Determination of Photosystem II Subunits by Matrix-assisted Laser Desorption/Ionization Mass Spectrometry*

Received for publication, September 5, 2000, and in revised form, January 30, 2001
Published, JBC Papers in Press, January 30, 2001, DOI 10.1074/jbc.M008081200

Ildikò Szabò‡§, Roberta Seraglia§, Fernanda Rigoni‡, Pietro Traldi**, and Giorgio Mario Giacometti‡ ‡‡

From the ‡Department of Biology, University of Padova, Via G. Colombo 3, 35121 Padova, †Consiglio Nazionale delle Ricerche, Research Area, Corso Stati Uniti 4, 35100 Padova, and **Consiglio Nazionale delle Ricerche, Center for Study of the Stability and Reactivity of Coordination Complexes, Via Marzolo 1, 35131 Padova, Italy

Photosystem II of higher plants and cyanobacteria is composed of more than 20 polypeptide subunits. The pronounced hydrophobicity of these proteins hinders their purification and subsequent analysis by mass spectrometry. This paper reports the results obtained by application of matrix-assisted laser desorption/ionization mass spectrometry directly to isolated complexes and thylakoid membranes prepared from cyanobacteria and spinach. Changes in protein contents following physiopathological stimuli are also described. Good correlations between expected and measured molecular masses allowed the identification of the main, as well as most of the minor, low molecular weight components of photosystem II. These results open up new perspectives for clarifying the functional role of the various polypeptide components of photosystems and other supramolecular integral membrane complexes.

Photosystem II is a pigment-protein complex of the thylakoid membrane of higher plants, eukaryotic algae, and cyanobacteria. It catalyzes light-induced electron transfer from water to plastoquinone, with associated production of molecular oxygen. PSII consists of a large complex with a number of polypeptide components, most of which are integral membrane proteins. A number of extrinsic proteins are also associated with it at the membrane surface. The entire set of electron transfer cofactors, including chlorophyll a, pheophytin a, plastoquinones, and non-heme iron, is associated with the D1/D2 heterodimer. These two proteins, together with the $\alpha$ and $\beta$ subunits of cytochrome $b_{563}$ and the PsbI protein, constitute the so-called reaction center II (RCII), which is the smallest PSII subparticle still able to perform light-induced charge separation (1). Two large integral membrane proteins, CP43 and CP47, each coordinating a number (12–15) of chlorophyll $\alpha$ molecules, and several low molecular weight (<10 kDa) polypeptides are constituents of the PSII core complex, which is very similar in higher plants and prokaryotic cyanobacteria.

During the last decade, considerable progress has been made in our understanding of the organization of polypeptides constituting the reaction center of PSII, but the topology and functional role of the small protein subunits are still largely unknown. Some of them are universal, whereas others are present only in cyanobacteria (PsbU and PsbV) or only in higher plants (e.g., PsbP, PsbQ, PsbS) (2).

Investigation of the structural and functional roles of the various PSII subunits requires, as a preliminary step, a suitable method to detect them in the thylakoid membrane or purified subparticle preparations. The detection and study of stimuli-induced modifications of several PSII components have mainly been based on the use of polyclonal antibodies. However, these are often not available and, when available, are time-consuming to use.

A different technique, capable of accurately detecting even small amounts of the various PSII subunits in an integrated system, could be of great help in studying the function of these proteins. Mass spectrometry has recently been used for structural biology studies, also in the field of photosynthesis. A reverse phase high performance liquid chromatography (HPLC) purification system has been developed for the separation of PSII reaction center proteins, and the molecular masses of the resulting purified, intact proteins have been determined by electrospary ionization mass spectrometry (ESI-MS) (3–6). These studies demonstrate that ESI-MS is indeed a useful technique for studying hydrophobic membrane proteins also. Whereas ESI-MS allows highly accurate measurement of the molecular mass, it suffers from the important limitation that it cannot be applied in the presence of detergents, and the proteins, free of salts and detergents prior to MS analysis, must be purified (7). This paper reports the results obtained by MALDI mass spectrometry for rapid determination of non-purified proteins of photosynthetic complexes isolated from spinach and cyanobacteria.

