Biochimica et Biophysica Acta, 724 (1983) 142–150
Elsevier

BBA 41324

POLYPEPTIDE COMPOSITION OF A PHOTOSYSTEM II CORE COMPLEX

PRESENCE OF A HERBICIDE-BINDING PROTEIN

K. SATOH *, H.Y. NAKATANI, K.E. STEINBACK, J. WATSON and C.J. ARNTZEN

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI, 48824 (U.S.A.)

(Received February 23rd, 1983)

Key words: Photosystem II; Chlorophyll fluorescence; Herbicide-binding protein; Atrazine; Polypeptide composition

The polypeptide composition of a Photosystem II (PS II) core complex from higher plant chloroplasts has been characterized by subjecting the isolated complex to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Two polypeptides in the 40–50 kDa size class, attributed to the chlorophyll *a*-binding apoproteins of PS II, were resolved when the urea concentration in the SDS-polyacrylamide gel electrophoresis was greater than 1 M. The two chlorophyll *a*-binding proteins were dissimilar in their primary structure based upon their different hydrolysis products on SDS-polyacrylamide gel electrophoresis following papain treatment. The core complex contained three additional polypeptides. Two polypeptides in the 30–34 kDa size class were resolved when the urea concentration in the gel system was increased to greater than 4 M. One of the polypeptides in this size class was identified as the herbicide-binding protein from azido[^{14}C]atrazine labeling studies. The herbicide-binding protein displayed an anomalous electrophoretic migration behavior in SDS-polyacrylamide gel electrophoresis in the presence or absence of urea; its apparent molecular weight decreased when the urea concentration increased. The fifth protein component of the core complex was attributed to cytochrome *b*-559 which was found to consist of the ascorbate- and dithionite-reducible forms in the samples prior to SDS solubilization.

Introduction

An isolation procedure has previously been described which utilized digitonin fractionation, ultracentrifugation and isoelectric focusing to yield a highly purified functionally active PS II reaction center preparation [1]. This complex was initially characterized by SDS-polyacrylamide gel electrophoresis analysis as being composed of three major polypeptides with estimated molecular masses of 43, 27 and 6 kDa [2]. The 43 kDa polypeptide was attributed to a Chl *a*-binding reaction center apoprotein, based on earlier studies [3–5] whereas the 27 kDa polypeptide was attributed to a structural and/or functional component of the reaction center. The 6 kDa polypeptide was correlated with the subunits of cytochrome *b*-559 [5,6].

Procedures have been introduced to improve the resolution of polypeptides on SDS-polyacrylamide gel electrophoresis, especially for the analysis of proteins thought to participate in PS II function (for example, see Ref. 3). We have, there-

* Present address: Department of Biology, Faculty of Science, Okayama University, Okayama 700, Japan.

Abbreviations: PS, photosystem, LHC, light-harvesting chlorophyll complex; DCIP, 2,6 dichlorophenolindophenol; Q, quencher, a quinone acting as the primary stable electron acceptor for PS II; P-680, reaction center of PS II; Chl, chlorophyll; dinoseb, 2-(1-methylpropyl)-4,6-dinotrophenol; terbutryn, 2-(*tert*-butylamino)-4-ethylamino-6-methylthio-*s*-triazine; Mes, 4-morpholineethanesulfonic acid.

fore, reinvestigated the protein composition of the PS II core complex.

Materials and Methods

PS II 'core complex' particles were isolated from spinach and peas as previously described [1], except that the thylakoids were treated with NaBr (one wash with 2 M NaBr) prior to detergent fractionation to remove extrinsic membrane proteins and coupling factor components [7].

Low-temperature (77 K) and room-temperature fluorescence emission spectra were obtained with a dual-channel acquisition method using a System 4800 scanning spectrofluorimeter (SLM Instruments, Urbana, IL). Room-temperature absorption spectra and cytochrome measurements were obtained using a Hitachi 110 double-beam spectrophotometer. DCIP photoreduction (diphenylcarbazide → DCIP) was monitored at 575 nm using a Hitachi 100-60 spectrophotometer fitted for actinic side illumination at 90° to the measuring beam. The actinic beam was provided by a high-intensity microscope illuminator projected through a Corning 2-58 filter in conjunction with a Corning 1-75 heat filter. The spectrophotometer phototube was protected from scattered actinic illumination through the use of Corning 4-96 filters. The output from the Hitachi 100-60 was recorded on a Soltec XY time base recorder.

