Identification of Surface-Exposed Domains on the Reducing Side of Photosystem I

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Photosystem I (PSI) is a multisubunit enzyme that catalyzes the light-driven oxidation of plastocyanin or cytochrome c₆ and the concomitant photoreduction of ferredoxin or flavodoxin. To identify the surface-exposed domains in PSI of the cyanobacterium Synechocystis sp. PCC 6803, we mapped the regions in PsaE, PsaD, and PsaF that are accessible to proteases and N-hydroxysuccinimidobiotin (NHS-biotin). Upon exposure of PSI complexes to a low concentration of endoproteinase glutamic acid (Glu)-C, PsaE was cleaved to 7.1- and 6.6-kD N-terminal fragments without significant cleavage of other subunits. Glu²⁰ and Glu²ⁱ, located near the C terminus of PsaE, were the most likely cleavage sites. At higher protease concentrations, the PsaE fragments were further cleaved and an N-terminal 9.8-kD PsaD fragment accumulated, demonstrating the accessibility of Glu residues in the C-terminal domain of PsaD to the protease. Besides these major, primary cleavage products, several secondary cleavage sites on PsaD, PsaE, and PsaF were also identified. PsaF resisted proteolysis when PsaD and PsaE were intact. Glu²⁰ and Glu²¹ of PsaF became susceptible to endoproteinase Glu-C upon extensive cleavage of PsaD and PsaE. Modification of PSI proteins with NHS-biotin and subsequent cleavage by endoproteinase Glu-C or thermolysin showed that the intact PsaE and PsaD, but not their major degradation products lacking C-terminal domains, were heavily biotinylated. Therefore, lysine-74 at the C terminus of PsaE was accessible for biotinylation. Similarly, lysine-107, or lysine-118, or both in PsaD, but not in PsaE could be modified by NHS-biotin.

PSI in cyanobacteria and chloroplasts is a Chl-binding membrane-protein complex that catalyzes light-dependent electron transfer from reduced plastocyanin or Cyt c₆ to oxidized Fd or flavodoxin (Chitnis and Nelson, 1991; Bryant, 1992; Golbeck, 1993). PSI contains at least 11 polypeptides in cyanobacteria (Mühlenhoff et al., 1993) and three additional proteins, PsaG, PsaH, and PsaN, in higher plants (Ookels et al., 1989, 1992; Knoetzel and Simpson, 1993). PsaA and PsaB form a heterodimeric core that harbors the primary electron donor, P700, and a chain of electron transfer centers, A₀, A₁, and F₆. PsaC binds two [4Fe-4S] clusters, F₆₅₃ and F₆₇₃ (Oh-oka et al., 1988). PsaD provides an essential Fd-docking site on the reducing side of PSI (Zanetti and Merati, 1987; Zilber and Malkin, 1988; Lelong et al., 1994; Xu et al., 1994a) and is also required for the stable assembly of PsaC and PsaE into the PSI complex (Li et al., 1991; Chitnis and Nelson, 1992). PsaE is required for Fd reduction (Rousseau et al., 1993; Sonoike et al., 1993; Strotmann and Weber, 1993; Xu et al., 1994a) and may be involved in cyclic electron flow around PSI (Yu et al., 1993). PsaL is essential for the formation of PSI trimers (Chitnis and Chitnis, 1993). PsaF is exposed to the p-side (lumenal) of the photosynthetic membranes and can be cross-linked with plastocyanin or Cyt c₆ (Hippler et al., 1989; Wynn et al., 1989a, 1989b), but is not essential for the photooxidation of Cyt c₆ (Xu et al., 1994b). Other subunits, such as PsaJ, PsaK, PsaL, and PsaM, are much smaller (<5.5 kD) and are conserved from cyanobacteria to higher plants, but their functions are not known (Ikeuchi et al., 1993; Xu et al., 1994b).