MATERIALS AND METHODS

MALDI Mass Spectrometry—Measurements were performed on a REFLEX time-of-flight instrument (Bruker-Franzen Analytik, Bremen, Germany) equipped with a SCOUT ion source operating in positive linear mode. Ions, formed by a pulsed UV laser beam (nitrogen laser, $\lambda = 337$ nm), were accelerated to 25 kV. The use of this beam did not result in degradation of photosynthetic proteins during measurements. Sinapinic acid (saturated solution in acetonitrile:water (50:50 v:v)) was used as a matrix. Samples, at chlorophyll concentrations of 9–250 $\mu$g/ml, were diluted 2- to 10-fold with a 0.1% trifluoroacetic acid aque-
ous solution. 5 μl of the diluted sample solution was mixed with the same volume of matrix solution, and 1 μl of the resulting mixture was deposited on a stainless steel sample holder and allowed to dry before introduction into the mass spectrometer.

Because of the wide mass range to be examined, two different instrumental conditions were used. For low molecular masses (2,000–20,000 Da), pulsed ion extraction was carried out applying a voltage of 22.3 kV for 300 ns to the second grid. External mass calibration was carried out using [M + H]^+ ions of bovine insulin and horse myoglobin at m/z 5734 and 16952, respectively, and the corresponding doubly charged species at m/z 2868 and 8476, respectively. For high molecular masses (20,000–80,000 Da), pulsed ion extraction was used applying a voltage of 21.9 kV for 400 ns to the second grid. External mass calibration was carried out using [M + H]^+ ions of horse myoglobin and bovine serum albumin at m/z 16952 and 66431, respectively, and the doubly charged species of bovine serum albumin at m/z 33216. The GuessProt algorithm was used to determine the predicted molecular masses of the PSIII subunits.

Preparation of PSII Particles and Isolated RCII from Spinach—PSII core complexes from spinach were prepared according to Ghantakos et al. (8), and isolation of RCII was performed as described in Ref. 9.

Preparation of Thylakoid Membranes and PSII from PSI-less Synechocystis—Thylakoids were prepared using a modified version of the procedure of Mayes et al. (10). Briefly, cells were cultured as previously described (11), harvested by centrifugation (2000 × g for 5 min at 4 °C), and resuspended in a breaking buffer containing the following: 20 mM Mes, 5 mM MgCl₂, 5 mM CaCl₂, 1 mM benzamidine, 1 mM aminocaproic acid, and 25% glycerol (pH 6.35 with NaOH). Cells were broken on ice using a bead beater (Biospec) by application of 15 30-s cycles at 5-min intervals. Unbroken cells were eliminated (5-min centrifugation at 2000 × g for 30 min). Pellets were then resuspended in 50 mM Mes and 20 mM sodium pyrophosphate (pH 6.5). Isolated thylakoids were solubilized with 2% (w/v) dodecyl maltoside for 30 min and loaded on a continuous sucrose gradient containing 25 mM Mes, 0.5 mM sucrose, 10 mM NaCl, and 25% glycerol (pH 6.35 with NaOH). Cells were broken on ice using a bead beater (Biospec) by application of 15 30-s cycles at 5-min intervals. Unbroken cells were eliminated (5-min centrifugation at 2000 × g for 30 min). Pellets were then resuspended in 50 mM Mes and 20 mM sodium pyrophosphate (pH 6.5). Isolated thylakoids were solubilized with 2% (w/v) dodecyl maltoside for 30 min and loaded on a continuous sucrose gradient containing 25 mM Mes, 0.5 mM sucrose, 10 mM NaCl, 5 mM CaCl₂, and 0.03% dodecyl maltoside (pH 6.5). Three bands, i.e. carotenoids, cytochrome b/f, and PSII, separated out after 17 h of centrifugation at 35,000 rpm. The PSII band was collected and concentrated in Centricon-100 tubes.

Preparation of RC47 from Synechocystis—RC47 particles, containing the PSII reaction center (RCII) and the chlorophyll a internal antenna CP47 but lacking the other chlorophyll a internal antenna CP43, were prepared from PSI-less Synechocystis following the procedure described in Szabo et al. (6). Briefly, thylakoid membranes from a PSI-less mutant strain of Synechocystis were solubilized in 10% dodecyl maltoside for 30 min, subjected to anion exchange chromatography, and eluted with a linear 0–200 mM gradient of MgSO₄. The elution profile comprised a single peak for RC47 at about 110 mM MgSO₄.