Photoaffinity labeling of the herbicide-binding protein was conducted with azido[^{14}C]atrazine as previously described [8]. This compound was kindly supplied by Dr. Gary Gardner of Shell Agricultural Chemicals, Modesto, CA, U.S.A. [^{35}S]Methionine incorporation into the thylakoid membrane proteins was accomplished by application of [^{35}S]methionine to the pea leaf surface 4 h prior to extraction of the chloroplasts as described in Ref. 9.

Polypeptides were analyzed by SDS-polyacrylamide gel electrophoresis using polyacrylamide gradients (10–17.5%) as described in Ref. 10. Urea or urea gradients in the slab gels were utilized as noted in the text. Samples were not heat-treated prior to electrophoresis. Polypeptides excised from slab gels were further analyzed by partial proteolysis with papain (Sigma Chemical Co.) according to the procedure of Cleveland et al. [11] for gel slices. Gel scans were obtained on a Gelman Automatic Computing Densitometer, ACD-18.

Results and Discussion

Spectral characteristics of the core complexes of PS II

Room-temperature absorption and fluorescence emission characteristic of the PS II core complex preparation are shown in Fig. 1. The Chl *a* absorption peak maximum was near 673 nm and, as expected for a reaction center particle, no absorbance due to Chl *b* was observed as a shoulder near 650 nm. The presence of carotenoid(s) species is indicated by the absorption shoulders in the Soret region (460–490 nm). These are likely to be due to β-carotene [12]. Mathis et al. [13] previously showed the presence of a carotenoid in the PS II particle from flash-illumination studies on a carotenoid triplet state. A minor absorption peak was always observed at 540 nm. This may be due to the presence of pheophytin which has recently been shown to act as the intermediate electron acceptor between P-680 and Q [14,15]. That this absorption was not due to simple degradation of of the chlorophyll was indicated by the fact that this absorption characteristic was not observed for any of the other pigmented fractions simultaneously purified with the PS II reaction center particle (PS I reaction center or LHC components). An upper estimate based on spectroscopic data of Vernon [16] indicated approx. 1 pheophytin molecule per 25 Chl *a* molecules. Earlier functional assays with this complex have indicated one reaction center per 50 Chl [13]; this may indicate 2

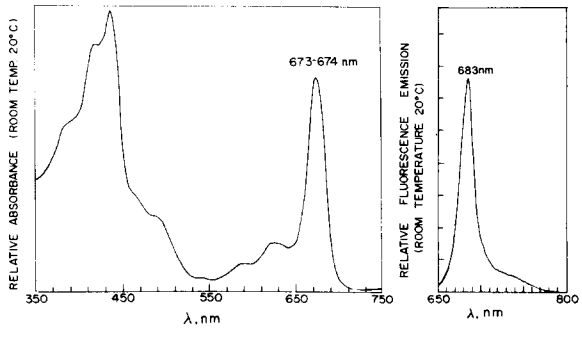

Fig. 1. Room-temperature absorption and fluorescence emission spectra of the purified PS II core complex.

pheophytins per reaction center.

A 695 nm fluorescence emission peak at 77 K is characteristically observed from thylakoids and has been attributed to PS II [17]. The 77 K fluorescence emission peak of the PS II reaction center preparation isolated in this study occurred near 685 nm at 77 K (Fig. 7). The addition of 1,10-phenanthroline to the preparation resulted in the appearance of a shoulder on the long-wavelength side of the fluorescence emission peak which could be resolved by differential spectroscopy to a component with a peak maximum near 697 nm (not shown, previously described in Ref. 18). We believe that the dominant 685 nm fluorescence emission of our preparation arises from the antennae chlorophylls which are constituents of the PS II core complex. We attribute the 695 nm fluorescence emission to the reaction center chlorophyll (P-680) and/or the pheophytin associated with P-680 which acts as the intermediate electron acceptor as has been hypothesized by Breton [19]. It cannot be due to fluorescence from aggregated forms of the light-harvesting complex serving PS II (LHC II), which does show a 695 nm emission at 77 K [20], since the LHC II is removed from our preparation. The mechanism by which 1,10-phenanthroline caused the appearance of the 695 nm fluorescence in the PS II core complex is unknown. It may result from an interaction directly with the PS II reaction center pigment proteins or indirectly through perturbations of the molecular environment surrounding the pigment-protein complex. Larkum and Anderson [21] have shown that aggregation and lipid dilution of a similar PS II reaction center preparation led to alterations in the 685 nm/695 nm fluorescence emission ratio, indicating that environmental factors play a definite role in the pigment-protein interactions.

