Effects of Proteolysis on the Photochemical Activity and Polypeptide Composition of the Photosystem II Core Complex Isolated from Spinach Chloroplasts

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ABSTRACT

The photosystem II core complex (TSF-IIa) is composed of polypeptides of molecular weight 54-, 47-, 42-, and 30 kilodaltons (kD) and cytochrome b-559. After treatment with trypsin or a-chymotrypsin for 20 hours, the TSF-IIa particles still retained their photochemical activity and the light-induced cytochrome b-559 signal, although all of the polypeptides of the complexes, except the 30 kD unit were extensively degraded. Proteolytic treatment decreased the apparent molecular weight of the complex from 250,000 to 100,000 daltons as determined by gel filtration, and also decreased the protein to chlorophyll ratio by 40%. Chlorophyll a appeared to be associated with the 47- and 42 kD polypeptides. Proteolysis of the complex produced a single chlorophyll a band with a slightly higher electrophoretic mobility. This band was not equivalent to the 30 kD polypeptide. Proteolysis also reduced the sensitivity of the TSF-IIa particles to 3-(3',4'-dichlorophenyl)-1,1-dimethyurea (DCMU), but did not completely abolish it.

It was concluded that although the polypeptides of the photosystem II core complex were cleaved by the proteases, many of the peptide fragments do not dissociate from the complex, particularly the chlorophyll a-containing portions, leaving the complex photochemically active. This supports the concept that chlorophyll has a structural function in chlorophyll-protein complexes.

Proteolytic enzymes have been used extensively to study the localization of thylakoid membrane proteins, particularly the Chl-protein complexes (3, 5, 18, 21-23). Although many of the electron transport components of the membrane are easily digested, the Chl-protein complexes are only minimally affected, suggesting that these proteins are buried entirely within the membrane. On the other hand, Jennings et al. (11) have found that the polypeptides associated with both PSI and the LHC\(^4\) can be digested upon prolonged proteolytic treatment of the thylakoid membrane. However, in neither case are the complexes denatured nor their photophysical properties altered. These authors suggest that the Chl present in the complexes stabilizes the structure of the complex. Unfortunately, their observations were not extended to include the effects of proteolysis on PSII.

The present study describes the effects of trypsin and \(\alpha\)-chymotrypsin on the photochemical activity and structure of the isolated PSII core complex. At low concentrations of either protease, the photochemical activity of the complex was not inhibited; however, the component polypeptides were extensively degraded. This suggests that the reaction center polypeptide of PSII was cleaved during proteolysis but the peptide fragments, possibly held in place by their associated Chl, did not dissociate from the complex.

MATERIALS AND METHODS

Preparation of TSF-IIa Particles. The initial purification procedures followed those of Vernon et al. (26) with some modifications. Spinach thylakoids prepared in 50 mM Tris-Cl (pH 7.6) containing 350 mM sucrose, were twice treated with 2.0 M NaBr as described by Nelson (12) to remove coupling factor, and then washed with the above buffer. The lamellae were resuspended in 50 mM Tris-Cl (pH 8.0) and 0.5 M sucrose at a concentration of 2.5 mg Chl/ml. Triton X-100 was added to give a final ratio of 40 mg Chl:1 g Triton X-100. The solution was stirred on ice for 1 h after which it was subjected to a low speed centrifugation (5,000g, 5 min.) to remove debris. The supernatant was centrifuged at 144,000g for 1 h (2 C) and the resultant supernatant discarded. The pellet was resuspended in 5.0 mM Tris-Cl (pH 8.0) containing 50 mM NaCl, and again centrifuged at 144,000g for 1 h. The supernatant of this centrifugation, designated TSF-IIa, was diluted 1:1 with water and placed on an aminoethyl cellulose column at 5 C. About 1.5 g dry weight of aminoethyl cellulose was used for each mg Chl of TSF-IIa particles applied. A Chl fraction (about 25% of the total Chl), which had no PSI activity, eluted directly from the column. The column was washed successively with two column volumes each of 20 mM Tris-Cl (pH 8.2) and 20 mM Tris-Cl (pH 8.2) containing 0.5 M NaCl. Occasionally a small amount of Chl eluted during the latter step. The remaining Chl was eluted with 20 mM Tris-Cl (pH 8.2), 0.5 M NaCl, and 0.1% Triton X-100. This Chl fraction was applied to a Sephadex G-100 column as described previously (17) to remove the salt and Triton X-100. Additional gel filtration of the particles on either Sepharose 4B (5.0 mM Tris-Cl [pH 8.5]) or Sepharose 6B (20 mM Tris-Cl [pH 8.2] and 0.05% Triton X-100) was also performed at 5 C as previously described (17).