The recent x-ray diffraction analysis of cyanobacterial PSI crystals at 6-Å resolution (Krauss et al., 1993), in combination with biochemical studies (Golbeck, 1993), provides a framework for understanding the overall architecture of PSI. PsaC, PsaD, and PsaE are peripheral subunits, located on the n-side (stromal in chloroplasts and cytoplasmic in cyanobacteria) of photosynthetic membranes, with PsaC positioned in the center of each monomeric PSI on a local pseudo-2-fold axis of symmetry (Krauss et al., 1993). Besides structural studies, the subunit interactions in PSI are also deciphered from biochemical experiments and characterization of PSI mutants. For example, the availability of subunit-deficient PSI complexes and improved Tricine-urea-SDS-PAGE has revealed structural interactions among PsaE, PsaF, and/or PsaJ (Xu et al., 1994b) or between PsaD and PsaL (Q. Xu, P.R. Chitnis, unpublished data). These findings are corroborated by chemical cross-linking of these subunits (T.S. Armbrust, J.A. Guikema, unpublished data).

The detailed architecture of PSI is yet to be determined. Protease accessibility studies have provided insights regarding organization and topology of PSI subunits (Zilber and Malkin, 1992). In higher plant PSI, PsaA, PsaB, PsaD, PsaE, PsaH, PsaK, and PsaL have stroma-exposed regions (Zilber and Malkin, 1992). Mapping of the proteolytic fragments of PsaD and PsaE indicates that N-terminal extensions of PsaD and PsaE are accessible to proteolysis (Lagoutte and Vallon, 1994).

Abbreviations: Glu-C, endoproteinase Glu-C; NHS-biotin, N-hydroxysuccinimidobiotin.

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reagents for Western blotting were purchased from Amer-
of the complex remain largely unknown. 

Here we treated PSI complexes with Glu-C and mapped the proteolytic peptides by N-terminal amino acid sequencing. We also labeled the PSI complexes with NHS-biotin and mapped the biotinylation sites on PsaD and PsaE. Based on these data, we identified the surface-exposed domains of PsaD and PsaE in cyanobacterial PSI.

MATERIALS AND METHODS

Materials

Prestained protein molecular mass standards were from Gibco-BRL. The centrifugal filtration apparatus (Centricon-

Modification of the PSI Subunits with NHS-Biotin

PSI complexes at 150 μg Chl/mL were incubated with 58 μM NHS-biotin, 10 mM Mops (pH 7.0), 0.05% Triton X-100, and 0.05% DMSO for 30 min at room temperature. The reaction was quenched with 50 mM ammonium bicarbonate (pH 7.8). The labeled PSI subunits were separated by Tricine-urea-SDS-PAGE and electroblotted to Immobilon-P membranes. The blot was probed with an avidin-peroxidase conjugate and developed with hydrogen peroxide and 4-chloro-1-naphthol (Frankel and Bricker, 1992).

Analytical Gel Electrophoresis and Immunodetection

PSI complexes were solubilized with 1% SDS and 0.1% 2-mercaptoethanol at room temperature for 1 h. Polypeptides were resolved by Tricine-urea-SDS-PAGE (Xu et al., 1994a). After electrophoresis, gels were stained with Coomassie blue or silver nitrate. Alternatively, proteins were electro-transferred to Immobilon-P membranes. Immunodetection was performed using enhanced chemiluminescence reagents. The antibody against PsaD of Synechococcus sp. PCC 7002 was a gift from Dr. John H. Golbeck at the University of Nebraska. Other antibodies were raised against Synechocystis sp. PCC 6803 proteins.

N-Terminal Amino Acid Sequencing

Peptides were blotted to Immobilon-P membranes, stained with Coomassie blue containing 1% acetic acid for 2 min, destained with 50% methanol, and rinsed extensively with deionized water. N-terminal sequences were determined on an Applied Biosystems (Foster City, CA) 477A sequencer at the Biotechnology Core Facility of Kansas State University.

