dept. of Biology, Institute of Plant Biochemistry and Biotechnology, University of Muenster, Hindenburgplatz 55, 48143 Muenster, Germany. Tel.: 49–251-8324790; Fax: 49–251-8328371; E-mail: mhippler@uni-muenster.de.

2 The abbreviations used are: PSI, photosystem I; pc, plastocyanin; cyt c₆, cytochrome c₆.
Recognition Sites between Cytochrome c₆ and Photosystem I

It is of note that the hydrophobic interaction site of the PSI core formed by PsaA and PsaB with the electron transfer donors is conserved between prokaryotic and eukaryotic organisms. The high resolution structural data of cyanobacterial and pea PSI (24, 25) show that a Trp residue (corresponding to Trp651 in C. reinhardtii) from a luminal helix of PsaA together with a Trp residue (corresponding to Trp651 in C. reinhardtii) from a luminal helix of PsaA form a sandwich-like structure directly situated above P700. The importance of the luminal loop j of the PSI electron transfer between PSI and the soluble donors has been demonstrated in Synechocystis PCC 6803. A double mutation in the luminal loop j of the PsaB (W622C/A623R) caused a highly photosensitive phenotype and exhibited a severe defect in the interaction and electron transfer with pc or cyt c₆ (26). The function of the two Trp residues in electron transfer between the two donors and PSI was directly analyzed in a reverse genetic experiment in C. reinhardtii. Alteration of the Trp residues by site-directed mutagenesis and transformation of PsaA- and PsaB-deficient strains resulted in the generation of PsaA and PsaB mutant strains PsaA-Trp651Phe, PsaA-Trp651Ser, and PsaB-Trp657Phe (corresponding to PsaA-Trp655 PsaB-Trp631 in Synechococcus) (13, 27). The change of either of the two PsaA or PsaB Trp residues to Phe abolished the formation of an intermolecular electron transfer complex between the altered PSI and pc, indicating that PsaATrp651 as well as PsaBTrp657 of loops i/j form the hydrophobic recognition site required for binding of pc to the core of PSI.

In respect to electrostatic and hydrophobic recognition sites of the eukaryotic donor molecules, only pc has been investigated in detail. Site-directed mutagenesis of cyanobacterial pc has demonstrated that the “southern” conserved negatively charged patch and the northern hydrophobic flat site of pc are required for electrostatic attraction and hydrophobic contact between pc and PSI, respectively, to promote binding and efficient electron transfer between pc and PSI (8–10, 28).

The precise electrostatic recognition site of eukaryotic cyt c₆ that enables specific binding to the positively charged domain of PsaF is unknown. To identify the amino acid on cyt c₆ responsible for binding to PsaF, we isolated a chemically cross-linked complex between cyt c₆ and PSI from C. reinhardtii that was competent in fast and efficient electron transfer. The active cross-linked complex was digested with trypsin, and peptides were analyzed by mass spectrometry. The mass spectrometric analysis identified cross-linked peptides and amino acids on cyt c₆ and PsaF that, in turn, allows defining the precise recognition sites on both proteins. Using the crystal structures for eukaryotic PSI (25) and eukaryotic cyt c₆ (22) and the genetic and biochemical data ((12, 13, 27) and this study), we present and discuss a model of the intermolecular complex between the two electron transfer partners.

MATERIALS AND METHODS

Strains and Media—C. reinhardtii wild-type strain was grown as described in Tris acetate phosphate medium with or without copper (29).

Isolation of pc and Cyt c₆—The isolation of pc and cyt c₆ followed published procedures (4, 11). The concentrations of pc and cyt c₆ were determined spectroscopically using an extinction coefficient of 4.9 mM⁻¹ cm⁻¹ at 597 nm for the oxidized form of pc (30) and 20 mM⁻¹ cm⁻¹ at 552 nm for the reduced form of cyt c₆ (2). Samples were tested for contamination of each other by immunodetection using anti-pc or anti-cyt c₆ antibodies.

Isolation of Thylakoid Membrane PSI Complex—The isolation of thylakoid membranes purified by centrifugation through a sucrose step gradient and the isolation of PSI particles was as described previously (11). Chlorophyll concentrations were determined according to Porra (31).

SDS-PAGE (15.5% T, 2.66% C; % T is the polycrylamide concentration at a constant concentration of the cross-linking agent bisacrylamide (% C)) was carried out according to Laemmli (32). After the electrophoretic fractionation, the protein bands were visualized by Coomassie staining, and bands corresponding to the PsaF-cyt c₆ cross-link product were excised.