Activity Measurements. The DPC-dependent reduction of DCIP by TSF-IIa particles was assayed by measuring the absorption decrease at 600 nm. The assay medium consisted of 20 mM Tris-maleate (pH 6.0), 0.5 mM DPC, and 25 \(\mu\)M DCIP. Determined...
nations were made using an Aminco DW-2a spectrophotometer operated in the double-beam mode. Illumination of the sample compartment was provided by a projector lamp with a variable voltage supply. The output was passed through a water filter and a red cutoff filter (Corning CS 2-64). A 620 nm cutoff filter (Bausch and Lomb No. 90-1-620) protected the photomultiplier from the actinic light source. Light-induced Cyt b-559 reduction was monitored using the spectrophotometer in the dual-wavelength mode of operation. The measuring and reference wave-lengths were 559 and 570 nm, respectively. Illumination was provided using the same filter combination as described above. The light intensity at the cuvette surface was $1 \times 10^5$ erg/cm$^2$-s. Light intensities were measured using a Kettering-Yellow Springs instruments radiometer. The reaction mixture contained 10 mM Tris-maleate (pH 6.0), 0.5 mM DPC, and 20 $\mu$g Chl/ml. The light-induced P700 signal was measured as described in Gross (8). Absorption and difference spectra were also recorded on an Aminco DW-2a spectrophotometer.

180° Light Scattering Measurements. Light scattering experiments to determine the isoelectric point of the TSF-IIa particles before and after proteolysis were performed in a stirred cuvette with a 1-cm pathlength. The sample contained 15 $\mu$g Chl/ml in 2.5 mM Tris-Cl and 0.04% Triton X-100. The pH of the solution was adjusted in a stepwise fashion using 0.1 M HCl. The A at 540 nm due to 180° light scattering was measured as a function of pH using a Model 139 Hitachi Perkin-Elmer spectrophotometer.

Protease Treatment. Proteolysis of the PSII particles was performed at 5 C in a stirred container with either $\alpha$-chymotrypsin (TLCK-treated) or trypsin (DPCC-treated) in 5.0 mM Tris-Cl (pH 8.0). The Chl concentration was approximately 50 $\mu$g/ml. Photochemical assays were performed on the samples by directly diluting an aliquot into the assay buffer (a 1:50 dilution). To prepare samples for SDS-gel electrophoresis, 100-$\mu$l aliquots were removed from the incubation mixture, to which 10 $\mu$l of 50 mM phenylmethanesulfonyl fluoride (in 1-propanol) was added. This was incubated on ice for 15 min, after which the SDS and electrophoresis buffer were added. The mixture was left at 5 C until just prior to electrophoresis. The samples were not boiled. For experiments with the protease-treated PSII particles other than SDS electrophoresis and photochemical assays, the sample was again applied to an aminoethyl cellulose column and isolated in an identical fashion to that described above.

Electrophoresis. Electrophoresis under denaturing conditions was performed at room temperature according to Kirchanski and Park (14) on either 10% polyacrylamide slab or tube gels. SDS was added to the samples to give a final concentration of 2.0%. The samples were not heated prior to electrophoresis. Electrophoresis under non-denaturing conditions was performed following the procedure of Camm and Green (2) except LiDS was substituted for SDS in the gel and reservoir buffers. Detergent was omitted entirely from the samples. Samples were electrophoresed for 6 h at 3 C then scanned immediately for Chl at 670 nm using a Gilford Gel Scanner. The polypeptide bands were stained with Coomassie brilliant blue G-250 following the procedure of Holbrook and Leaver (10).

The apparent molecular weights of the polypeptides were calculated from a standard curve constructed using BSA (68,000), glutamate dehydrogenase (33,000), aldolase (40,000), chymotrypsinogen (25,700) RNase A (13,700), and Cyt c (11,700).

Chl and Protein Determinations. Chl concentrations were determined using the equations of Arnon (1). Protein concentrations were determined using the Bio-Rad Dye Binding Assay method with catalase as the standard.