RESULTS

Accessibility of PSI Subunits to Glu-C

We treated PSI complexes with different concentrations of Glu-C and subsequently examined PSI subunits by SDS-PAGE to estimate their relative accessibility to proteolysis (Fig. 1). Upon exposure of PSI complexes to 1 μg Glu-C/mg Chl, most of the PsaE subunit was degraded without a significant cleavage of other subunits and with simultaneous appearance of 7.1- and 6.7-kD peptides, termed EI and EII (Fig. 1A). A polyclonal antibody against PsaE of Synechocystis sp. PCC 6803 recognized both EI and EII fragments (data not shown). At Glu-C concentrations of 5 μg/mg Chl or higher, additional PSI subunits were degraded. Notably, a 9.8-kD fragment (labeled DI) accumulated, with a concomitant decrease in the relative amount of PsaD. This fragment was
Table I. N-terminal sequence analysis of prominent PSI fragments produced using Glu-C as described in Figure 1

<table>
<thead>
<tr>
<th>Proteolytic Fragment</th>
<th>Apparent Mass* (kD)</th>
<th>Sequencing Cycle</th>
<th>Identification and Position of the First Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td>7.1</td>
<td>ALNRC</td>
<td>2ALNRC of PsaE (Chitnis et al., 1989a)</td>
</tr>
<tr>
<td>EII</td>
<td>6.7</td>
<td>ALNRC</td>
<td>2ALNRC of PsaE (Chitnis et al., 1989a)</td>
</tr>
<tr>
<td>DI</td>
<td>9.8</td>
<td>TELSG</td>
<td>2TELSG of PsaD (Reilly et al., 1988)</td>
</tr>
</tbody>
</table>

* Average of three independent estimates from the electrophoretic mobility.

Accessibility of PSI subunits to Glu-C. A, Purified PSI complexes were treated with 0, 1, 5, 20, 50, and 100 µg Glu-C/mg Chl at 15°C for 20 min. The polypeptides in PSI complexes containing 5 µg of Chl were separated by Tricine-urea-SDS-PAGE and visualized by silver staining. The subunits labeled in the figure have been identified using immunodetection and N-terminal amino acid sequencing (Xu et al., 1994b), and their molecular masses were determined from migration of the following protein markers: insulin (2.9 kD), bovine trypsin inhibitor (6.2 kD), lysozyme (14.3 kD), β-lactoglobulin (18.4 kD), carbonic anhydrase (29 kD), ovalbumin (43 kD), and BSA (68 kD). The PsaA-PsaB, PsaD, PsaF, PsaJ, PsaK, PsaL, PsaM of Synechocystis sp. PCC 6803 migrated on a Tricine-urea-SDS-polyacrylamide gel with apparent molecular masses of 66, 18.2, 15.7, 14.2, 8.9, 8.0, 5.2, 3.4, 3.1, and 2.9 kD. B, The PSI complexes were treated with 0 and 20 µg Glu-C/mg Chl as in A (lanes 0 and 20). An identical sample of protease-treated PSI complexes was ultrafiltered using a Centricon-100. The retentate was resuspended with 12% Suc, 0.05% Triton X-100, and 10 mM Mops-HCl, pH 7.0 (lane 20). The samples containing 10 µg of Chl were solubilized and proteins were separated by Tricine-urea-SDS-PAGE and visualized by staining the gel with Coomassie blue.