Cross-linking Procedure—Cross-linking was performed as described previously (11). Cross-linked particles were fractionated by SDS-PAGE separation, and the cross-linked band was excised and digested with trypsin as described previously (33). For kinetic analysis, the cross-linking reaction was stopped by dilution, and particles were collected as described previously (11).

Flash Absorption Spectroscopy—Kinetics of flash-induced absorbance changes at 817 nm were measured essentially as described previously (6, 12). The measuring light was provided by a luminescence diode (Hitachi HE8404SG, 40 milliwatt, full width at half-maximum 30 nm) supplied with a stabilized battery-driven current source. The light was filtered through a 817-nm interference filter (full width at half-maximum 9 nm) and passed through a cuvette containing 200 µl of the sample with an optical path-length of 1 cm.

Mass Spectrometry—For mass spectrometric identification of the peptides, the excised protein bands were destained with 70% ethanol, 10% acetic acid, cut in small pieces, and subjected to trypsin digestion as described previously (34). Liquid chromatography-tandem mass spectrometry analysis was performed on a LCQ Deca XP Plus ion trap mass spectrometer (Thermo Electron, San Jose, California) in combination with a Famos autosampler, Switchos valve machine, and an Ultimate high pressure liquid chromatography machine (LC-Packings, Sunnyvale, California) as described previously (34). For reversed phase chromatography, a linear gradient over 90 min with an initial aqueous phase of 0.1% (v/v) formic acid in 5:95 acetonitrile:water and a final organic phase of 0.1% (v/v) formic acid in 80:20 acetonitrile:water was used. Acquisition of mass spectra and sequence identification using Sequest software (Thermo Electron, San Jose, California) were done as described previously, except that the range of peptide masses (MH +) was 200–7500. The data base used for the peptide search was constructed of photosynthetic components of Chlamydomonas. To detect cross-linked peptides, Glu or Asp residues were allowed up to six different additional masses per run when searching the spectra against the data base with the Sequest software. The masses corresponded to various tryptic fragments of PsaF, allowing up to two missed cleavages and searching in different combinations of the different additional masses.
Model Construction—The structure of C. reinhardtii cyt c₆ is already solved (Protein Data Bank (PDB) entry 1cyj) (22) and was used for the model presented here. To create a model of the electron transfer complex between PSI and cyt c₆, the C. reinhardtii PsaA, PsaB, and PsaF sequences were modeled to the eukaryotic PSI structure (PDB entry 1yo9), which is a model derived from the crystal structure of a eukaryotic PSI and a cyanobacterial PSI structure (35). Sequences for each PSI subunit were obtained from the Chlamydomonas genome project version 3.0 and homology models for each subunit were constructed individually using the Swiss model online server (36) and the Swiss PDB viewer (37). The cyt c₆ was then docked by hand to the PSI complex taking the biological data of a distance of ~14 Å between the redox cofactors and the data presented here into account. The model was energy-minimized using the Swiss PDB viewer implemented GROMOS96.

RESULTS

Cross-linking between PSI and Cyt c₆—Cross-linking of PSI with cyt c₆ resulted in a cross-linked product visible as an additional band after SDS-PAGE separation and Coomassie staining of the reaction mixture (Fig. 1). The band appeared in the mass range of the Psaf-cyt c₆ cross-linked product, as described previously, by immunodetection with anti-PsaF antibodies (13). The band was excised, digested with trypsin, and used for further mass spectrometric analysis.

The Cross-linked Complex between PSI and Cyt c₆ Is Competent in Fast Electron Transfer as Revealed by Flash-induced Absorption Spectroscopy—To investigate the functionality of the cross-linked complex between PSI and cyt c₆, the isolated cross-linked particles were analyzed by flash-induced absorption spectroscopy. The resulting kinetic traces (Fig. 2) can be deconvoluted by a bi-exponential decay revealing three distinct populations of PSI in the analyzed sample. The fast phase shows the same properties as the intermolecular electron transfer complex reaction between PSI and cyt c₆ with a half-time of 4 μs. The amplitude of this fast phase corresponds to 60% of the total amplitude. Because this kinetic component is extremely sensitive to changes in distance and geometry within the electron transfer complex, we conclude that, in more than half of the cross-linked particles, PSI is cross-linked in a functional way that resembles the in vivo native complex. The second, slower phase with a half-time of 42 μs and a minor amplitude of 6.8% in respect to the total amplitude might relate to a cross-link product obeying a distorted geometry of the electron transfer partners. The constant fraction with 33.2% of the total amplitude is reduced in a different timescale and corresponds to not cross-linked or not functionally cross-linked PSI, which is reduced by ascorbate.