Reaction center polypeptides

The PS II core complex was previously characterized [2] as being composed of three polypeptides, i.e., 43, 27 and 6 kDa. The polypeptide migrating electrophoretically in the 43 kDa region had been attributed to the apoprotein of the PS II reaction center Chl *a*-binding protein. In preliminary studies following the earlier electrophoresis procedure, a Coomassie-stained band was observed in the 43 kDa region; however, a diffusely stained area was also present in the size range of 43–50 kDa. When the PS II particle was subjected to SDS-polyacrylamide gel electrophoresis (10–17.5%) in the presence of urea, this diffusely stained area altered its migration pattern to give rise to a distinctly staining band of 47–50 kDa. The apparent molecular weight increased with increasing urea concentration (see Figs. 3 and 4). Two Chl *a*-binding proteins have previously been assigned to the PS II reaction center [3,4]; in the present study, the presence of urea (SDS-polyacrylamide gel electrophoresis) was required to resolve their presence (see Refs. 5 and 18). This requirement may be due to residual nonionic detergent bound to the isolated core complex, which prevents their complete dissociation by SDS in the absence of urea.

Since there was a possibility that one of the proteins which we resolved in the 40–50 kDa size range could be an artifact of sample preparation (i.e., degradation of a single protein), we have analyzed the relationship of the 43 kDa polypeptide to the 47–50 kDa polypeptide. This was

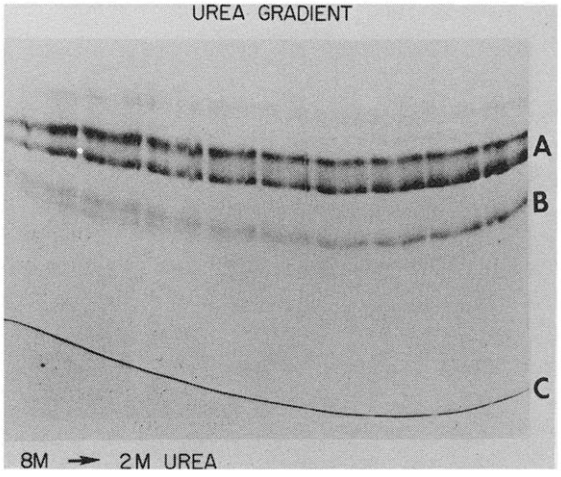

Fig. 2. SDS-polyacrylamide gel electrophoresis polypeptide (12.5% acrylamide) profile for PS II reaction center particle in the presence of a continuous urea gradient (approx. 2 M to 8 M urea from right to left across the gel). Note the separation of polypeptide species in both the 40–50 kDa (A) region and the 32 kDa (B) region into two distinct polypeptides. (C) The region of migration of the low-molecular mass protein suggested to be cytochrome *b*-559.

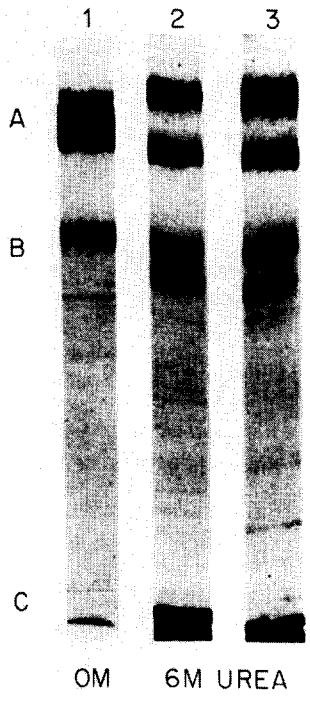

Fig. 3. Resolution and comparison of the SDS-polyacrylamide gel electrophoresis polypeptide profile of the PS II reaction center particle in 0 and 6 M urea. A comparison of the polypeptide profile for preparations obtained from peas (lanes 1 and 2) and spinach (lane 3) are provided. Regions A, B and C are as indicated in Fig. 2.

