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Identification of the photochemically iodinated amino-acid residue on D1-protein in the Photosystem II core complex by peptide mapping analysis

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The D1-protein is exclusively iodinated in the Photosystem II core complex (Takahashi, Y., Takahashi, M. and Satoh, K. (1986) FEBS Lett. 208, 347–351) as a result of photooxidation of iodide in the secondary electron donor (Z). The photochemically iodinated protein was subjected to a peptide mapping analysis after partial chemical and proteolytic cleavages in order to identify the iodo-labeled amino-acid residue(s). The cleavage at the C-terminal side of tryptophan by *N*-chlorosuccinimide, methionine by CNBr, and glutamic acid by *Staphylococcus aureus* V8 proteinase indicated that the iodination site is restricted, in each case, to specific peptide fragments of apparent molecular mass of 14.5, 3 and 8 kDa, which presumably correspond to Ile-143–Trp-278, Arg-140–Met-172 and Leu-133–Glu-189, respectively, in the deduced sequence of spinach D1-protein. The conclusion reached from this analysis is that the overlapping sequence among these three fragments, which starts at Ile-143 and ends at Met-172, and which contains two tyrosine residues of potential iodination site at the position of 147 and 161, carries the specific photochemical iodination site(s). When the surface-exposed tyrosine and histidine residues on D1-protein in the Photosystem II core complex were subjected to iodination in the dark using lactoperoxidase/H_2O_2 system, the Arg-140–Met-172 fragment was not iodinated, indicating that the photochemical iodination site is present in the interior part of the complex, in accordance with the folding model of D1-protein in the Photosystem II reaction center. Since Tyr-161 on D1-protein is predicted to be close to the putative His ligands for P-680 on the lumenal side of thylakoid membrane, it was concluded that this Tyr, rather than Tyr-147, which is present on the stromal side, was the photochemical iodination site. Symmetry consideration of the sequence of D1- and D2-proteins, together with EPR spectral shape, led us to conclude further, in agreement with the proposal based on the recent site-directed mutagenesis for the signal IIs species on D2-protein, that the Tyr-161 on D1-protein alone constituted the secondary electron donor (Z) of the Photosystem II reaction center.

Introduction

The PS II core complex capable of driving photochemical electron transport from the secondary electron donor, Z, to the primary qinone acceptor, Q_A, is composed of six protein subunits with apparent molecular masses of 47, 43, 32, 30, 9 and 4.5 kDa (see review, Ref. 1). The 30 kDa subunit (D1-protein) is a chloroplast gene (*psbA*) product [2], functioning on both sides of the PS II reaction center; photoaffinity labeling experiments [3–5] have identified this component as a herbicide- and Q_B-binding protein on the acceptor side, while specific iodination experiments have identified this protein on the donor side at the iodide oxidation site, which presumably corresponds to the Z-binding site [6,7]. The functioning of the D1-protein on both sides of PS II has also been indicated by the analysis of a *Scenedesmus* mutant [8,9] and is consistent with the proposal that this component, together with the 32 kDa component (D2-protein), forms the site for primary photochemistry of PS II [10,11], in the same way that the L and M subunits of purple bacteria form the reaction center [12–14]. The recent isolation and characterization of a PS II complex consisting of D1, D2-

Abbreviations: Bchl, bacteriochlorophyll; CBB, Coomassie Brilliant Blue; Chl, chlorophyll; DCIP, 2,6-dichlorophenol-indophenol; PS I, Photosystem I; PS II, Photosystem II; SDS, sodium dodecyl sulfate; N-CSI, *N*-chlorosuccinimide.

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proteins and cytochrome *b*-559 have strongly supported this proposal [15–18].

On the donor side of PS II, there exists an EPR species (D^+) with a signal shape similar to that of Z^+, although the connection of this component to the photosynthetic electron transport chain seems to be indirect [19]; Z exhibits a photoreversible signal which decays rapidly (signal IIf or IIvf), whereas D exhibits a dark-stable signal (signal IIs). In the previous iodination experiment [6], it was shown that the iodination of D1-protein takes place only in the light, whereas the D2-protein is iodinated even in the dark. The chemical entity responsible for the activation of iodide in the dark was concluded to be the dark-stable EPR species (signal IIs), which might be closely associated with, or be part of, the D2-protein [20]. The identification of the Tyr-160 on the D2-protein as the source for D^+ has been established in *Synechosystis* based on several independent lines of evidence [21–23]. For reasons of sequence symmetry and EPR signal shape, it is now presumed that Z is the corresponding Tyr residue on the D1-protein. However, experimental data supporting this belief are totally lacking at present.

In the present study, we have carried out peptide mapping analysis of the fragments of photochemically iodinate dD1-protein after various chemical and proteolytic cleavages in order to identify the iodinated amino-acid residue and concluded that the Tyr-161 on the deduced protein sequence of spinach is the site of photochemical iodination and that this residue most probably corresponds to the secondary electron donor, Z, in PS II.

Materials and Methods

PS II core complex was purified from digitonin extracts of spinach chloroplasts following the method described in Ref. 24.