Mass Spectrometric Analysis Reveals Specific Sites of Protein-Protein Interaction—Mass spectrometric analysis of the excised and tryptic digested protein band revealed Psaf and cyt c₆ as expected. The identified peptide sequences and recovery rates are summarized in Table 1.

For the identification of cross-linked peptides using Sequest, Glu and Asp residues were allowed additional masses that corresponded to tryptic peptides of Psaf, allowing up to two missed cleavages (minus H₂O for the cross-link peptide bound). The rationale behind this is that we expected to find cyt c₆ peptides that were cross-linked via Lys residues of Psaf. Because the Sequest software allows only up to six modifications, the mentioned additional masses were searched in different combinations.
Recognition Sites between Cytochrome c₆ and Photosystem I

In complete digests, we found no significant hits for queries that included PsaF-peptides that contained the C-terminal Lys residue as the only Lys residue. This was expected, because cross-linking the ε-amino group of a Lys residue to the γ/δ-carboxyl group of Asp/Glu residue results in loss of the recognition site for peptide hydrolysis by trypsin.

For queries with PsaF peptides that include one missed cleavage site, we found peptides 24TLEKR²⁸ and 2¹ELKTLEK²⁷ cross-linked with the cyt c₆ peptide ⁶⁷LSEEIQAVAYEVFK³¹. Allowing two missed cleavage sites, we identified PsaF peptide ²⁸KE₂₇ cross-linked to the same cyt c₆ peptide (summarized in Table 2). Because of the fact that all PsaF peptides were linked to the same cyt c₆ peptide ⁶⁷LSEEIQAVAYEVFK³¹ and this peptide was also found by Sequest by itself (see Fig. 3A), additional cross-linking/interaction sites between cyt c₆ and the basic patch of PsaF could be predicted. It should be of note that the cross-linking products were also found by Sequest searches when looking for PsaF trypsic peptides modified on Lys with the mass corresponding to the cyt c₆ peptide ⁶⁷–⁸¹ (data not shown), although the Xcorr factors were significantly lower. This demonstrates that peptide length and query identity strongly influences the outcome of the Sequest algorithm. This is not surprising, because these cutoff values were designed for simple linear peptides and have less significance for the more complex cross-linked peptides.

Unfortunately, Sequest analysis did not allow for discrimination of the specific sites of cross-linking (Glu⁶⁹/Glu⁷⁰/Glu⁷¹ on cyt c₆ or Lys⁵⁸/Lys⁶⁶ on PsaF for ²⁰KE₂₇) and gave significant values for the different proposed products. Therefore, we manually examined the tandem mass spectra for discriminating y and b ions. We did not find specific tandem mass spectrometry fragments to discriminate between Glu⁶⁹ and Glu⁷⁰ for the cross-link with ²⁰KE₂₇ and ²¹ELKTLEK²⁷ of PsaF. However, for the peptide Psalys⁷⁰–Lys²⁷, we found the series y₂⁺⁴ – y₂⁺⁷, indicating Lys²³ to be the linkage residue. For the PsaF peptide ²⁴TLEKR²⁸ cross-linked to the cyt c₆ peptide ⁶⁷LSEEIQAVAYEVFK³¹, we found y₁²⁺, y₁³⁺, b₁⁵⁺, and b₁²⁻ ions indicating a cross-link between Glu⁶⁶ and Lys²⁷ (see Fig. 3C).

As stated above, we expected other cross-linked peptides. Therefore, we carefully re-examined other Sequest hits that were below the significance cutoff and checked their significance with an extended tandem mass spectrometry product table, including y and b ions as well as y’ and b’ ions. Indeed we found an additional cross-linking product, cyt c₆⁵₈GAMPAWADR⁶⁶ cross-linked to PsaF⁻¹³AYAKLEK¹⁹, implying a cross-link between Asp⁶⁶ of cyt c₆ and Lys⁵₈ of PsaF. The peptide cyt c₆⁵₈GAMPAWADR⁶⁶ was not found in the tandem mass spectrometry analysis of the total cross-linked peptides. However, this is not related to cross-linking efficiency but rather to a feature of the “flyability” of the peptide, because it was not detected in an tandem mass spectrometry experiment with only purified and trypsin-digested cyt c₆ (data not shown). Looking at the resulting geometry of the PSI and cyt c₆ cross-linked complex, it seems rather unlikely that this product is part of the proper electron transfer complex (see “Discussion”).