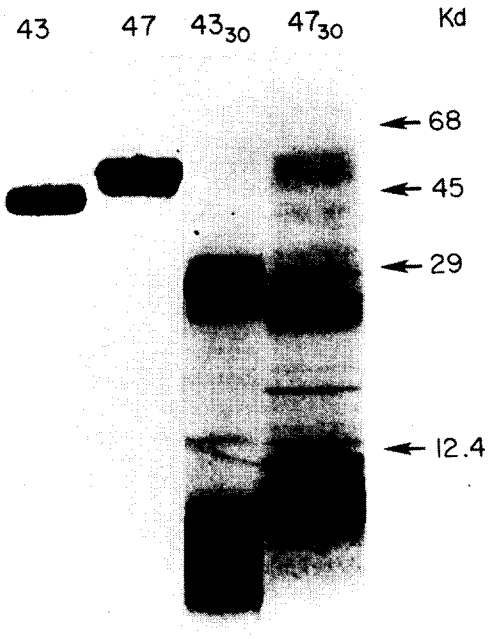

Fig. 4. Comparative partial hydrolysis patterns (papain, 0.1 μg/ml) of the excised gel slices from the A region (approx. 47 and 43 kDa polypeptides from SDS-polyacrylamide gel electrophoresis, 4 M urea gels) resubjected to SDS-polyacrylamide gel electrophoresis in the presence of urea. Kd denotes the molecular mass marker proteins: bovine serum albumin, ovalbumin, carbonic anhydrase and cytochrome *c*. Subscript refers to incubation time (in min) of the excised polypeptide with papain.

carried out by excising gel slices containing the different proteins and re-subjecting them to SDS-polyacrylamide gel electrophoresis following partial proteolysis with papain. The digestion pattern following this procedure is shown in Fig. 4. We conclude that the two polypeptides are dissimilar in their primary structure. Our PS II core complex, therefore, contains both PS II 'reaction center' Chl *a*-binding proteins previously identified from genetic studies [3,4]. The topographical relationship of these two polypeptides in the PS II core complex was studied by exposing the particle to trypsin. The 43 kDa polypeptide was found to be more sensitive to trypsin hydrolysis (not shown). The simplest explanation of these data is that the 43 kDa component is more surface-exposed than the 47 kDa polypeptide. Another possibility may be that the 47 kDa polypeptide lacks exposed arginine or lysine peptides.

Polypeptides of 30–34 kDa in the core complex

Previous analysis of the core complex [2] identified a protein with an apparent molecular mass of 27 kDa. In the present study, the molecular mass of this component was determined to be approx. 32 kDa at low urea concentrations (see Fig. 2 and Ref. 5). The presence of urea greater than 4 M) in the SDS-polyacrylamide gel electrophoresis led to the separation of this band into two distinct components (Fig. 2). The lower (faster migrating) band had an apparent molecular mass of 30 kDa while the apparent molecular mass of the slower migrating band was approx. 34 kDa (see Fig. 3). A comparison of the polypeptide profile of the PS II core complex isolated from peas and spinach (lanes 2 and 3, respectively) is also included in Fig. 3, indicating the similarity of the core complex isolated from different higher plant material.

Pertinent to functional aspects of the PS II core

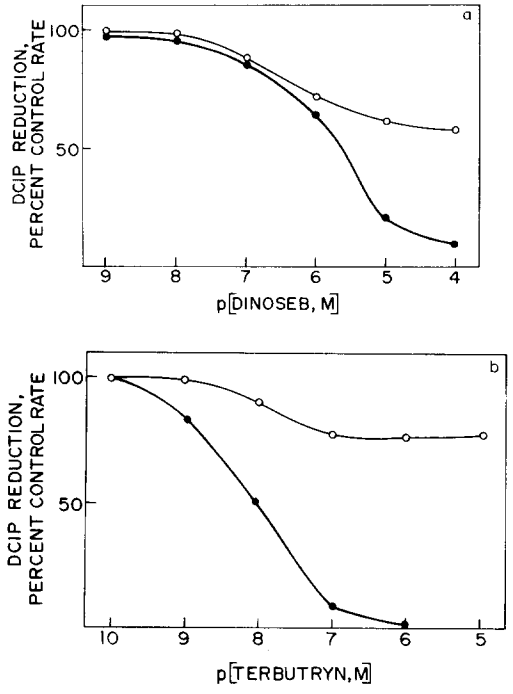

Fig. 5. Comparative sensitivity of the PS II reaction center particle (diphenylcarbazide → DCIP) and thylakoids (H_2O → DCIP) from peas to a phenolic-type PS II herbicide, dinoseb (a) and a triazine herbicide, terbutryn (b). At high concentrations (10^{-4} M) of terbutryn, secondary nonspecific inhibition was observed (not shown). (○——○) Particles, (●——●) thylakoids.