The D1-protein in the core complex (0.3 mg Chl/ml) was specifically iodinated in the light for 1 min in the presence of 0.05 mM DCIP/0.2 mM KI (0.2 mCi $^{125}I^-$, NEM Research Products)/100 mM NaCl/0.2% digitonin/50 mM Tris-HCl (pH 7.2) as described previously [6]. After iodination, the reaction mixture was diluted two-fold with a medium comprising 0.2% digitonin/50 mM Tris-HCl (pH 7.2) and subsequently adsorbed onto a small DEAE-Toyopearl 650S (Toyo Soda, Tokyo) column. After washing, the iodo-labeled core complex was eluted from the column with a medium comprising 0.2% digitonin/150 mM NaCl/50 mM Tris-HCl (pH 7.2), and thus separated from the unreacted iodide.

The PS II core complex was also enzymatically iodinated with lactoperoxidase using radioiodination system (NEM Research Products). The core complex (0.035 mg Chl/0.1 ml), 0.2 mCi $^{125}I^-$ and 0.025% glucose were added to iodination reagent beads and the reaction mixture was incubated for 30 min at room temperature. After terminating the iodination reaction by adding sodium metabisulfite, the reaction beads and the unreacted iodide were removed from the core complex chromatographically after dilution of the reaction mixture 10-times as described above.

After solubilization of the iodinated core complex with a medium comprising 2.5% SDS/2.5% 2-mercaptoethanol/125 mM Tris-HCl (pH 6.8), the iodo-labeled peptides were separated by SDS-polyacrylamide gel electrophoresis according to Laemmli [25], with a resolving gel containing 15% acrylamide and 6 M urea. Chemical cleavages of the D1-protein with N-CSI and CNBr were performed according to Refs. 26 and 27, respectively, in the gel slices excised after stained with CBB. After chemical cleavage, the gel slices were equilibrated with a small amount of 50 mM Tris-HCl (pH 6.8) and then incubated in a solution comprising 5% SDS/5% 2-mercaptoethanol/125 mM Tris-HCl (pH 6.8) for 10 min and subsequently applied onto sample wells of the second SDS-polyacrylamide gel for analysis. Proteolytic digestion of D1-protein was carried out in the stacking gel [28]; the gel slices containing D1-protein were incubated in 5% SDS/5% 2-mercaptoethanol/125 mM Tris-HCl (pH 6.8) for 10 min and then placed in the sample wells and overlayed with proteinase solution. After D1-protein and proteinase were mixed electrophoretically in the stacking gel, the current was turned off for 30 min in order to subject the protein to proteolytic digestion, and then the proteolytic fragments were electrophoretically separated. Proteinases were dissolved in a medium comprising 20% glycerol/50 mM Tris-HCl (pH 7.2). The second electrophoresis was carried out according to Laemmli [25], except that the resolving gel contained 18% acrylamide and 6 M urea. For molecular weight determination of peptide fragments, an electrophoresis calibration kit which contained myoglobin digests (17.2, 14.6, 8.24, 6.38 and 2.56 kDa) was obtained from Pharmacia. For the fluorography, Fuji RX X-ray film was exposed to the dried gel in the presence of intensifying screen (Du Pont Cronex) at $-80\,^\circ C$ for 1 or 2 weeks. The radioactivity incorporated into D1-protein was determined with an Aloka auto well gamma system JDC-751 as described previously [20]. Silver staining of proteins was carried out according to the method of Oakley et al. [29].

Results

When the water-splitting activity of thylakoid membranes is inhibited, the externally added iodide can donate electrons to the secondary donor of PS II [30], and this results in the iodination of the protein which carries the donor component [31]. The PS II core complex used in this study has no oxygen-evolving activity,

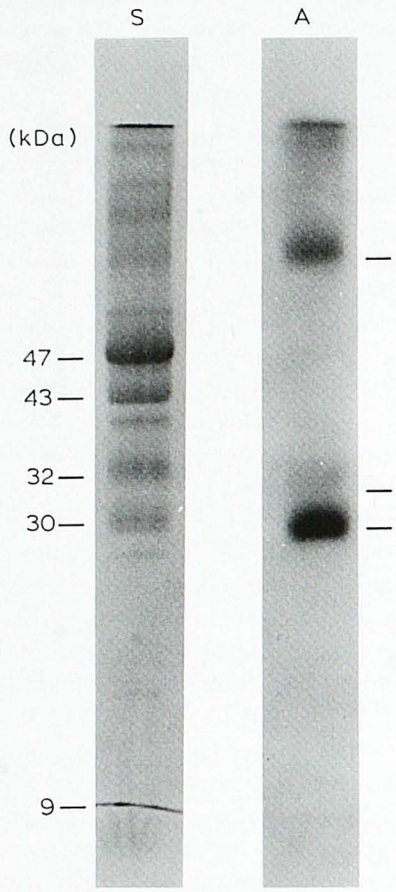

Fig. 1. Labeling of PS II core complex with ^{125}I in the presence of DCIP during illumination for 1 min. (A) Stained with CBB, (B) autoradiography. Arrows on the right side indicate the positions of aggregate of D1- and D2-proteins, D2-protein and D1-protein, from top to bottom. A band of approx. 60 kDa, which is ascribed to the aggregated form of D1- and D2-proteins, is also labeled under the present conditions [6].

but drives photochemical electron transport from the artificial donors, e.g., DPC and KI, to the artificial acceptor, e.g., DCIP [5]. As already described in Ref. 6, the illumination of the core complex in the presence of DCIP and KI results in the specific iodination of D1-protein, which is inhibited by the presence of DPC, as shown in Fig. 1. In contrast, the D2-protein is iodinated even in the dark, due to the iodide oxidation by a dark-stable electron donor, D^+, which gives rise to EPR signal IIs [6,20].