SDS-PAGE—SDS-polyacrylamide gel electrophoresis in the presence of urea (6x) was performed as described previously (12).

Irradiation Conditions—PSII samples at 90 μg/ml chlorophyll were irradiated in a cuvette with a 1-cm light path using a visible light intensity of 700 μmol of photons m⁻² s⁻¹ and a UV-B light intensity of 5 μmol of photons m⁻² s⁻¹. A Vilbert-Lourmat 215M lamp was used as a UV-B source, wrapped in cellulose diacetate foil (0.15 mm thick) to screen out any UV-C component emitted by the UV-B source.

RESULTS

PSII Core and RCII Complexes from Spinach—The representative MALDI spectrum of a PSII core complex from spinach in the low molecular mass range (3000–6000 Da) is shown in Fig. 1A. At least 11 peaks are detected, and some of them, identifiable as known PSII subunits, are listed in Table I. It is well known that PSII core complexes of higher plants contain several low molecular weight polypeptides, as demonstrated by analysis of the plastidial genome (13, 14). Morris and co-workers (3, 4, 6) studied polypeptides purified from preparations of PSII reaction center (RCII) and the RC47 complex of higher plants (pea and spinach, respectively) by HPLC purification/separation followed by ESI-MS analysis. RC47 is a PSII subcomplex, resulting from detergent-induced dissociation of internal antenna CP43 from the PSI core complex. To better identify the MALDI peaks of Fig. 1A and to compare our results with those reported by Sharma et al. (3, 4), the RCII from spinach was prepared and analyzed by MALDI.

MALDI mass spectra for low and high molecular masses of RCII are shown in Fig. 1, B and C, respectively. SDS-PAGE analysis and the absorption spectra of the PSII core and RCII preparations, generally used to identify and characterize the various PSII subparticles obtained by detergent extraction, are shown in Fig. 2. A small amount of light-harvesting complex II (LHCII) still present in the preparations is observable in both the SDS-PAGE and the 20–30-kDa range of the MALDI spectra (data not shown).

Table I compares our data with the expected mass values of unprocessed precursors of PSII components and with the results obtained by ESI-MS (3–6). As may be seen, MALDI data on subunit composition and MW values in intact spinach PSII cores and RCII complexes closely match those obtained by Morris and co-workers (3–6) on polypeptides purified from RCII and RC47. However, in the case of RCII in the low molecular mass region, we clearly detected an additional peak at m/z 5928, assigned to the PsbW protein. Accordingly, this protein has recently been suggested (15) to be the sixth component of the RCII complex purified from higher plants. PsbW has been detected by ESI-MS only in spinach RC47 (6) but not in pea RCII (3). A peak at m/z 5928 assigned to PsbW is also visible in the spectrum of the PSII core complex (Fig. 1A).

Altogether, these results show that, in particular in the low molecular mass range, direct measurements on PSII and RCII
preparations are quite accurate and that MW values are comparable with those of isolated proteins obtained by ESI-MS.

**PSII Core Complexes from Cyanobacteria—**Study of the structural-functional role of the subunit components of supramolecular complexes has been greatly advanced by the development of molecular genetic techniques such as the generation of mutants carrying site-specific modifications or gene deletions. In this respect, the identification of an easily transformable photosynthetic organism, with high frequency of homologous recombination, able to survive and propagate without functional photosystems, has been of great help. The cyanobacterium *Synechocystis* sp PCC 6803 fulfils these requirements, and, moreover, its whole genome has been sequenced. However, preparation of purified PSII subparticles and their isolated polypeptide components is even more difficult from cyanobacteria than from higher plants. A method to reveal and identify PSII components in cyanobacteria quickly, with no need for extended, time-consuming purification, would be useful, and it was for this reason that we focused our attention on *Synechocystis*.