complex was the recent identification of a 32 kDa protein as the PS II herbicide-binding protein [8]. This protein has been suggested to be the apoprotein of the secondary electron acceptor 'B' [22]. That the herbicide-binding protein was likely to be present in the core complex was indicated by the sensitivity of the particle (diphenylcarbazide → DCIP) to the PS II herbicides (terbutryn and dinoseb, triazine and nitrophenol herbicides, respectively; Fig. 5). Analysis of different core complex preparations indicated that a population was sensitive to PS II-directed herbicides (maximal inhibition of 40–60% of control activity). Most importantly, the results showed that the particles retaining herbicide sensitivity have similar relative sensitivities to the herbicides as do intact thylakoids. With terbutryn the I_{50} value (the concentration of inhibitor required to obtain 50% inhibition) for the particle was estimated to be $1.5 \cdot 10^{-8}$ M whereas for thylakoids it was $8 \cdot 10^{-9}$ M (Fig. 5). A component of inhibition occurring at 10^{-4} M herbicide was not included, since this was thought to be due to nonspecific binding.

The presence of the 32 kDa herbicide-binding protein in our particles was confirmed by photoaffinity labeling studies. Ultraviolet irradiation of the core complex in the presence of azido[^{14}C]atrazine resulted in the covalent binding of this inhibitor to the faster migrating polypeptides (approx. 30 kDa) shown by a gel scan of the autoradiogram (see Fig. 6).

The identity of the slower migrating polypeptide in the 30–34 kDa size region (in 6 M urea gels) cannot yet be established. However, it is clearly not the atrazine-receptor protein, since it is not tagged by the photoaffinity label.

In previous studies, Kuwabara and Murata [24] reported that a 33 kDa polypeptide in thylakoids was associated with PS II function. This protein was found to be enriched in lysine. We have reported [25] that this lysine-rich polypeptide is lost from thylakoids early in the detergent extraction steps such as is used in preparation of our PS II core complex. We therefore conclude that the polypeptide described by Kuwabara and Murata [24] is different from either of the components in the 30–34 kDa size class present in the PS II core complex. In support of this conclusion we note that DNA sequencing of the herbicide receptor protein indicated the lack of lysine (McIntosh, L. and Hirschberg, J., personal communication). Kuwabara and Murata [26] have independently concluded that the protein in their studies is not the herbicide-binding protein.

The herbicide-binding protein had previously been shown to be identical to the product of the chloroplast-encoded 'photogene 32' [9]. To verify that the polypeptide of approx. 30 kDa which we retain in our PS II core complex is indeed this chloroplast gene product, we have utilized the fact that the photogene 32 product is rapidly turned over in mature leaves. Intact pea leaves were supplied with high specific activity [^{35}S]methionine and allowed to carry out protein synthesis in the light. The photogene 32 product was found to be heavily labeled after only 4 h incubation (for details see Ref. 9). We have, therefore, isolated chloroplasts from the [^{35}S]methionine-treated leaves