Chemicals. The following chemicals were obtained from Sigma: Amino Ethyl Cellulose; Trypsin Type XI; $\alpha$-Chymotrypsin Type VII; SDS; and LiDS. All other chemicals were reagent grade or better.

**RESULTS**

Purification of TSF-IIa Particles. Our first step was to purify the PSII particles. Thus, our isolation procedure differs from that of Vernon et al. (25, 26) in several respects. Since coupling factor is a common contaminant in these preparations, it was removed from the chloroplast membranes by NaBr washes before isolation of the particles. Also, an ion exchange step on aminoethyl cellulose was substituted for either their sucrose density gradient centrifugation (25) or Bioglas absorption chromatography (26). The aminoethyl cellulose step is preferable to either method because it allows for the purification of large quantities of particles in a short period of time.

The aminoethyl cellulose column separated the TSF-IIa particles into two Chl fractions. The first Chl fraction had no PSII activity. Its absorption spectrum showed a blue-shifted absorption maximum at 667.5 nm and a greater Chl b content, compared to the original TSF-IIa particles (Fig. 1). This fraction may be comparable to the inactive TSF-IIa′ fraction obtained by Vernon et al. (25) on sucrose gradients. The second Chl fraction, which was photochemically active, had a slightly red-shifted absorption maximum and a specific activity 10 to 15% higher than the original TSF-IIa particles.

Generally, aminoethyl cellulose chromatography simplified the polypeptide composition of these particles. The major polypeptides present in these particles have mol wt of 54, 47, 42, and 30 kD (Fig. 2). The particles contain a variable amount of LHC as indicated by one or more polypeptides of 25,000 daltons. The particles also contained Cyt b-559 (about 50 Chl per Cyt b-559) as reported by others (13, 16, 25, 26). The relative amounts of the various polypeptides were somewhat variable from preparation to preparation. This may be due in part to the tendency of both the 47- and 42-kD polypeptides to form diffuse bands on SDS gels. The reason for this is unknown.

The mol wt of the 54 kD band changes slightly after purification of the TSFIIa particles. These are real changes and not artifacts of the gel system. They may reflect a change in the binding of SDS or in the extent of denaturation.

After chromatography on aminoethyl cellulose, the particles were in a highly aggregated state. They eluted in the void volume of a Sepharose 4B column in the absence of detergent, unlike the original TSF-IIa particles which eluted with an apparent mol wt of 460 kD (17). However, in the presence of 0.05% Triton X-100 these particles eluted as a single Chl-containing peak at 250 kD (17). After this latter column, the TSF-IIa particles were completely photochemically inactive. This contrasts with control particles which retain 50% of their activity after incubation in 0.05% Triton X-100 for the same period of time. Neither gel filtration procedure significantly altered the polypeptide composition, Cyt content or absorption spectrum of the particles. The reason for the inactivation of the particles by filtration in the presence of
Triton X-100 is unknown but may be due to the loss of a nonprotein component from the complex.

The P700 content of these particles was also somewhat variable. We found values of 1,800 to 2,400 Chl/P700. This suggests that in some preparations as much as 10% of the total Chl was actually associated with PSI. This PSI content is comparable to that found by Lach et al. (16) in their TSF-IIa preparations. Although the light-induced P700 signal is barely detectable, the presence of the PSI complex is clearly shown on the LiDS gels, as will be discussed below.

The Effect of Aging on PSII Activity. We determined the effect of time of storage of the particles on the photochemical activities to determine the proper conditions for the proteolytic digestions. Immediately after isolation, the TSF-IIa preparations showed rates of DCIP reduction between 150 and 490 μmol·mg Chl⁻¹·h⁻¹. The photochemical activity decreased with approximately first order kinetics (Fig. 3). The time required for the activity to decay 50%, or half-life, is a good measure of the stability of the photochemical activity. The half-life was a function of both the temperature of incubation and the concentration of Triton X-100. For example, at 5°C, the half-life was between 5 and 10 h (Fig. 3). This decreased to 20 min at 20°C and increased to 22 h at 0°C (not shown). Removal of the Triton X-100 by gel filtration prior to storage at 5°C increased the half-life to 34 h.

For these reasons, we decided to remove the Triton X-100 from the preparations prior to protease treatment and to carry out the incubations with the protease at 5°C. Nonetheless, the activity of the particles had decreased to approximately 150 μmol·mg Chl⁻¹·h⁻¹ before the start of the protease treatment. After 20 h of incubation under control conditions (i.e. without protease) the activity had fallen to approximately 70 μmol·mg Chl⁻¹·h⁻¹.