PSII cores were isolated from a PSI-less mutant cyanobacterium. Part of the *psaAB* operon coding for the core complex of PSI was deleted from the genome of *Synechocystis* wild-type strain (16). PSI-less cells have approximately six times more PSII than do wild-type cells, as estimated on a chlorophyll basis. The MALDI spectra of Fig. 3 were obtained from two different preparations. In the 20,000–70,000 *m/z* range, seven main peaks were detected at *m/z* 55,276 ± 6, 51,470 ± 9, 39,268 ± 9, 38,049 ± 17, 34,399 ± 9, 31,030 ± 7, and 25,750 ± 9 (*n* = 16 spectra from 8 different preparations). Each MALDI measurement was highly reproducible for the same sample, and good reproducibility was observed in the *m/z* values of the peaks for samples deriving from different preparations (compare A and B of Fig. 3). However, the relative intensity of the peaks depended on sample preparation, due to the presence of varying amounts of interfering materials, such as salts and lipids (17).

Molecular weight values obtained by MALDI were compared with protein masses calculated from the nucleotide sequences of PSII components. Good correlations between calculated and measured values revealed the following correspondence between measured *m/z* values and known PSII components:

| Putative PSII subunit (spinach) | Expected mass of unprocessed precursor | Measured mass from spinach PSII or RCII (this work) | Measured mass from pea or spinach RCII or RC47 (3–6) |
|--------------------------------|--------------------------------------|------------------------------------------------------|------------------------------------------------------|
| psbTc                          | 3,822                                | 3,853                                                | 3,849.6 sp.                                          |
| psbI                           | 4,168                                | 4,201                                                | 4,195.5 sp.                                          |
| PsbK                           | 6,914                                | 4,296                                                | 4,292.1 sp.                                          |
| psbL                           | 4,366                                | 4,369                                                | 4,365.5 sp.                                          |
| β cytochrome b<sub>559</sub>    | 4,367                                | 4,412                                                | 4,409.1 sp.                                          |
| PsbW                           | 14,177                               | 5,928                                                | 5,927.4 sp.                                          |
| α cytochrome b<sub>559</sub>    | 9,255                                | 9,260                                                | 9,255.1 sp.                                          |
| D1                             | 38,819                               | 37,998                                               | 38,040.9 pea                                         |
| D2                             | 39,376                               | 39,379                                               | 39,456.1 pea                                         |

Fig. 3. MALDI spectra of two distinct preparations of PSII core complex from *Synechocystis*. *m/z* values are indicated in A; assignment of the main peaks is shown in B.
species of the ion at $m/z$ 51,470. The peaks at $m/z$ 31,030 and 34,399 may represent subunits of ATP synthase (predicted masses of 30,698 and 34,605 Da, respectively). Alternatively, the peak at $m/z$ 31,030 may correspond to the phycobilisome 32-kDa linker peptide (predicted mass of 30,797 Da). Clear-cut identification of these peaks would require the use of mutants lacking these proteins.

It is worth noting that the $m/z$ peaks assigned to the apoproteins of the two internal antennae, CP43 ($m/z$ 51,460) and CP47 ($m/z$ 55,276), are reproducibly very different in their amplitude, despite the fact that both components have a 1:1 stoichiometric ratio to the reaction center. This may be related to the different ionization probability of the two subunits when embedded in the PSII core complex. It might be speculated that CP43, which is less closely associated with the core and is located in a more external position with respect to CP47 (18), offers a higher cross-section for ionization to incoming laser photons. To confirm unequivocally the assignment of the $m/z$ peaks to the internal antennae, we prepared an RC47 particle from the Synechocystis PSII core. This PSII subparticle, obtained by dissociating the CP43 subunit by partial detergent solubilization, is the cyanobacterial analogue of the RC47 obtained from higher plants by other groups (6). An immunoblot of its polypeptide components is shown in Fig. 4C. Whereas the D1, D2, and CP47 subunits of the PSII core are left unchanged in the RC47 particle (Fig. 4C, lanes 2, 4, and 8), only a tiny amount of CP43 is contained in the latter (lane 6). If the proposed assignments are correct, we expect the $m/z$ peak at 51,470 to disappear from the MALDI spectrum of the RC47 particle. Fig. 4 compares the spectrum of RC47 (B) with that of the core complex (A). The expectation is fully matched, because the $m/z$ peak at 51,470 is highly reduced with respect to the intensity of the D1 and D2 peaks. It may be noted that the peak at 55,276, attributed to CP47, maintains approximately the same relative intensity. Therefore, an eventual shadowing action on CP47 must be exerted by core proteins other than CP43, in accordance with the recent finding that the two internal antennae are on opposite sites of the D1-D2 heterodimer, rather than sequentially located within the PSII core (20). It may also be noted that detergent treatment on sample A to produce RC47 (Fig. 4B) also eliminates the impurities giving rise to $m/z$ peaks in the range of 25–35 kDa.