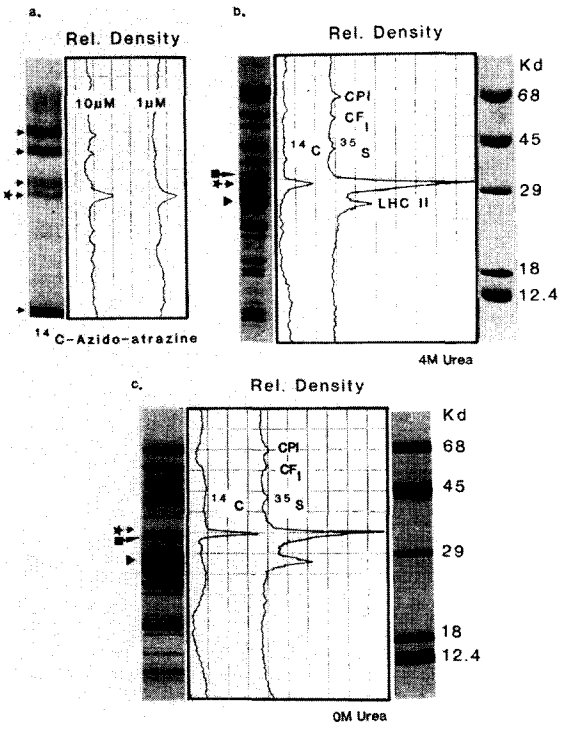

Fig. 6. Analysis of polypeptide labeling with either azido[^{14}C]atrazine or [^{35}S]methionine in PS II particles (a) or thylakoids (b,c). SDS-polyacrylamide gel electrophoresis was conducted in gels containing 6 M urea (a), 4 M urea (b) or 0 M urea (c). (a) Isolated PS II core complex particles were incubated with either 1 or 10 μM azido[^{14}C]atrazine with 10 min ultraviolet illumination (further details in Ref. 8). A photograph of the stained gel, with the five polypeptides of the complex indicated by arrows, is shown to the left of a recorder trace obtained by scanning the autoradiogram of this gel. The radioactivity of the sample was solely associated with the 30 kDa polypeptide (the peak in the densitometer scan is indicated by a star) for the 1 μM azidoatrazine treatment. At higher (10 μM) herbicide levels, where nonspecific binding occurs, the densitometer scan revealed slight radioactive labeling of the 47 and 43 kDa proteins. (b and c) Thylakoids isolated from leaves fed [^{35}S]methionine to label radioactively the photogene 32 product (details in Ref. 9) are compared with respect to labeling patterns to control thylakoids incubated in ultraviolet light with azido[^{14}C]atrazine (details in Ref. 8). Each figure compares a photograph of the Coomassie-blue stained polypeptides (left) and the protein standards (right) used in the gel procedures to a densitometer scan of the autoradiogram prepared from the radiolabeled gels in 0 M urea gels (c), azido[^{14}C]atrazine was detected in a single peak (labeled with star) at about 34 kDa; this coincided with the distribution of the dominantly labeled ^{35}S-polypeptide known as the photogene 32 product [8]; ^{35}S labeling was also detected in other thylakoid polypeptides including Chl-protein complex I (CP I), the α- and β-subunits of the coupling factor (CF_1) and in polypeptides of the Chl *a*/*b* light-harvesting complex (LHC II; identified on the stained gel by a triangle). A polypeptide which stains densely with Coomassie blue at an apparent molecular mass of 32 kDa is indicated by a square). In gels containing 4 M urea (b), the densely staining polypeptide near 32 kDa is still evident (labeled with a square). In contrast, the ^{14}C and ^{35}S radiolabel was shifted to an apparent molecular mass of 30 kDa, coincident with a diffusely stained polypeptide region (indicated by a star).

and compared the [^{35}S]methionine labeled product with the azido[^{14}C]atrazine-labeled thylakoids from gel scans of the autoradiograms after subjecting the samples to SDS-polyacrylamide gel electrophoresis with varying urea concentrations (0 and 4 M urea).

The [^{35}S]methionine-labeled photogene 32 product was found to comigrate with the azido-[^{14}C]atrazine-labeled polypeptide (see Fig. 6b and c). In the 0M urea gels of thylakoid membranes, the labeling pattern indicated that the labeled polypeptides (either ^{35}S- or ^{14}C-labeled) migrated slower than the densely stained 32kDa protein. We conclude that the '32 kDa' protein which stains intensely with Coomassie blue is identical to the lysine-rich protein of Kuwabara and Murata [24] and is not the photogene 32 product. In support of this idea, it was observed that addition of 4 M urea caused both the [^{35}S]methionine- and azido[^{14}C]atrazide-labeled polypeptide to migrate with a lower apparent molecular mass (near 30 kDa, Fig 6c) whereas the densely staining polypeptide ran at a higher apparent molecular mass. The parallel anomalous migration behavior for both the [^{35}S]methionine- and the azido[^{14}C]atrazine-labeled proteins in SDS-polyacrylamide gel electrophoresis ($\pm$ urea) clearly indicated that this is the same protein observed for the azido[^{12}C]atrazine-labeled protein isolated in our PS II core complex.

Our results indicate that there are at least three polypeptides associated with thylakoids which migrate electrophoretically in the 30–34 kDa region: the densely staining polypeptide isolate earlier by Kuwabara and Murata [24] plus two polypeptides retained in our PS II core complex, one of which is the herbicide-binding protein.