The residual activity represents a true PSII-reaction rather than a reaction catalyzed by free Chl because it is inhibited by boiling the particles as well as by the addition of DCMU (see below) or Triton X-100.

The Effect of Protease Treatment on PSII Activity. We determined the effect of various concentrations of trypsin and chymotrypsin on the activity of the PSII particles after 20 h of incubation (Fig. 4). An incubation time of 20 h was chosen because it was the time required to observe maximal changes in the polypeptides. As can be seen, the PSII particles are remarkably stable to proteolytic digestion. At a concentration of 5 μg/ml (Chl:protease ratio of 10),

little or no inactivation was observed after 20 h of incubation. Even at higher concentrations of the proteases, up to 100 μg/ml (Chl:protease ratio of 0.5), the activity was inhibited only 40% compared to an untreated sample. We also determined the activity as a function of time of incubation (Fig. 5). The rate of DCIP reduction of the control decreased 40% during the 20 h of incubation as described above. However, the activity of the samples treated with 5 μg/ml trypsin or chymotrypsin were equal to or greater than the untreated controls at all times studied. In fact, there was a 10 to 20% increase in activity after short times of incubation.

Note, if proteolysis was conducted on the complex without prior removal of Triton X-100 from the sample, activity was lost relatively quickly. As we reported earlier (17), Triton X-100 inhibits the photochemical activity of the TSF-IIa particles. This inhibition is reversible for short periods of time. However, 0.05% Triton X-100 also greatly accelerates an irreversible decay of activity. Apparently, proteases further accelerate this decay rate, possibly by increasing the accessibility of the labile component to Triton X-
FIG. 5. The effect of α-chymotrypsin (5 μg/ml) and trypsin on the photochemical activity of TSF-IIa particles, as a function of the time of incubation. The initial control rate of DCIP reduction was 150 μmol-mg⁻¹ Chl-h⁻¹.

FIG. 6. The polypeptide composition of TSF-IIa particles on SDS gels after different times during proteolysis. (A) The chymotrypsin concentration was 5 μg/ml except for the bottom trace where 100 μg/ml was used. (B) The trypsin concentration was 5 μg/ml. Other conditions are given under "Materials and Methods."

100. Or conversely, Triton X-100 may increase the accessibility of a labile component of the complex to the proteases. The Effect of Proteases on the Polypeptide Composition of the TSF-IIa Complex. Unlike the photochemical activity, the polypeptide composition of the TSF-IIa particles was considerably altered by protease treatment. SDS-gels of samples treated with α-chymotrypsin for different incubation periods (Fig. 6A) showed that all of the major polypeptides were extensively digested, with the exception of the 30 kD polypeptide. After 1 h, there was a complete loss of the 47 kD polypeptide and a considerable loss of the 54, 43, and 25 kD polypeptides. This corresponds to the same time period in which the activity of the particles was actually increasing. After 20 h, essentially only the 30 kD polypeptide remained intact. Trypsin treatment of the TSF-IIa particles gave nearly identical results (Fig. 6B).

After proteolytic treatment, the TSF-IIa particles could still be isolated by the aminoethyl cellulose procedure. All of the Chl in the sample was bound to the column in low salt, suggesting that no free Chl had been released by the proteolytic digestion. The protease was removed from the column with a 5 μM NaCl wash and the Chl was removed with 0.5 μM NaCl and 0.05% Triton X-100. This material was still photochemically active. After a further gel filtration step in Sephadex G-100, SDS-gels of the protease-treated samples showed only the 30 kD polypeptide (Fig. 6A, bottom trace). However, there was a considerable amount of Coomassie blue staining material of higher mobility (mol wt <15,000) which was not resolved into discrete bands (see also Fig. 8B). This material, which was not present in untreated samples, probably consists of polypeptide fragments, heterogeneous in size, which were not removed or resolved from the complex by the chromatography procedures.

We found that proteolysis had no effect on either the visible absorption spectrum or the Chl fluorescence emission or excitation spectra (not shown). These results show that the environment of the Chl molecules was not altered. This agrees with the results of the activity studies.