MALDI Spectrum of High MW Subunits of PSII Core Complex in Light Stress Conditions—It is known that the D2 and, to an even greater extent, D1 proteins of higher plants and cyanobacteria are characterized by high turnover and that their degradation rate is increased by UV-B radiation (21–25) or an excess of photosynthetic active radiation (26–29). The usual way to reveal these phenomena is to measure the D1 and D2 contents of the thylakoid membrane after exposure to radiation by SDS-PAGE and immunoblotting and then to search for the generated fragments by the same methods. The following experiment aimed at checking whether the MALDI technique could be used as an alternative, direct method to study the phenomenon. Fig. 5 shows an example of this application. PSII

![Figure 4](image_url)

**Fig. 4.** MALDI spectra of PSII core complexes (A) and RC47 subparticle (B) from *Synechocystis*. Immunoblots (C) of PSII core complexes (lanes 1, 3, 5, and 7) and RC47 (lanes 2, 4, 6, and 8). Lanes 1 and 2, 3 and 4, 5 and 6, and 7 and 8 were probed with antibodies specific for D1, D2, CP43, and CP47, respectively. PSII samples were loaded at 0.25 μg of chlorophyll.

![Figure 5](image_url)

**Fig. 5.** MALDI spectra of PSII core complexes from *Synechocystis*: untreated sample (A) and samples irradiated with visible (B) and UV-B light (C). See "Materials and Methods" for irradiation conditions.
isolated from PSI-less organisms was left untreated (Fig. 5A) or was irradiated with visible light (B) or UV-B light (C) for 30 min. The intensity of each peak was measured in the three samples derived from the same preparation and expressed as a percentage of the intensity of the CP43 peak (m/z 51,470), which is known to be unaffected by the light intensities used here (30). Whereas the relative intensities of most of the peaks did not change upon exposure to radiation, those corresponding to D1 and D2 (m/z 38,048 and 39,268, respectively) showed marked variations. In particular, the intensities of the D1 peak decreased by 63% in visible light-illuminated PSIII and by 13% in the UV-B-treated sample. The intensities of the D2 peak decreased by 45 and 35%, respectively. It is worth stressing the point that the three spectra (Fig. 5, A–C) were obtained from aliquots of the same sample, thus ensuring that evaluation of the peak areas was accurate enough to make quantitative comparisons significant. An unidentified peak at m/z 31,071 was also found to be slightly affected by both kinds of irradiation, suggesting that this protein is involved in the stress response.

Low MW Components of PSIi from Cyanobacteria—Because the low MW subunits of cyanobacteria and spinach PSII differ in their predicted masses, the low molecular mass region of the MALDI spectra of PSIi core complex prepared from the PSI-less strain of Synechocystis was examined (Fig. 6). Detected peaks were tentatively identified with the main known subunits, as listed in Table II.

The identity of a few peaks is still unresolved. For example, the nature of the peaks at m/z 3570 and 8170 is unknown, whereas the peak at m/z 8008 may correspond either to a phycobilisome 7.8-kDa linker peptide or to a subunit of ATP synthase (calculated masses are 7805 and 7968 Da, respectively).

In an intermediate molecular mass region (data not shown), one peak at m/z 12,474 may correspond to the psbW precursor (unprocessed protein mass of 12,590 Da), and another peak at m/z 14,468 may correspond to cytochrome c550, also called PsbY (predicted mass of 15,119 Da).

In view of our study of the structural-functional role of the PsbH subunit of PSIi, we were particularly interested in its identification by MALDI, because this protein cannot be revealed by SDS-PAGE or immunoblotting in cyanobacteria, in which it is not phosphorylated. The predicted molecular mass of PsbH for cyanobacteria is 6985 Da. As may be observed in Fig. 6 and Table II, a peak corresponding to PsbH was found in the PSIi core complex preparation from Synechocystis.