Cytochrome b-559

A low-molecular mass protein was associated

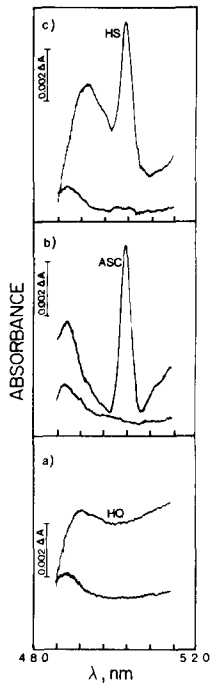

Fig. 7. Spectrophotometric determination of cytochrome *b*-559 content of the PS II core complex preparations from peas. Difference spectra were obtained after initially oxidizing the sample with small amounts of solid potassium ferricyanide. The baseline was obtained and a small amount of solid hydroquinone (HQ) was added to the sample cuvette (a). The sample and reference cuvettes were then equilibrated with hydroquinone to obtained a new baseline and solid sodium ascorbate (ASC) was added to the samples cuvettes (b). The sample and reference cuvette were then equilibrated with ascorbate and sodium dithionite (HS) was added to the sample cuvette (c). Further detailed explanations of this procedure were presented in Ref. 27.

with the core complex by SDS-polyacrylamide gel electrophoresis (see Figs. 2 and 3, region C) [2]. In the purified complex, before SDS treatment, cytochrome *b*-559 was determined spectrophotometrically [27]. Cytochrome *b*-559 has been known to exist in a number of redox forms [27,28]. The core complex in this study was found not to contain any high-potential form (Fig. 7c) which is likely due to the fact that the detergent fractionation procedure utilized to isolate this particle resulted in the alteration of this high-potential form [27,28]. The cytochrome component of the core complex preparation was found to be composed of the 'middle' and 'low' potential forms of cytochrome *b*-559; i.e., ascorbate and dithionite reducible (Fig. 7b and c). The relative proportions of these two forms were found to vary with storage time of the purified complex at either 4 or −60°C. The storage resulted in an increase in the relative amount of the low-potential form. The relative amount of cytochrome *b*-559 to Chl *a* in the PS II particle was found to be approx. 40–50. Although cytochrome *b*-559 appeared to be ubiquitous to PS II preparations, its functional role in the PS II reaction center has not been determined (see Refs. 29 and 30). Previously, only the dithionite-reducible form was observed in the core PS II preparations [1].

Electron donation to the photochemically active complex

The PS II core complex was found to be capable of reducing DCIP in the presence of diphenylcarbazide [2]. In addition to diphenylcarbazide, NH_2OH and I^- were also found to donate electrons to P-680 (donor → DCIP; see Table I). The lack of any observed electron donation via benzidine and *p*-phenylenediamine may have been due to their high redox potentials. PS II electron donors are assumed to function through D_1 or Z (secondary electron donor to the reaction center; see Refs. 31 and 32). While we have no direct evidence for the involvement of a unique polypeptide in determining the functional properties of D_1 or Z see (Ref. 32), it is tempting to speculate that the 34 kDa polypeptide of the PS II complex

TABLE I

ELECTRON DONATION TO PS II

PS II particles purified from peas (approx. 5 μg Chl *a*/ml) were suspended in 0.1 M sorbitol, 50 mM Mes-NaOH, pH 6.5, 10 mM NaCl and 5 mM $MgCl_2$. DCIP reduction was monitored at 575 nm with a Hitachi 100-60 spectrophotometer. (No reduction of potassium ferricyanide was observed in the presence of KI, not shown.)

Electron donor	DCIP reduction (μequiv./mg Chl per h)
Diphenylcarbazide (1 mM)	140
NH_2OH (20 mM)	130
KI (10 mM)	26
Benzidine (1 mM)	–
p-Phenylenediamine (1 mM)	–

(SDS-polyacrylamide gel electrophoresis for urea) (Fig. 3) may be involved in these reactions.

Conclusion

This report was shown that a functional PS II core complex from higher plants is composed of five polypeptides. Four of these can be correlated to functional activities. The polypeptides of 43 and 47 kDa correspond to the Chl *a*-binding PS II reaction center proteins previously identified in genetic studies [3,4]. Recently, the 695 nm fluorescence emission band (77 K) was found to originate from the 47 kDa Chl *a*-binding protein; fluorescence data indicated that this polypeptide was associated with reaction center properties and relegated the 43 kDa Chl *a*-binding protein to a light-harvesting role (Nakatani, H.Y., Li, K., Kuang, T.Y. and Arntzen, C.J., unpublished observations). A protein with an anomalous migration behavior (dependent upon urea content of the gels) is identified as the rapidly turned over photogene 32 product which binds the PS II herbicide, azidoatrazine. The presence of a low-molecular mass polypeptide (approx. 6 kDa) has been correlated with the presence of low- and mid-potential forms of cytochrome *b*-559 in the isolated complex. The fifth protein in the core complex has an apparent molecular mass of 34 kDa. The latter polypeptide is not the atrazine receptor protein nor can it be correlated with the 33 kDa protein observed by Kuwabara and Murata [24], since the lysine-rich polypeptide is lost during the isolation of the PS II core complex in this study [25]. We have suggested that this 32 kDa protein may be associated with Z or D_1, unless direct donation from exogenous donors to P-680 can occur.