In order to identify the photochemically iodinated residue(s) on the protein, the radiolabeled D1-protein was isolated from the illuminated core complex as described in Materials and Methods and then subjected to the peptide mapping analysis using SDS-polyacrylamide gel electrophoresis. To avoid nonspecific labeling, the core complex was iodinated for the short time of 1 min in the light, as compared with that in the previous report [6]. Under this condition, the iodide incorporated into D1-protein was less than 1 mol per mol reaction center.

In the first experiment, the isolated, iodinated protein was cleaved selectively at the C-terminal side of Trp with N-CSI as described in Materials and Methods (Fig. 2). In the experiment shown in Fig. 3, the gel slices containing D1-protein were incubated with N-CSI solution at 37°C for 15 min and subsequently subjected to the second SDS-polyacrylamide gel electrophoresis for peptide mapping. When the iodinated D1-protein was re-electrophoresed without cleavage (0 mM), small amounts of aggregated bands with slower mobilities together with a major band at 30 kDa were observed as silver-stained and radioactive bands. Digestion of D1-protein at 3.8 mM N-CSI resulted in the production of more than 20 stained peptide fragments. Among them, about 10 peptide fragments of apparent molecular masses larger than 14 kDa, were found to be iodinated.

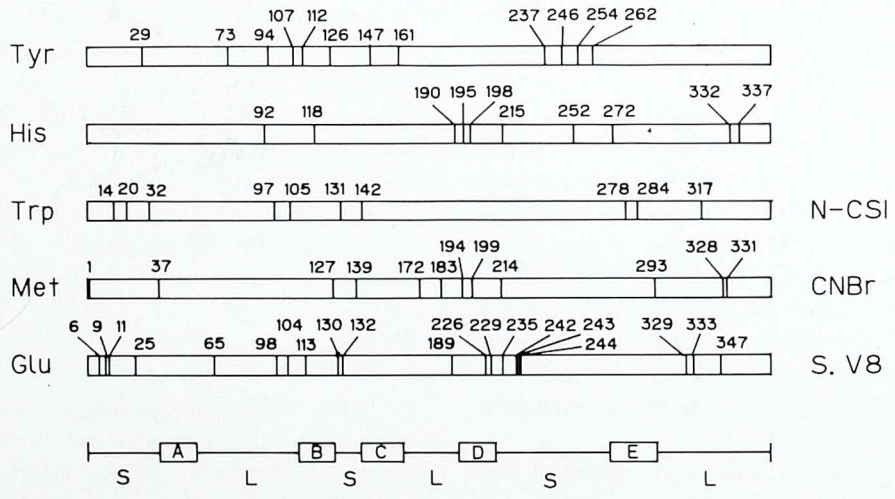

Fig. 2. Positions of amino-acid residues on D1-protein and the cleavage sites with different treatments. Positions of tyrosine (Tyr), histidine (His), tryptophan (Trp), methionine (Met) and glutamic acid (Glu) residues are indicated on different columns [2]. At the bottom, the position of putative transmembrane helices (A–E) are shown by boxes [10]. S and L segments are predicted to be exposed in stromal and lumenal matrices, respectively.