MALDI Measurements on Whole Thylakoid Membrane—We checked for the presence of PsbH protein in the whole thylakoid membrane of Synechocystis. A peak at m/z 7094 was observed in the PSIi-less preparation (Fig. 7A). For comparison, thylakoids from a PsbH deletion mutant, IC7 (31, 32), were also examined (Fig. 7B), but no significant differences could be observed between the two MALDI spectra except for the lack of the peak at m/z 7094 in the deletion mutant. It may thus be concluded that this peak actually corresponds to PsbH. The m/z value of PsbH measured in whole thylakoids is ~100 Da higher than expected and measured in the PSIi core complex. Although we have no simple explanation for this difference, it may be due to the lower precision of m/z values when measured in a rich lipid environment such as that of whole thylakoids. Although the possibility that PsbH in thylakoids is phosphorylated cannot be ruled out (33), it is not probable, because the known phosphorylation site in cyanobacteria is lacking (34).

DISCUSSION

Analysis of protein components in the membrane multisubunit complex of photosystem II has traditionally been performed by various versions of SDS-PAGE and immunoblotting. A great amount of information has been obtained by these techniques, but their application has been limited by poor gel resolution and the difficulty of producing suitable antibodies, especially for the low molecular weight components. The development of HPLC procedures for the purification of low MW components and the D1 and D2 proteins of PSIi allowed Morris and co-workers (3–6) to apply mass spectrometry to analysis of PSIi components, using electron spray as the ionization method. This technique is accurate in determining protein molecular masses and allowed these authors to characterize post-translational modifications of some PSIi polypeptides. However, it does depend to a great extent on the proper purification of each subunit, which must be free of salts and detergents; so it is a rather cumbersome procedure for the extremely hydrophobic proteins of PSIi. We present here the results of the application of an alternative mass spectrometric technique, which, although somewhat less accurate in determining exact molecular masses, does allow quick and simple analysis of the protein components of the PSIi complex, in a single step, on integrated samples such as the entire PSIi core complex or, in some cases, even untreated photosynthetic membranes.

The first part of the work aimed at comparing our MALDI analysis on different detergent-extracted subparticles of PSIi from higher plants with results obtained by ESI-MS on isolated subunits. To assign the observed peaks in the PSIi core complex from spinach to the corresponding proteins, we took into account the masses of unprocessed precursors and/or comparisons with the MALDI spectra of purified preparations of RCII. The results shown in Table I compare favorably with those
obtained by ESI-MS, and the molecular mass differences found are within measurement uncertainty. One interesting result of these experiments was that, in our isolated RCII preparation, also known as the D1-D2-cytochrome b559 complex, we also found the PsbW protein (molecular mass, 5928 Da) as well as the PsbI protein, which has long been known to belong to this complex, thus confirming the recent report of Irrgang et al. (15). PsbW usually escapes detection by SDS-PAGE but was easily clearly visible in the MALDI spectrum (Fig. 2B). The lack of detection of this protein in ESI-MS suggests that PsbW is loosely bound to the RCII complex and may easily be lost during purification.

A number of peaks in the 4500–5500 m/z region of PSII core complexes (Fig. 1A) were not identified; they may have been impurities or, more probably, D1 fragments still associated with the PSII core (35). Some unidentified minor peaks were also found in the high molecular mass region of the purified RCII spectrum, showing the sensitivity of MALDI analysis to impurities in preparations (Fig. 1C).

The second part of our work was application of the MALDI technique to the photosynthetic membrane of Synechocystis, to check whether it could identify PSII protein components in this organism, which is a particularly interesting system frequently adopted for studies applying the molecular genetic approach to structural and functional studies of PSII. Attribution of the peaks observed in the MALDI spectra was made easier by knowledge of the molecular masses of all the precursors of thylakoid proteins in this organism.

When the PSII complex was isolated from the PSI-less strain of Synechocystis, the MALDI spectrum turned out to be highly reproducible even in the high MW region, which is generally the most unfavorable for this technique. Such good resolution and reproducibility was not obtained, in the same region, for PSII cores isolated by detergent treatment of the thylakoid membrane of spinach. This may have been because of the large quantity of detergent used in the latter case, which is known to lead to signal suppression in the MALDI ionization process (36). Most of the known components in both high and low molecular mass regions were identified using the expected molecular masses, and by comparing subparticles and/or mutant strains lacking one PSII protein component.