Figure 1. Accessibility of PSI subunits to Glu-C. A, Purified PSI complexes were treated with 0, 1, 5, 20, 50, and 100 µg Glu-C/mg Chl at 15°C for 20 min. The polypeptides in PSI complexes containing 5 µg of Chl were separated by Tricine-urea-SDS-PAGE and visualized by silver staining. The subunits labeled in the figure have been identified using immunodetection and N-terminal amino acid sequencing (Xu et al., 1994b), and their molecular masses were determined from migration of the following protein markers: insulin (2.9 kD), bovine trypsin inhibitor (6.2 kD), lysozyme (14.3 kD), β-lactoglobulin (18.4 kD), carbonic anhydrase (29 kD), ovalbumin (43 kD), and BSA (68 kD). The PsaA-PsaB, PsaD, PsaF, PsaJ, PsaK, PsaL, PsaM of Synechocystis sp. PCC 6803 migrated on a Tricine-urea-SDS-polyacrylamide gel with apparent molecular masses of 66, 18.2, 15.7, 14.2, 8.9, 8.0, 5.2, 3.4, 3.1, and 2.9 kD. B, The PSI complexes were treated with 0 and 20 µg Glu-C/mg Chl as in A (lanes 0 and 20). An identical sample of protease-treated PSI complexes was ultrafiltered using a Centricon-100. The retentate was resuspended with 12% Suc, 0.05% Triton X-100, and 10 mM Mops-HCl, pH 7.0 (lane 20). The samples containing 10 µg of Chl were solubilized and proteins were separated by Tricine-urea-SDS-PAGE and visualized by staining the gel with Coomassie blue.

The results in Figure 1A would not distinguish between proteolytic fragments that were released from PSI and those that remained as peripheral PSI components. The EI, EII, and DI fragments were prominent species during cleavage of PSI complexes with 20 µg Glu-C/mg Chl (Fig. 1B). To determine their relative association with the PSI core, the protease-treated PSI complexes were filtered using the Centricon-100, and retentates were analyzed by Tricine-urea-SDS-PAGE. The EI and EII cleavage products of PsaE were more tightly associated with the PSI complexes than was the 9.8-kD DI fragment of PsaD (Fig. 1B, lane 20*). The stability of these associations was further examined by incubation with 1 mM NaI prior to filtration. The EI and EII fragments were relatively resistant to removal by NaI (data not shown).

When in solution, both PsaE (Rousseau et al., 1993) and PsaD (data not shown) are completely cleaved by Glu-C. Therefore, the data in Figure 1 demonstrated that specific Glu residues of PsaD and PsaE are shielded from the protease after their assembly into PSI complexes. These results prompted identification of EI, EII, and DI peptides by N-terminal amino acid sequencing (Table I). Both EI and EII fragments had N termini that matched the 2ALNRC terminus of PsaE (Chitnis et al., 1989). Therefore Glu-C cleaved PsaE at the Glu residues 1.8 and 2.3 kD from the C terminus to generate the EI and EII fragments. The N terminus of the DI fragment had a sequence that matched the 2TELSG of PsaD (Reilly et al., 1988) (Table I). Thus, a 6-kD C-terminal domain of PsaD was cleaved by Glu-C. A 6-kD polypeptide was observed on the polyacrylamide gel (Fig. 1A) but did not accumulate to the same extent as the DI fragment. This fragment may be more rapidly cleaved to smaller fragments. These data showed that the Glu residues in the C-terminal domains of PsaD and PsaE were accessible to Glu-C and thus were exposed to the aqueous phase.

To identify secondary sites of Glu-C cleavage, we subjected PSI complexes to extensive proteolysis using 100 µg Glu-C/mg Chl. Increased protease concentration yielded numerous proteolytic fragments from PSI subunits, including FI, FIIL, DII, DIV, DVI, and EIII, with apparent molecular masses of 13.3, 9.0, 6.4, 6.2, 4.4, 3.8, 3.5, and 2.5 kD, respectively (Fig. 2). Although some of these fragments were initially identified by western blotting (data not shown), we determined amino acid sequences of their N termini for accurate identification and mapping (Table II). These studies revealed that all sequenced cleavage products were derived from PsaD, PsaE, and PsaF. In contrast, PsaL and PsaK did not decrease in intensity after extensive proteolysis, and none of the peptides identified in this study (Tables I and II) were derived from these subunits. This suggested that Glu residues in PsaL and PsaK were not easily accessible to the protease.