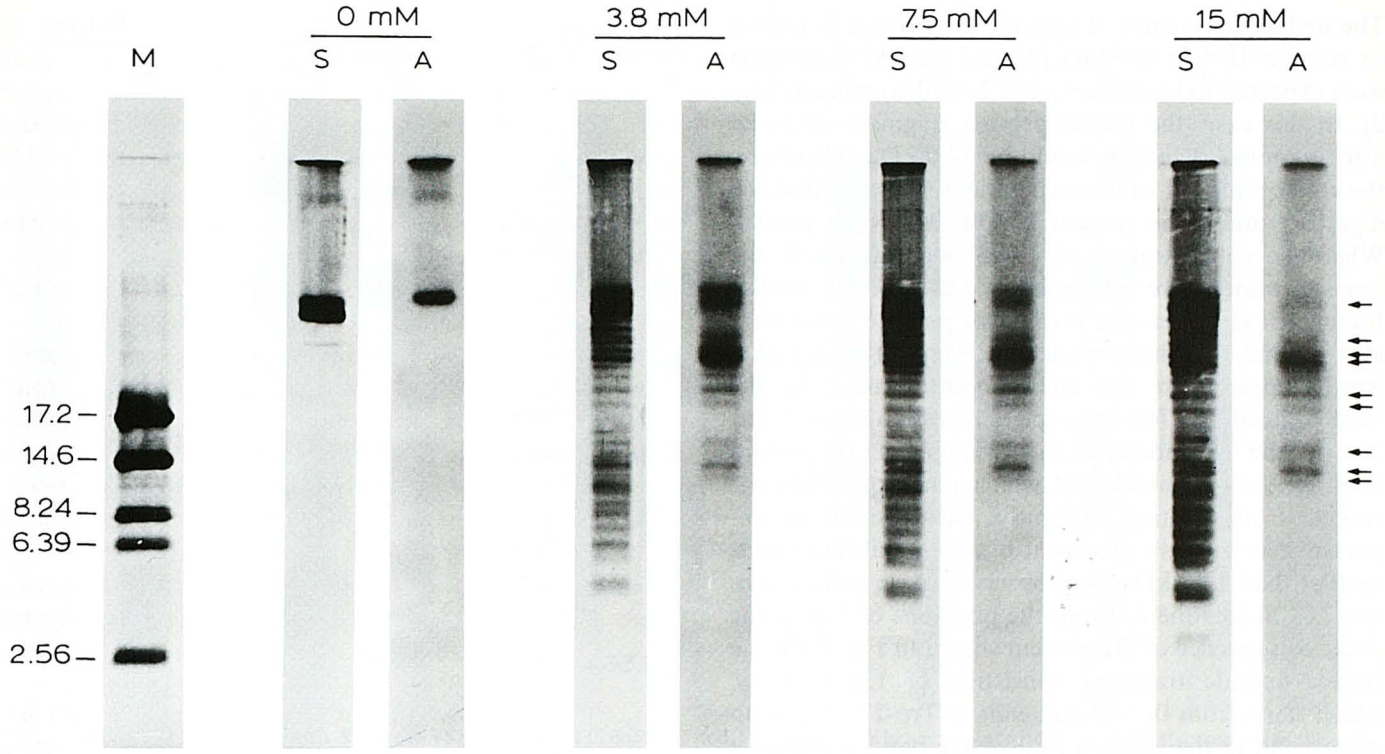

Fig. 3. Cleavage of D1-protein with various concentrations of N-CSI. Gel slices containing D1-protein were incubated at 37 °C for 15 min with N-CSI solution at 0, 3.8, 7.5 and 15 mM. Peptide fragments were separated by SDS-polyacrylamide gel electrophoresis on 18% acrylamide gels containing 6 M urea. (M) Molecular weight standards, (S) stained with silver, (A) autoradiography.