### Table 1

**Recovery of peptides from PsaF and cytochrome c₆ by MS**
The underlined sequences were found by MS, and the italic sequences were found as cross-linked products. Gly⁶⁸-Arg⁷⁶ of cytochrome c₆ and Ala¹³-Arg⁸⁶ of PsaF were only found in cross-linked peptides.

| Recovery | Sequence |
|----------|----------|
| Cytochrome c₆ | 53 | ADLALGAQVFGNCGCAACHMEGRNSVNPETLKDAALEQYLDGGFGEVSTYQVENKKGAMPWADRSLSEEIQAVAYEVFKYQATD |
| PsaF | 55 | DIAGLTPCSESAAYAKLEKELKTLEKRLQYEVADSPAVALQATRMERTKAPANYAKAGLHNDGGLPLIDPGLAKYHGAEKETVLEKLDVPLATKLAVQACAVPLAVQELQRTPLEKKEINITYSPR |

**Table 2**

**Identified cross-linked peptides in one representative MS-MS experiment**
Listed are scan numbers; z (charge of the precursor ion); dM, calculated mass, measured mass of precursor; XCorr, correlation factor for Sequest analysis (only ions with a XCorr ≥ 1.5, 2.5, or 3.5 for singly, doubly, or triply charged ions, respectively, were taken into account); ΔCn, difference in XCorr between best and second best hit; ions, fragments from MS-MS experiment/maximal expected number of fragments (only y- and b-ions of the cytochrome c₆ peptide are accounted for); sequence, proposed sequence from Sequest (the signs behind the Glu/Asp correspond to the respective cross-linked PsaF peptide shown on the bottom of the table).

| Scan     | z | dM  | MH+  | XCorr | ΔCn | Ions | Sequence |
|----------|---|-----|------|-------|-----|------|----------|
| 1497–1499 | 3 | 0.0 | 2725.6 | 3.9096 | 0.000 | 26/56 | (R)LSE⁶⁷EEIQAVAYEVFK⁷⁰ |
| 1500–1504 | 2 | 1.5 | 2724.0 | 3.7797 | 0.032 | 25/56 | (R)LSE⁶⁷EEIQAVAYEVFK⁷⁰ |
| 1583–1587 | 3 | 0.1 | 2597.3 | 4.3184 | 0.000 | 28/56 | (R)LSE⁶⁷EEIQAVAYEVFK⁷⁰ |
| 1592–1595 | 2 | 1.4 | 2596.1 | 4.2510 | 0.015 | 27/56 | (R)LSE⁶⁷EEIQAVAYEVFK⁷⁰ |
| 1532–1535 | 3 | 0.7 | 2384.0 | 4.6149 | 0.000 | 29/56 | (R)LSE⁶⁷EEIQAVAYEVFK⁷⁰ |
| 1540–1543 | 2 | 0.9 | 2382.4 | 4.1799 | 0.000 | 19/28 | (R)LSE⁶⁷EEIQAVAYEVFK⁷⁰ |
| 1007–1019 | 2 | 0.7 | 1778.7 | 4.0482 | 0.011 | 18/28 | (R)LSE⁶⁷EEIQAVAYEVFK⁷⁰ |
| 1008–1012 | 1 | 0.6 | 1777.4 | 3.8124 | 0.087 | 16/28 | (R)LSE⁶⁷EEIQAVAYEVFK⁷⁰ |

* ²⁰KE₂₇, ²¹ELKTLEK²⁷, ²⁸KE₂₇, ³¹AYAKLEK¹⁹.
FIGURE 3. Mass spectrometry of peptide LSEEEIQVAEYVFK of cyt c₆ alone (A) and cross-linked to peptide ELKTELEK (B) or TLEKR of PsaF (C). The insets show the mass peaks from which the tandem mass spectra were achieved. In B and C, only the y and b peaks unique to the cross-linked peptide are indicated; all other major peaks derive from the peptide fragments, which do not carry the cross-linking site. In C, the peaks indicative for the specific cross-link between Glu₆₉ (cyt c₆) and Lys₂₇ (PsaF) are in italic.
propose that this PSI and cyt $c_6$ cross-linked complex corresponds to the fraction of PSI with a slower reduction half-time of 42 $\mu$s and a distorted geometry.