The pattern of fragment appearance upon increasing proteolysis suggested a sequence of subunit accessibility to Glu-
Figure 2. Extensive cleavage of PSI complexes with Glu-C. The PSI complexes (lane 0) were treated with 100 \( \mu \text{g Glu-C/mg Chl} \) at 37°C for 1 h (lane 100). The complexes containing 15 \( \mu \text{g of Chl} \) were solubilized. The proteins were separated by Tricine-urea-SDS-PAGE and electroblotted on Immobilon-P membranes. The membranes were stained with Coomassie blue in 1% acetic acid.

C. For example, PsaE was readily cleaved at Glu-C concentrations that have minimal impact on PsaD (Fig. 1). When levels of PsaD and PsaF were immunodetected after cleavage of PSI with increasing concentrations of Glu-C (Fig. 3), PsaF fragments were observed only after extensive degradation of PsaA-PsaB, PsaD, and PsaE, which may expose Glu-C-sensitive sites on PsaF.

Table II. N-terminal sequence analyses of the proteolytic fragments produced by extensive cleavage of PSI complexes by Glu-C as described in Figure 2

<table>
<thead>
<tr>
<th>Proteolytic Fragment</th>
<th>Apparent Mass</th>
<th>Sequencing Cycles</th>
<th>Identification and Position of the First Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>13.3 kD</td>
<td>DDFANL</td>
<td>1DDFANL of mature PsaF (Chitnis and Nelson, 1991)</td>
</tr>
<tr>
<td>DI</td>
<td>9.8 kD</td>
<td>TELSG</td>
<td>2TELSG of PsaD (Reilly et al., 1988)</td>
</tr>
<tr>
<td>FII(^b)</td>
<td>9.0 kD</td>
<td>DDFAN</td>
<td>3DDFANL of mature PsaF (Chitnis and Nelson, 1991)</td>
</tr>
<tr>
<td>DIII</td>
<td>6.4 kD</td>
<td>KYAT</td>
<td>27KYATT of PsaD (Reilly et al., 1988)</td>
</tr>
<tr>
<td>DIII(^c)</td>
<td>6.2 kD</td>
<td>VQYLH</td>
<td>99VQYLH of PsaD (Reilly et al., 1988)</td>
</tr>
<tr>
<td>DIV</td>
<td>4.4 kD</td>
<td>GENLL</td>
<td>33GENLL of PsaD (Reilly et al., 1988)</td>
</tr>
<tr>
<td>DV</td>
<td>3.8 kD</td>
<td>AQGTK</td>
<td>114AQGTK of PsaD (Reilly et al., 1988)</td>
</tr>
<tr>
<td>DVI</td>
<td>3.5 kD</td>
<td>QCLAL</td>
<td>614QCLAL of PsaD (Reilly et al., 1988)</td>
</tr>
<tr>
<td>EII(^c)</td>
<td>2.5 kD</td>
<td>ALNRG</td>
<td>8ALNRG of PsaE (Chitnis et al., 1989a)</td>
</tr>
</tbody>
</table>

\(^a\) Average of three independent estimates from the electrophoretic mobility.  
\(^b\) To facilitate proper identification of FII, PSI complexes from a PsaE-less mutant strain (Chitnis et al., 1989a) were digested under the same condition as for the wild-type PSI.  
\(^c\) The fragment EII migrated below 2.9-kD insulin marker and its apparent mass was calculated by extrapolation of the calibration curve.
DISCUSSION