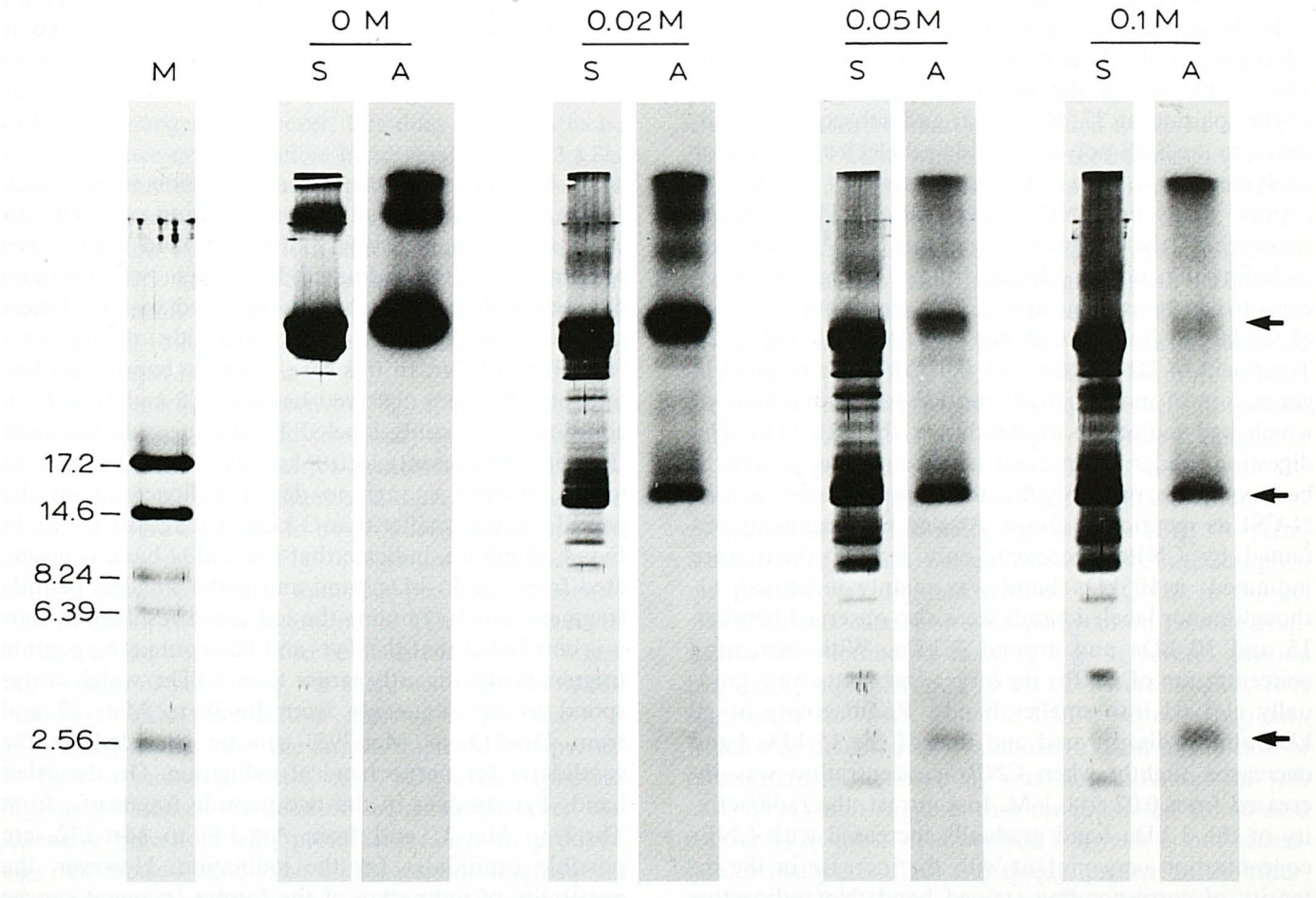

Fig. 4. Cleavage of D1-protein with various concentrations of CNBr. Gel slices containing D1-protein were incubated at 37 °C for 2 h with CNBr solution at 0, 0.02, 0.05 and 0.1 M. (M) Molecular weight standards, (S) stained with silver, (A) autoradiography.

The deduced sequence of spinach D1-protein is known to contain 10 Trp residues [2] and thus 11 fragments were expected to be present after N-CSI treatment (Fig. 2). In this case, the largest peptide fragment obtained after complete digestion would be 14.5 kDa. Therefore, the result obtained in this experiment indicates that the digestion under the present condition is only partial. When the concentration of N-CSI was increased, the larger peptides were further cleaved to produce smaller fragments. However, the profiles of peptide maps were almost similar to the previous one, except that a 3 kDa band appeared in the higher concentration in the silver-stained gel. The incomplete digestion seems to be due to the conformational restriction of D1-protein fixed in the acrylamide gel which partly prevents the reagent from reacting efficiently. Nevertheless, an important fact in this experiment is that no labeled bands smaller than 14.5 kDa were observed, even after extensive N-CSI treatment. From the positions of Trp in the deduced sequence of D1-protein shown in Fig. 2 [2], the largest peptide fragment consisting of 136 residues, which starts from Ile-143 and ends at Trp-278, seems to correspond to the smallest radioactive band and thus it is concluded that this fragment contains the specific amino-acid residue(s) iodinated in the light. This fragment contains 6 Tyr and 6 His residues, which are expected to be the targets for iodination [30].

In the second experiment, the D1-protein was cleaved selectively at the C-terminal side of Met with CNBr. The D1-protein in the gel slices was incubated with CNBr solution at 37°C for 2 h and subsequently subjected to the SDS-polyacrylamide gel electrophoresis for analysis. Fig. 4 shows the dependence of profiles of peptide maps on CNBr concentration. The deduced sequence of the D1-protein contains 12 Met residues, including the starting Met, and thus 12 fragments were expected to be present after CNBr treatment. The size of the largest fragment, in this case, is 9.9 kDa (Fig. 2). Treatment of D1-protein with 0.02 M CNBr resulted in generation of more than 20 peptide fragments, most of which had molecular masses larger than 10 kDa. The digestion, under the present condition, thus proved to be incomplete, probably due to the same reason as with N-CSI as mentioned above. Among the fragments obtained by CNBr treatment, only few of them were iodinated; a 15 kDa band was mainly iodinated, although minor labeled bands were also observed between 15 and 30 kDa and around 3 kDa. With increasing concentration of CNBr, the larger fragments were gradually cleaved into smaller bands. Radioactivity at 30 kDa almost disappeared and that of the 15 kDa band decreased slightly when CNBr concentration was increased from 0.02 to 0.1 M. In contrast, the radioactivity of the 3 kDa band gradually increased with CNBr concentration concomitant with the increase in the intensity of corresponding stained band. No radioactive

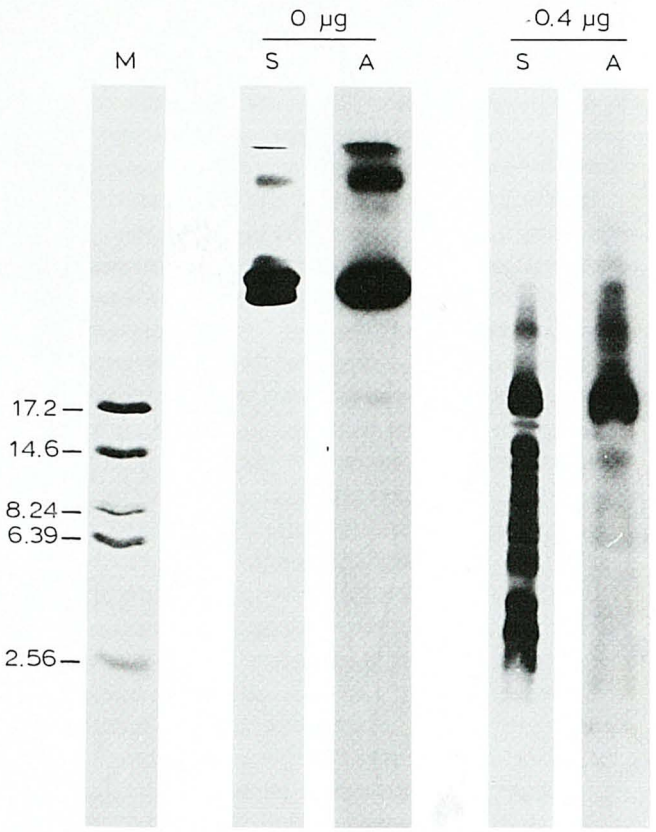

Fig. 5. Cleavage of D1-protein with papain at 0 and 0.4 μg per well. D1-protein was electrophoretically mixed with the proteinase and incubated for 30 min in the stacking gel. Electrophoresis conditions wre the same as Fig .2. (M) Molecular weight standards, (S) stained with silver, (A) autoradiography.