We also determined the effect of proteolysis on Cyt b-559 (7). Both the oxidized minus reduced difference spectrum and the total amount of the Cyt were unchanged. The photochemical reduction was also unchanged (Fig. 7). We observed that 3.5 to 5% of the total amount of the cytochrome was reduced in the light which is consistent with the results of others (16). Moreover, the signal was DPC-dependent but DCMU-sensitive which shows that it is a bona fide Cyt b-559 signal. It was inhibited by 0.05% Triton X-100 which is in accord with our previous results concerning the effect of Triton X-100 on the photochemistry of PSII (17).

Although no Chl was lost after proteolysis, the amount of protein decreased by 40% (Table I). This is consistent with a decrease in the size of the particles from 250 to 100 kD as determined by gel filtration in the presence of 0.05% Triton X-100. The isoelectric point as determined by turbidity (180° light-scattering) was only slightly altered (from pH 4.55 ± 0.1 in the control to pH 4.4 ± 0.05 after 20 h of treatment with 5 μg/ml trypsin or chymotrypsin.

We also examined the effect of proteolysis on the sensitivity of the PSII particles to DCMU. Trypsin treatment of chloroplasts decreases their sensitivity to DCMU, raising the Kᵣ from 50 nm to 3 μM and also allowing direct donation of PSII electrons to ferricyanide (24). In addition, Horton and Croze (5) found that trypsin completely abolished DCMU inhibition in PSII membrane fragments (TSF II particles). We find that our PSII particles as isolated have a biphasic response to DCMU (Fig. 8). Approximately 50% of the particles have a low Kᵣ of less than 0.5 μM, whereas the remainder have a higher Kᵣ (approximately 20 μM). These results suggest that there are two sites of action of DCMU.

FIG. 7. Light-induced cytochrome b-559 reduction of control and α-chymotrypsin-treated samples of TSF-IIa particles.

Table I. Effects of Proteolysis on Protein to Chl Ratio of TSF-IIa Particles

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Protein:Chl (w:w)</th>
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<tr>
<td>A. Control</td>
<td>7.0</td>
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<tr>
<td>Chymotrypsin-treated</td>
<td>4.3</td>
</tr>
<tr>
<td>B. Control</td>
<td>9.3</td>
</tr>
<tr>
<td>Trypsin-treated</td>
<td>5.7</td>
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in these particles. After the aminomethyl cellulose and Sephadex G-100 steps, only the high \( K_d \) site remained. Treatment with chymotrypsin caused only a small decrease in the DCMU sensitivity. Our results do not conflict with those of other workers (5, 24) because we are probably looking at a different DCMU binding site which is less sensitive to proteolytic digestion. The DCMU-sensitive site had already been lost prior to proteolysis.

As may be expected, neither the purification nor protease treatment altered the sensitivity of the activity to Triton X-100 (not shown). Similarly, the \( K_m \) values for DPC (about 50 \( \mu M \)) and DCMU (about 5.0 \( \mu M \)) were not altered. This indicates that the accessibility of the donors and acceptors to their reaction sites was not changed.

The sum of these results indicates that the TSF-IIa complex, as a whole, can undergo extensive proteolytic cleavage without significantly altering its photochemical properties. This leads to either one of two conclusions. Either the reaction center polypeptide, and therefore the Chl-containing species, is the 30 kD polypeptide, which does not undergo cleavage, or the reaction center polypeptide does undergo cleavage without denaturing or loss of activity.

To differentiate between these two possibilities, identification of the Chl-containing polypeptide(s) was necessary. To achieve this, the particles were subjected to electrophoresis on LiDS gels under non-denaturing conditions (4). Figure 9A shows the Chl pattern prior to the staining of the gels. The untreated TSF-IIa particles showed five Chl bands. The Chl band of lowest mobility (apparent mol wt of 90 kD) consists of PSI contamination. The next two Chl bands (apparent mol wt of 47 and 43 kD) were barely resolved from each other. These we believe are authentic PSI Chl-containing polypeptides. The third Chl band consists of LHC contamination and the fourth Chl band consists of free pigments, the latter representing the vast majority of the total Chl molecules present. The apparent amount of both PSI and LHC Chl contamination of the TSF-IIa particles is greatly exaggerated on these gels because the Chl associated with PSI is much more readily dissociated under these conditions, compared to the Chl associated with either PSI or LHC. Stained LiDS gels (Fig. 9B) show that the Chl is associated with polypeptides in the 40 to 50 kD range. No Chl was found to be associated with the 30 kD polypeptide.