We also attempted the resolution of whole, untreated photosynthetic membrane by MALDI-MS. One particularly interesting result was identification of the PsbH protein in the photosynthetic membrane of Synechocystis. This small subunit of the PSII core was originally detected as a 9-kDa phosphoprotein in pea chloroplasts (37). The physiological roles proposed for it include regulation of photoinduction (38), control of the electron transfer from Qa to Qb (10), and stabilization of the PSII dimer in higher plants (39, 40). Interestingly, the psbh gene product from cyanobacteria is truncated at the N terminus site and lacks the phosphorylation site (19). This renders its detection in cyanobacteria extremely difficult by immunoblotting using a polyclonal antiserum produced against the spinach phosphoprotein; however, no specific antibodies against PsbH from Synechocystis have yet been obtained. Furthermore, the PsbH protein cannot be detected by staining of SDS-PAGE. Thus, the reported clear-cut detection of PsbH in the thylakoid membrane of Synechocystis will greatly help further work in progress on this important subunit.

Assignment of the various m/z peaks to PSII polypeptide subunits, based on the expected molecular masses, was confirmed in all cases in which an independent check could be applied, as in the case of D1, D2, CP43, CP47, and H subunit. This confirmation and the favorable comparison of MALDI results with those obtained by ESI mass spectrometry on higher plant PSII particles provide good proof that this technique can be of great help in the identification and analysis of the polypeptide subunits of large integral membrane complexes. In addition to structural information concerning the composition of complexes from a number of different organisms, MALDI can also be applied to study the effects of various stimuli or stress conditions. This is demonstrated by the changes induced by exposure of a PSI and UV-B radiation or excessive visible light in their MALDI spectra. As expected, a significant decrease in the relative abundance of the peaks due to D1 is observed after visible light stress and of those of both D1 and D2 proteins upon exposure to UV-B, in agreement with previous results (21–29). The observed significant increase in the number of peaks in the low molecular mass region (data not shown) also indicates the possibility of detecting fragments originating from radiation-induced degradation of PSII proteins.

In conclusion, the present work provides evidence that the MALDI technique may be applied directly to photosynthetic protein complexes without the need to purify subunits prior to MS analysis. This novel approach, in combination with genetic engineering, may greatly contribute to unraveling the role of the various protein subunits of photosystems and other supramolecular membrane complexes.

Acknowledgments—We are grateful to Des. E. Bergantino and E. Bergo and Prof. R. Barbato for discussions, Dr. M. Bianchetti for help in preparing some samples, and Prof. J. Barber and W. F. J. Vermaas for the IC7 and PSI-less mutants of Synechocystis, respectively.

REFERENCES
1. Nanba, O., and Satoh, K. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 109–112
2. Barber, J., Nield, J., Morris, E. P., Zheleva, D., and Hankamer, B. (1997) Physiol. Plant. 106, 817–827
3. Sharma, J., Panico, M., Barber, J., and Morris, H. R. (1997) J. Biol. Chem. 272, 3935–3943
4. Sharma, J., Panico, M., Barber, J., and Morris, H. R. (1997) J. Biol. Chem. 272, 32153–32157
5. Sharma, J., Panico, M., Shipton, C. A., Nilsson, F., Morris, H. R., and Barber, J. (1997) J. Biol. Chem. 272, 33158–33166
6. Zheleva, D., Sharma, J., Panico, M., Morris, H. R., and Barber, J. (1998) J. Biol. Chem. 273, 16122–16127
7. Barnidge, D. R., Drate, E. A., Jesaitis, A. J., and Sunner, J. (1999) Anal. Biochem. 10, 1–9
8. Ghanotakis, D. F., Demetriou, D. M., and Yocum, C. F. (1987) Biochim. Biophys. Acta 801, 15–21
9. Chapman, D. J., Gounaris, K., and Barber, J. (1990) Methods in Plant Biochemistry (Rogers, L. J., ed) Vol. V, pp. 171–193, Academic Press, London
10. Mayes, S. R., Dubbs, J. M., van S., Hideg, E., Nagy, L., and Barber, J. (1995) Biochemistry 32, 1454–1465