band, however, was detected in the region smaller than 3 kDa, even at the highest concentration of CNBr. In order to test the sensitivity of the method used in the present study, the iodinated D1-protein was partially digested with papain which is reported to have broad specificity to digest the C-terminal side of Arg, Lys, His, Gly and Tyr. In this case, multiple bands with low radioactivity were observed between 1.5 and 18 kDa in addition to a mainly labeled 18 kDa peptide fragment (Fig. 5). The present electrophoresis system thus proved to be sensitive enough to detect radioactivity of the peptide bands smaller than 3 kDa. The results shown in Fig. 4, therefore, indicate that the 3 kDa band is generated from the 15 kDa band and is the smallest peptide fragment which contains the iodinated residue(s). Thus it is concluded that the Tyr- and His-containing peptide fragments significantly larger than 3 kDa, which correspond to the sequences from Ile-38 to Met-127 and from His-215 to Met-293, can be excluded as the candidates for photochemical iodination. On the other hand, Tyr residues in the two peptide fragments, from Thr-2 to Met-37 and from Arg-140 to Met-172, are possible candidates for the iodination. However, the possibility of iodination of the former fragment can be

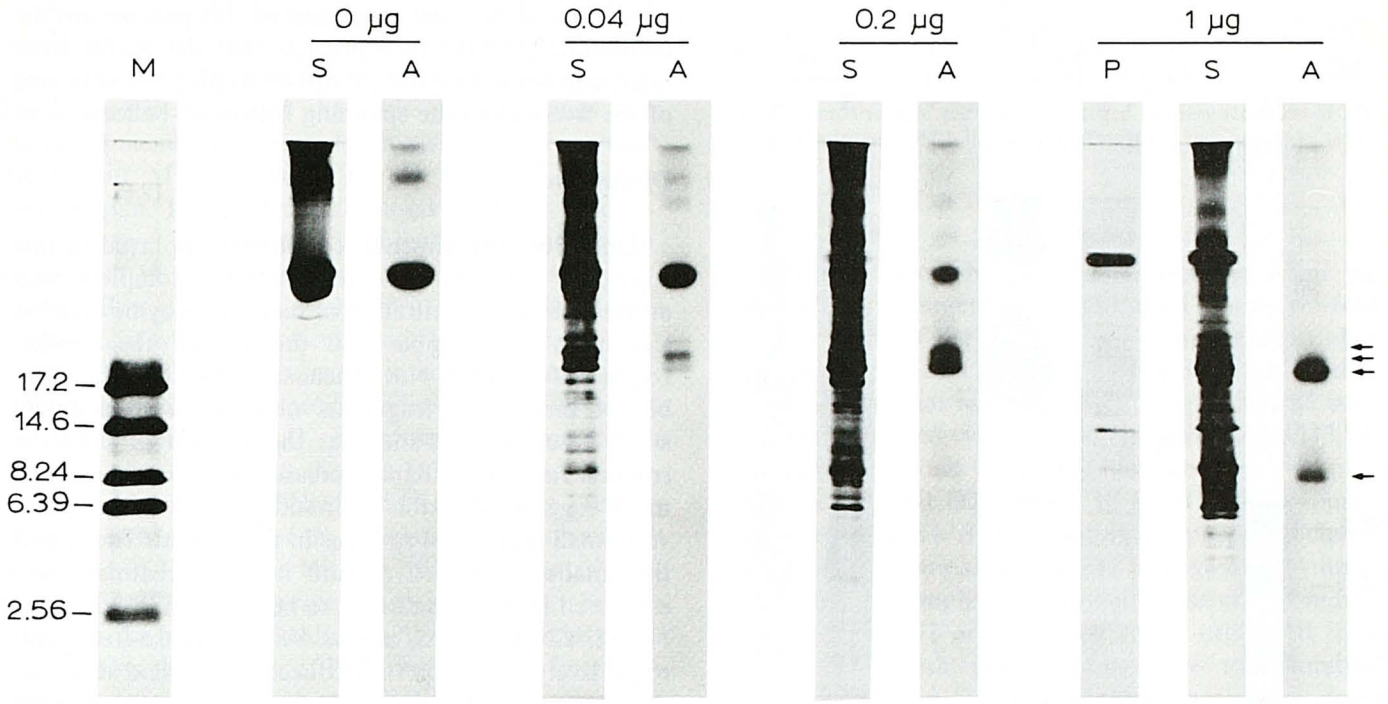

Fig. 6. Cleavage of D1-protein with *S. aureus* V8 proteinase at 0, 0.04, 0.2 and 1 μg per well. The condition of digestion of D1-protein was the same as Fig. 5. Electrophoresis conditions were the same as Fig. 2. (M) Molecular weight standards, (S) stained with silver, (A) autoradiography.