TSF-IIa samples treated with chymotrypsin showed a similar Chl pattern on LiDS gels except that there was a single Chl band between the PSI and LHC bands. This Chl band had a slightly higher mobility than the original PSI Chl-containing polypeptides. After staining with Coomassie blue, a diffuse protein band was apparent under the Chl. This protein band was clearly different from the 30 kD polypeptide which was not digested. As with the SDS gels, none of the higher mol wt polypeptides (42, 47-, and 54 kD) were observed on the LiDS gels of the samples treated with proteases. The diffuse protein band and its associated Chl are present only under very mild non-dissociating conditions. From this we conclude that the reaction center polypeptide of PSI Chl molecules may have a structural role in holding the complex together.

DISCUSSION

We found the PSI complex to be composed of four principle polypeptides (54, 47, 43 and 30 kD) plus Cyt b-559. The NaBr treatment plus chromatographic steps have eliminated many of the contaminants observed in other preparations (15). In fact, the peptide composition is nearly identical to that observed by Wessels and Borchert (27, 28) for a PSI preparation isolated using digitonin with the exception that Wessels and Borchert observed additional low molecular weight polypeptides in their preparations. Our preparation contains a greater number of polypeptides than the preparation of Satoh (19) who observed only the 43 and 27 kD polypeptides in addition to Cyt b-559. The 27 kD peptide of Satoh is probably identical to our 30 kD peptide.

Several investigators have reported finding PSI associated Chl \( \alpha \)-containing polypeptides isolated from detergent-fractionated chloroplasts on either SDS or LiDS gels (2, 4, 9, 20). These Chl \( \alpha \) complexes have mol wt in the 40 to 50 kD region. For example, Camm and Green reported two Chl \( \alpha \) complexes in addition to others, with mol wt of 47 and 43 kD (2). Additionally, Satoh has reported that the Chl \( \alpha \) is associated with the 43 kD polypeptide in his digitonin-prepared particles (20). Our results generally concur with these findings. However, we can not unhesitatingly state that there are two separate Chl \( \alpha \)-containing polypeptides in the PSII complex. The two Chl \( \alpha \) bands observed in our LiDS gels may actually be due to a single Chl \( \alpha \)-containing polypeptide in two slightly different states of denaturation.

Limited proteolysis of the PSII particles resulted in the following: (a) A loss in protein without a concomitant loss in Chl; (b) no change in the Chl absorption or fluorescence spectra; (c) retention of activity including DCIP reduction and Cyt b-559 reduction; (d)
no change in the $K_a$ values for either DCIP or DPC; (e) retention of the 40 kD Chl-protein bands on LiDS gel electrophoresis but the loss of the apoproteins corresponding to these pigmented bands after SDS gel electrophoresis; (f) extensive degradation of all polypeptides with the possible exception of the 30 kD polypeptide.

We can explain these results based on the following model. The loss in protein and the decrease in molecular weight can be explained by the disappearance of the 42, 47, and 54 kD bands. These bands are not thought to be necessary for activity since they are also missing in the preparation of Satoh (19, 20) which also retains its activity. Moreover, we postulate that the PSII complex has a tertiary structure similar to that previously observed for the bacteriochlorophyll a protein from the green photosynthetic bacteria (6). In this structure, there is a cylinder of protein wrapped around a Chl core. The tertiary structure would be maintained by strong hydrophobic interactions between the Chl molecules and the protein. As long as these remained intact, the protein would hold together. Thus, the molecule could be nicked in various places by the protease but would still hold together until the Chl was removed. This would explain the retention of activity, Chl absorption, and emission spectra and the appearance of the Chl protein in the LiDS gels but would also explain why the 40 kD polypeptides are not observed after SDS treatment which extracts the Chl. A similar explanation was proposed by Jennings et al. (11) to explain their observations that chymotrypsin treatment of chloroplast lamellae did not alter the electrophoretic mobility of PS I or the LHC although both polypeptides were extensively degraded. A similar situation also occurs in the case of the enzyme ribonuclease which can be cleaved in one place without a loss in activity.

There also remains the question of the 30 kD polypeptide. Either it is insensitive to protease treatment or it is the degradation product of one of the larger polypeptides. In either case, it must be buried on the inside since it is resistant to protease treatment. If it were exposed to the medium, it should have been digested by either trypsin or chymotrypsin due to their different specificities.

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LITERATURE CITED