Chemical cross-linking, protease accessibility, and in vitro reconstitution experiments have revealed that PsaD, PsaE, and PsaC are exposed on the n-side of photosynthetic membranes and that a considerable part of PsaC is buried under PsaD and PsaE (Oh-oka et al., 1989; Zilber and Malkin, 1992). PsaF is substantially exposed on the p-side of the photosynthetic membranes (Wynn and Malkin, 1988; Hippler et al., 1989; Wynn et al., 1989a, 1989b). Here we examined the surface exposure of PsaD, PsaE, and PsaF in a topological assessment of PSI. For this purpose we used proteolytic cleavage and chemical modification experiments that probe different reactive groups with different levels of accessibility from the aqueous phase. Glu-C, a 34-kD protease, recognizes peptide bonds that are C terminal to glutamyl residues, and NHS-biotin, a smaller molecule, recognizes primary amines in a protein.

The solution conformation of Synechococcus sp. PCC 7002 PsaE, proposed from NMR analysis, reveals that the protein has an antiparallel five-stranded β-sheet structure (Falzone et al., 1994a, 1994b). Because of high sequence identity among cyanobacterial PsaE, the structure of Synechococcus sp. PCC 7002 PsaE provides a reliable model for Synechocystis sp. PCC 6803 PsaE. There are five glutamyl residues at positions of 15, 29, 63, 65, and 67 in PsaE of Synechocystis sp. PCC 6803 (Chitnis et al., 1989). Among them, glutamyl residues at positions 15, 63, 65, and 67 are conserved between the PsaE subunits from the two cyanobacterial species. The peptide mapping of the major PsaE fragments (Table I) and
predicted Glu-C fragments of PsaE (Table III) indicate that the primary Glu-C cleavage sites may be in a cluster of Glu residues at positions 63, 65, and 67. Examination of the structure of *Synechococcus* sp. PCC 7002 PsaE revealed that the C-terminal peptide bonds of Glu62 and Glu66 (corresponding to Glu63 and Glu67 in *Synechocystis* sp. PCC 6803 PsaE), but not of Glu64 (corresponding to Glu65 in *Synechocystis* sp. PCC 6803 PsaE), are accessible from the surface. Therefore, Glu63 and Glu67 in *Synechocystis* sp. PCC 6803 PsaE are the most likely sites of primary cleavage by Glu-C. The remaining Glu residues in PsaE are not readily accessible to Glu-C and therefore may be shielded by the other PSI subunits. The loop of PsaE, located opposite the surface-exposed Glu63-Glu67 region, varies in length depending on the source of PsaE and may anchor PsaE in the PSI complex. Interestingly, a 2.5-kD PsaE fragment with N termini of PsaE was accumulated when the PSI complexes were extensively degraded by Glu-C. This implies that the Glu15, which is near the N-terminal domain of PsaE, may be relatively shielded from aqueous phase. It is possible that proteolytic cleavage of the C-terminal domain of PsaD exposed the Glu69 of PsaE.