**DISCUSSION**

To elucidate the electrostatic interaction between cyt $c_6$ and PSI on the molecular level, we took advantage of chemical cross-linking, which resulted in a zero-length cross-linked complex between PsaF and cyt $c_6$. Importantly, the cross-linked complex was competent in fast and efficient electron transfer, indicating that cross-linking conserved the authentic orientation of the electron transfer partners in the complex. Mass spectrometric analyses of tryptic peptides from the cross-linked product between cyt $c_6$ and PsaF revealed specific interaction sites between residues Lys27 of PsaF and Glu69 of cyt $c_6$ and between Lys23 of PsaF and Glu69/Glu70 of cyt $c_6$. Interestingly, this identifies the Lys residues of PsaF, in particular Lys23, which are implicated in binding of pc and cyt $c_6$, as demonstrated by the reverse genetics experiments (12). This recognition site is also consistent with pea PSI crystal structure (25). In respect to the soluble electron donor and binding partner, this recognizes Glu69/Glu70 of cyt $c_6$ as electrostatic partner sites. Glu69 and Glu70 are located at the “eastern face” of cyt $c_6$.

In cyanobacteria, a positively charged amino acid located at the northern face of either pc or cyt $c_6$ is crucial for the interaction with the reaction center (19–21). Interestingly, an equivalent positively charged, exposed amino acid is present in cyt $c_6$ from *C. reinhardtii* (22). Although the residue on PSI interacting with this positively charged, exposed amino acid is unknown, our result demonstrates that the negatively charged residues Glu69/Glu70 of cyt $c_6$ bind to Lys23 and Lys27 of PsaF and represent the electrostatic recognition site of eukaryotic cyt $c_6$.

In Fig. 4, we present a molecular model of the intermolecular electron transfer complex between eukaryotic cyt $c_6$ and PSI. In this model, the distance between the redox cofactors is $\sim 14$ Å, as estimated by Hippler et al. (10), and the Glu69 and Glu70 of cyt $c_6$ are able to form a strong salt bridge with Lys27 and Lys23 of PsaF, respectively, as the mass spectrometric data indicate. Lys20 and Lys16 of PsaF form weaker salt bridges with Glu71 of cyt $c_6$ and Glu613 of PsaB, respectively. This is in accordance with the finding that Glu613 of PsaB functions to keep the N-terminal part of PsaF in a proper position to enable an effective electron transfer complex (13). Interestingly, in this model, the conserved positive charge on the northern face of cyt $c_6$ (Arg66) and the adjacent Asp65 can form a strong salt bridge with the pair Arg623/Asp624 of PsaB.

**FIGURE 4. Model of the electron donor docking sites.** Shown are the sites of the complex between PSI (green ribbons) and cyt $c_6$ (white). In yellow are the heme group of cyt $c_6$, the Trp pair B627/A651, and the special chlorophyll pair P700. The distance between the redox cofactors is $\sim 14$ Å. The Glu69 and Glu70 of cyt $c_6$ are able to form a strong salt bridge with Lys27 and Lys23 of PsaF, respectively, as the mass spectrometric data indicate. Lys20 and Lys16 of PsaF form weaker salt bridges with Glu71 of cyt $c_6$ and Glu613 of PsaB, respectively. Interestingly, in this model, the conserved positive charge on the northern face of cyt $c_6$ (Arg66) and the adjacent Asp65 can form a strong salt bridge with the pair Arg623/Asp624 of PsaB.

**Acknowledgment**—We thank Mia Terashima for critical reading of the manuscript.

**REFERENCES**

1. Hippler, M., Rimbault, B., and Takahashi, Y. (2002) *Protist* 153, 197–220
2. Wood, P. M. (1978) *Eur. J. Biochem.* 87, 9–19
Recognition Sites between Cytochrome c₆ and Photosystem I