E. Bergantino, personal communication.
Analysis of Composition of Photosynthetic Complexes

11. Barbato, R., Polverino de Laureto, P., Rigoni, F., De Martini, E., and Giacometti, G. M. (1995) *Eur. J. Biochem.* 234, 459–465
12. Barbato, R., Friso, G., Giardi, M. T., Rigoni, F., and Giacometti, G. M. (1991) *Biochemistry* 30, 10220–10226
13. Douglas, S. E. (1998) *Curr. Opin. Genet. Dev.* 8, 655–661
14. Shimada, H., and Sugiura, M. (1991) *Biochemistry* 30, 10220–10226
15. Douglas, S. E. (1998) *Curr. Opin. Genet. Dev.* 8, 655–661
16. Shimada, H., and Sugiura, M. (1991) *Biochemistry* 30, 10220–10226
17. Xiang, F., and Beavis, R. C. (1993) *Org. Mass Spectrom.* 28, 1424–1428
18. Barber, J. (1998) *Biochim. Biophys. Acta* 1365, 269–277
19. Chiaramonte, S., Giacometti, G. M., and Barber, J. (1999) *Eur. J. Biochem.* 260, 1–12
20. Hankamer, B., Morris, E. P., and Barber, J. (1999) *Nat. Struct. Biol.* 6, 560–564
21. Friso, G., Barbato, R., Giacometti, G. M., and Barber, J. (1994) *FEBS Lett.* 339, 217–221
22. Giacometti, G. M., Barbato, R., Chiaramonte, S., Friso, G., and Rigoni, F. (1996) *Eur. J. Biochem.* 242, 799–806
23. Mater, Z., Sass, L., Szabó, L., Vass, L., and Nagy, P. (1998) *J. Biol. Chem.* 273, 17439–17444
24. Vass, I., Kirilovsky, D., Perewoska, I., Mate, Z., Nagy, F., and Etienne, A. L. (2000) *Eur. J. Biochem.* 267, 2640–2648
25. Barbato, R., Bergo, E., Szabó, L., Dalla-Vecchia, F., and Giacometti, G. M. (2000) *J. Biol. Chem.* 275, 10976–10982
26. Greenerg, B. M., Gaba, V., Canaani, O., Malkin, S., Mattoo, A. K., and Edelman, M. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 86, 6617–6620
27. Barbato, R., Friso, G., de-Laureto, P. P., Frizzo, A., Rigoni, F., and Giacometti, G. M. (1992) *FEBS Lett.* 311, 33–36
28. Aro, E. M., Virginia, I., and Andersson, B. (1995) *Biochim. Biophys. Acta* 1143, 113–134
29. Kettunen, R., Tyystjarvi, E., and Aro, E. M. (1996) *Plant Physiol.* 111, 1183–1190
30. Rajagopal, S., Murthy, S. D., and Mohanty, P. (2000) *J. Photochem. Photobiol. B Biol.* 54, 61–66
31. Mayes, S. R., and Barber, J. (1991) *Plant Mol. Biol.* 17, 289–293
32. Komenda, J., and Barber, J. (1995) *Biochemistry* 34, 9625–9631
33. Race, H. L., and Gounaris, K. (1993) *FEBS Lett.* 323, 35–39
34. Abdel-Mawgued, A. L., and Dilley, R. A. (1990) *Plant Mol. Biol.* 14, 445–446
35. Barbato, R., Friso, G., and Giacometti, G. M. (1996) *Eur. J. Biochem.* 242, 799–806
36. Worrall, T. A., Cotter, R. J., and Woods, A. S. (1998) *Anal. Chem.* 70, 750–756
37. Bennett, J. (1977) *Nature* 269, 344–346
38. Kuhn, M., Thiel, A., and Broger, P. (1988) *Z. Naturforsch.* 43, 413–417
39. Morris, E. P., Hankamer, B., Zheleneva, D., Friso, G., and Barber, J. (1997) *Structure* 5, 837–849
40. Michel, H. P., and Bennett, J. (1987) *FEBS Lett.* 212, 103–108