Glycosyl residues in the C-terminal domain of PsaD are the primary cleavage sites for Glu-C (Table I). The C-terminal domain of PsaD is also accessible to thermolysin (Xu et al., 1994a). Similarly, the proteolytically cleaved PsaD fragment with an N terminus identical to that of intact PsaD was found after chaotrope-induced release of peripheral proteins from purified PSI of *Synechococcus* sp. PCC 6301 (Li et al., 1991). When PSI complexes were extensively cleaved with Glu-C, Glu5' and Glu3' were accessible. The peptide mapping of the observed PsaD fragments (Tables I and II) and fragments predicted from the deduced amino acid sequence (Table III) indicated that Glu106, Glu107, and Glu113 were readily accessible to Glu-C. Interestingly, Lys107 of PsaD in *Synechocystis* sp. PCC 6803 can be cross-linked with Glu97 of Fd by a hydrophilic, zero-length cross-linker, N-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Lelong et al., 1994). The glutamyl residues in the C-terminal domain of PsaF
its C-terminal hydrophilic domain on the n-side of the membranes. Thus, it is possible that extensive cleavage of the membranes with its N terminus on the lumenal side and (Chitnis et al., 1991), suggesting that PsaF is positioned in integral membrane protein. First, the amino acid sequences to the lumenal side (Hippler et al., 1989; Wynn et al., 1989a, reconstitution studies have demonstrated that PsaF is exposed membrane. Although chemical cross-linking and functional were the likely cleavage sites. A hydrophobic domain preceding Glu" were also accessible to Glu-C. The 13.3- and 9.0-kD PsaF fragments with intact N termini gradually accumulated when PsaE and PsaD had been cleaved by Glu-C. Based on the apparent masses of these proteolytic fragments and deduced amino acid sequence (Chitnis et al., 1991), Glu" were the likely cleavage sites. A hydrophobic domain preceding Glu" is 20 amino acids in length and may span a membrane. Although chemical cross-linking and functional reconstitution studies have demonstrated that PsaF is exposed to the luminal side (Hippler et al., 1989; Wynn et al., 1989a, 1989b), several lines of evidence suggest that PsaF is an integral membrane protein. First, the amino acid sequences of PsaF deduced from the gene sequences reveal two moderately hydrophobic regions in this protein (Chitnis et al., 1991). Second, PsaF resists removal from wild-type PSI by chaotropic agents (Xu et al., 1994a, 1994b). However, it could be released from PSI core upon exposure to a high concentration of detergent (Bengis and Nelson, 1977). Third, a cross-linked product is recognized by both anti-PsaE and anti-PsaF antibodies and can be obtained by treatment of PSI with glutaraldehyde, showing that PsaE and PsaF are in close proximity (T.S. Armbrust, J.A. Guikema, unpublished data). PsaF is synthesized as a precursor protein with a transit sequence typical of proteins targeted to the thylakoid lumen (Chitnis et al., 1991), suggesting that PsaF is positioned in the membranes with its N terminus on the luminal side and its C-terminal hydrophilic domain on the n-side of the membranes. Thus, it is possible that extensive cleavage of the n-side components of PSI, most notably PsaE and PsaD, expose the C-terminal domain of PsaF. Alternatively, the C-terminal domain of PsaF may be shielded by PsaA-PsaB loops that are degraded after extensive proteolysis.

There is an apparent difference in the sensitivity of PSI subunits to proteolysis. PsaE was selectively cleaved by Glu-C before detectable degradation of PsaD, PsaC, or other PSI subunits (Fig. 1). A similar sensitivity pattern of the n-side components of PSI to extraction by NaI has been observed (Xu et al., 1994a, 1994b). Upon treatment of PSI complexes with NaI, a significant amount of PsaE was released before removal of PsaD and PsaC. The absence of PsaE leads to an enhanced cleavage of PsaD by thermolysin (Xu et al., 1994a). Both PsaD and PsaE are required for efficient Fd-mediated NADP⁺ photoreduction by PSI (Xu et al., 1994a). PsaD can also be chemically cross-linked with Fd (Merati and Zanetti, 1987; Zilber and Malkin, 1988; Lelong et al., 1994). Thus, PsaD and PsaE may partially overlap and form the docking site for the electron acceptors of PSI. These functionally important docking domains of PsaD and PsaE are expected to be surface exposed. Hence, the domains of PsaD and PsaE that were identified in this study may form the Fd-docking region on the reducing side of PSI.

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LITERATURE CITED


Figure 6. PSI modification by NHS-biotin and cleavage with thermolysin. The biotinylated PSI complexes (lane 1) were cleaved with thermolysin (20 μg protease/mg Chl) for 20 min to yield the PsaD fragments lacking the C terminus (lane 2). The preparations equivalent to 10 μg of Chl were solubilized and the proteins were separated by Tricine-urea-SDS-PAGE, electroblotted on Immobilon-P membranes, and stained with Coomassie blue. A replica blot of lanes 1 and 2 was probed with avidin-peroxidase conjugate peroxide and 4-chloro-1-naphthol.
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