3. Ho, K. K., and Krogmann, D. W. (1984) Biochim. Biophys. Acta 766, 310–316
4. Merchant, S., and Bogorad, L. (1986) Mol. Cell. Biol. 6, 462–469
5. Sandmann, G. (1986) Arch. Microbiol. 133, 6366–6375
6. Drepper, F., Hippler, M., Nitschke, W., and Haehnel, W. (1996) Biochemistry 35, 1282–1295
7. Finazzi, G., Sommer, F., and Hippler, M. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 7031–7036
8. Nordling, M., Sigfridsson, K., Young, S., Lundberg, L. G., and Hansson, O. (1991) FEBS Lett. 291, 327–330
9. Sandmann, G. (1986) Arch. Microbiol. 133, 6366–6375
10. Hippler, M., Drepper, F., Haehnel, W., and Rochaix, J. D. (1996) Biochemistry 35, 1282–1295
11. Hippler, M., Drepper, F., Farah, J., and Rochaix, J. D. (1997) J. Biol. Chem. 272, 6343–6349
12. Hippler, M., Drepper, F., Haehnel, W., and Rochaix, J. D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7339–7344
13. Sommer, F., Drepper, F., and Hippler, M. (2002) J. Biol. Chem. 277, 6573–6581
14. Farah, J., Rappaport, F., Choquet, Y., Joliot, P., and Rochaix, J. D. (1995) EMBO J. 14, 4976–4984
15. Hippler, M., Drepper, F., Rochaix, J. D., and Muhlenhoff, U. (1999) J. Biol. Chem. 274, 4180–4188
16. Haldrup, A., Simpson, D. J., and Scheller, H. V. (2000) J. Biol. Chem. 275, 31211–31218
17. Chitnis, P. R., Purvis, D., and Nelson, N. (1991) J. Biol. Chem. 266, 20146–20151
18. Xu, Q., Yu, L., Chitnis, V. P., and Chitnis, P. R. (1994) J. Biol. Chem. 269, 3205–3211
19. Molina-Heredia, F. P., Diaz-Quintana, A., Hervas, M., Navarro, J. A., and De La Rosa, M. A. (1999) J. Biol. Chem. 274, 33565–33570
20. Molina-Heredia, F. P., Hervas, M., Navarro, J. A., and De la Rosa, M. A. (2001) J. Biol. Chem. 276, 601–605
21. De la Cerda, B., Diaz-Quintana, A., Navarro, J. A., Hervas, M., and De la Rosa, M. A. (1999) J. Biol. Chem. 274, 13292–13297
22. Kerfeld, C. A., Anwar, H. P., Interante, R., Merchant, S., and Yeates, T. O. (1995) J. Mol. Biol. 250, 627–647
23. Redinbo, M. R., Cascio, D., Choukair, M. K., Rice, D., Merchant, S., and Yeates, T. O. (1993) Biochemistry 32, 10560–10567
24. Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W., and Krauss, N. (2001) Nature 411, 909–917
25. Ben-Shem, A., Frolow, F., and Nelson, N. (2003) Nature 426, 630–635
26. Sun, J., Xu, W., Hervas, M., Navarro, J. A., Rosa, M. A., and Chitnis, P. R. (1999) J. Biol. Chem. 274, 19048–19054
27. Sommer, F., Drepper, F., Haehnel, W., and Hippler, M. (2004) J. Biol. Chem. 279, 20009–20017
28. Lee, B. H., Hibino, T., Takabe, T., Weisbeek, P. J., and Takabe, T. (1995) J. Biochem. (Tokyo) 117, 1209–1217
29. Harris, E. H. (1989) The Chlamydomonas Sourcebook, pp. 575–581, Academic Press, San Diego, CA
30. Katoh, S., Shiratori, I., and Takamiya, A. (1962) J. Biochem. (Tokyo) 51, 32–40
31. Porra, R. J., Thompson, W. A., and Kriedemann, P. E. (1989) Biochim. Biophys. Acta 975, 384–394
32. Laemmli, U. K. (1970) Nature 227, 680–685
33. Stauber, E. J., Fink, A., Markert, C., Kruse, O., Johannsmeier, U., and Hippler, M. (2003) Eukaryotic Cell 2, 978–994
34. Naumann, B., Stauber, E. J., Busch, A., Sommer, F., and Hippler, M. (2005) J. Biol. Chem. 280, 20431–20441
35. Jolley, C., Ben-Shem, A., Nelson, N., and Fromme, P. (2005) J. Biol. Chem. 280, 33627–33636
36. Schwede, T., Kopp, J., Guex, N., and Peitsch, M. C. (2003) Nucleic Acids Res. 31, 3381–3385
37. Guex, N., and Peitsch, M. C. (1997) Electrophoresis 18, 2714–2723