Acknowledgements

This research was supported, in part, from funds obtained under contract No. DE-AC02-76ERO-1338 (DOE) to Michigan State University and paper No. 10868 from the Michigan State University Experiment Station.

References

1 Satoh, K. and Butler, W.L. (1978) Plant Physiol. 61, 373–379
2 Satoh, K. (1979) Biochim. Biophys. Acta 546, 84–91
3 Delepelaire, P. and N.H. Chua (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 111–115
4 Machold, O., Simpson, D.T. and Moller, B.L. (1979) Carlsberg. Res. Commun. 44, 235–254
5 Satoh, K. (1981) in Photosynthesis III. Structure and Molecular Organization of the Photosynthetic Apparatus (Akoyunoglou, G.A., ed.) pp. 607–616, Balaban Philadelphia
6 Garewal, H.S. and Wasserman, A.R. (1974) Biochemistry 13, 4063–4071
7 Nelson, N. (1980) Methods Enzymol. 69, 301–313
8 Pfister, K., Steinback, K.E., Gardner, G. and Arntzen, C.J. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 981–985
9 Steinback, K.E., McIntosh, L., Bogorad, L. and Arntzen, C.J. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 7463–7467
10 Leto, K. and Miles, C.D. (1980) Plant Physiol. 66, 18–24
11 Cleveland, D.W., Fischer, S.G., Kuschner, M.W. and Laemmli, U.K. (1977) J. Biol. Chem. 252, 1102–1106
12 Searle, G.F.W. and Wessels, T.S.C. (1978) Biochim. Biophys. Acta 504, 84–99
13 Mathis, P., Butler, W.L. and Satoh, K. (1979) Photochem. Photobiol. 30, 603–614
14 Shutilova, N.I., Faludi-Daniel, A. and Klimov, K.V. (1982) FEBS Lett. 138, 255–257
15 Klimov, V.V., Dolan, E. and Ke, B. (1980) FEBS Lett. 112, 97–110
16 Vernon, L.P. (1960) Anal. Chem. 32, 1144–1150
17 Butler, W.L. (1977) in Encyclopedia of Plant Physiology, New Series, Vol. 5 (Trebst, A. and Avron, M., eds.), pp. 149–167, Springer-Verlag, Berlin
18 Satoh, K. (1980) FEBS Lett. 110, 53–56
19 Breton, J. (1982) FEBS Lett. 147, 16–20
20 Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117
21 Larkum, A.W.D. and Anderson, J.M. (1982) Biochim. Biophys. Acta 679, 410–421
22 Arntzen, C.J., Darr, S.C., Mullet, J.E., Steinback, K.E. and Pfister, K. (1982) in The Function of Quinones in Energy Conserving Systems (Trumpower, B., ed.), pp. 443–452, Academic Press, New York
23 Audus, L.T. (1976) Herbicides, Academic Press, New York
24 Kuwabara, T. and Murata, N. (1979) Biochim. Biophys. Acta 581, 228–236
25 Nakatani, H.Y., Satoh, K., Steinback, K.E. and Arntzen, C.J. (1983) in Pesticide Chemistry: Human Welfare and the Environment, Vol. 3 (Matsunaka, S., Hutson, D.H. and Murphy, S.D., eds.), pp. 171–176, Pergamon Press, Oxford
26 Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 663–667
27 Hind, G. and Nakatani, H.Y. (1970) Biochim. Biophys. Acta 216, 223–225
28 Cramer, W.A., Fan, H.M. and Boehme, H. (1971) J. Bioenerg. 2, 289–303

29 Bouges-Bocquet, B. (1980) Biochim. Biophys. Acta 594, 85–103
30 Butler, W.L. (1978) FEBS Lett. 95, 19–25
31 Yocum, C.F. and Babcock, G.T. (1981) FEBS Lett. 130, 99–102
32 Satoh, K. and P. Mathis (1981) Photobiochem. Photobiophys. 2, 189–198