ruled out, since this fragment is not included in the sequence of the radioactive polypeptide obtained by N-CSI cleavage (Ile-143 to Trp-278). The partial cleavage product which corresponds to the sequence from Ile-184 to Met-214 might be a candidate for the iodinated framgent. However, this does not seem to be the case, since no radioactive band smaller than 3 kDa was found in the present experiment. Based on the above considerations, it seems to be evident that the iodinated site is located in the common region betweenthe 14.5 kDa N-CSI fragment and the 3 kDa CNBr fragment, which corresponds to the polypeptide from Ile-143 to Met-172 on the deduced primary structure of spinach D1-protein.

In the experiment shown in Fig. 6, the D1-protein was proteolytically cleaved selectively at the C-terminal side of Glu with *S. aureus* V8 proteinase [28]. D1-protein contains 20 Glu residues and therefore 21 peptide fragments were expected to be produced after complete digestion. As shown in Fig. 6, digestion with the proteinase produced more than 30 peptide fragments. Among these, two bands around 19 kDa and one band around 8 kDa were found to be mainly iodinated. When the amount of proteinase was increased, the two bands around 19 kDa were further digested to produce a 8 kDa band. In this case, the largest fragment is expected to have a molecular mass of 9 kDa after complete digestion, based on the deduced sequence. However, the extensive digestion with proteinase failed to produce any new labeled band. The 8 kDa peptide fragment is, therefore, concluded to be the smallest peptide fragment which contains an iodinated amino-acid residue. The result is consistent with the above conclusion that the iodinated residue is present in the region from Ile-143 to Met-172. The expected fragment in the case of the proteinase digestion, which contains this region, is from Leu-133 to Glu-189 and is calculated to have a molecular mass of 7 kDa, which roughly corresponds with the size of iodinated fragment estimated by SDS-polyacrylamide gel electrophoresis.

In the control experiment, the D1-protein in the core complex was subjected to iodination with iodine enzymatically produced by the action of lactoperoxidase in the presence of H_2O_2. In this case, surface-exposed Tyr and His residues are expected to be preferentially iodinated. In order to investigate whether the photochemically iodinated site of CNBr peptide fragment of 3 kDa is present in the exterior or interior of the protein, the D1-protein enzymatically iodinated was subjected to peptide mapping analysis (Fig. 7). After iodination of the D1-protein in the native core complex, the protein was digested with 0.1 M CNBr in the gel slices. In this case, however, many more peptide fragments were iodinated than in Fig. 4; several peptide fragment ranging from 7 to 20 kDa were labeled. It is significant, however, that the 3 kDa peptide fragment, which was detected as a radioactive band in the case of photochemical iodination, was not iodinated in this case. This result suggests that the iodinated amino-acid residue(s) in the 3 kDa peptide fragment is not accessible to lactoperoxidase and thus is present in the interior part of PS II core complex. This is consistent with

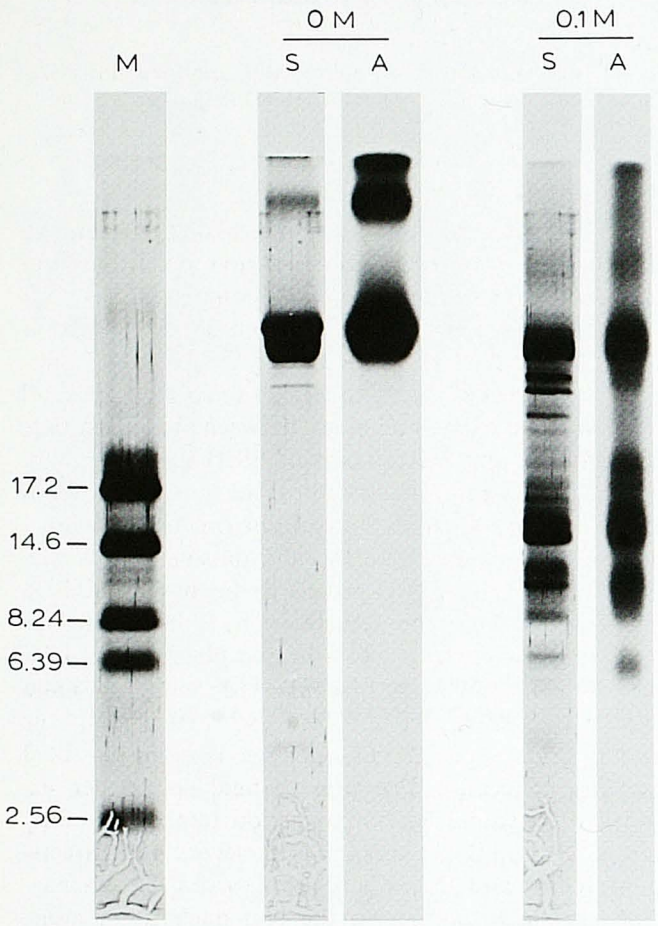

Fig. 7. CNBr cleavage of D1-protein iodinated with lactoperoxidase. PS II core complex was enzymatically iodinated with lactoperoxidase and subsequetly the D1-protein was purified from the core complex by SDS-polyacrylamide gel electrophoresis. The D1-protein was cleaved with 0.2 M CNBr. Electrophoresis conditions were the same as Fig. 2. (M) Molecular weight standards, (S) stained with silver, (A) autoradiography.

the proposal for the structure of D1-protein in the membrane [10,33] which predicts that the 3 kDa fragment corresponding from Arg-140 to Met-172 is in one of the five membrane-spanning helices (C-helices).

Discussion

Under the experimental conditions employed in this study, the cleavage of D1-protein was incomplete even at the highest concentration of reagent/enzyme used, as shown by the number and the size of the peptide fragments obtained. Nevertheless, the fact that the number of radioactive fragments obtained is exceedingly small in every case and that the amount of only the smallest radioactive band increases after extensive treatment suggests that the iodination site is limited to a very small number of residues in the protein. The size of the smallest radioactive band in each treatment was estimated in this experiment to be 14.5, 3 and 8 kDa, in N-CSI, CNBr and *S. aureus* V8 proteinase-treatment, respectively. Based on the deduced amino-acid sequence of spinach D1-protein [2] and the expected cleavage sites of the treatments, these fragments can best be ascribed to Ile-143–Trp-278, Arg-140–Met-172 and Leu-133–Glu-189, respectively. By comparing the overlapping sequence of the smallest radioactive fragments obtained by different chemical and proteolytic cleavages, it is concluded that the small fragment of about 3 kDa, which starts at Ile-143 and ends at Met-172, contains the specific iodination site(s) in D1-protein. Since it is established that the iodination sites in proteins are generally limited to Tyr and His residues [32], and it is known that the 3 kDa fragment contains only two Tyr residues, but no His, the potential iodination sites in D1-protein are deduced to be Tyr-147 and Tyr-161.

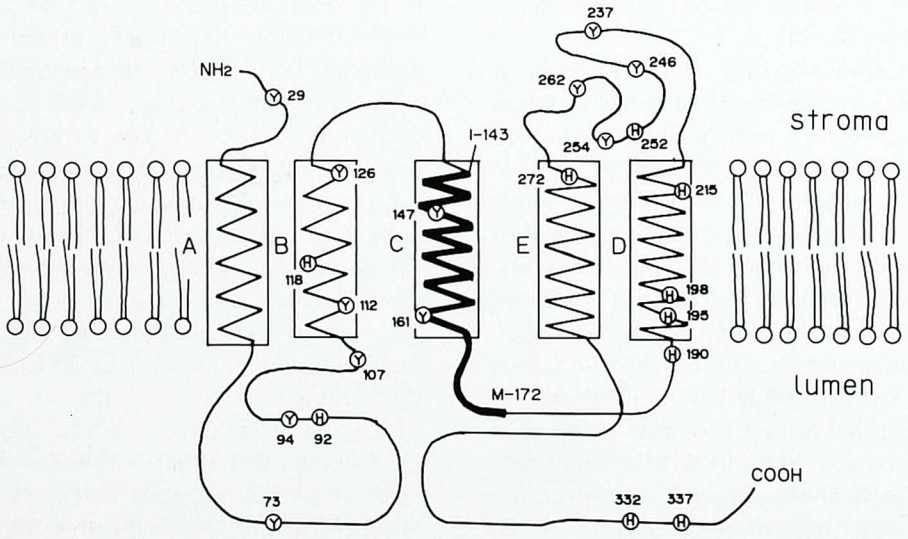

Fig. 8. Proposed structure of D1-protein modified from Ref. 10. The iodinated segment is indicated by the bold line and the positions of tyrosine (Y) and histidine (H) residues are indicated.

The structure of D1-protein has been analyzed based on the hydropathy index [10] and on the immunological cross-reactivity against site-directed antibodies [33]. These studies predicted that this protein spans the thylakoid membrane five times in a similar way to that the L and M subunits of purple bacterial reaction centers span. In the structure based on these predictions, the two Tyr residues of potential iodination site are located in the interior part of the third transmembrane helices (C-helices) as shown in Fig. 8. This figure is consistent with the present result that the iodination site(s) of the D1-protein in the intact core complex is protected from surface-specific iodination, as shown by the lactoperoxidase/H_2O_2 experiment. The predicted structure also shows that the Tyr-161 is located on the lumenal side of the C-helices and is probably present close to the Mn cluster of the oxygen-evolving system as illustrated in Fig. 8. The His-198 of D1-protein as well as the His-198 of D2-protein are assumed to be involved in the binding of chlorophyll dimer (special pair, P-680), from the analogy of PS II to the purple bacterial reaction center [34]. These two His residues are present on the lumenal side close to the Tyr-161 in the predicted folding structure of the PS II reaction center. On the other hand, the Tyr-147 is located on the stromal side of the C-helices and is close to the His-215 and His-272 of the D1-protein which, together with corresponding His residues in the D2-protein, are supposed to ligand the ferrous iron in the quinone-Fe acceptor ocmplex. Based on these considerations, it seems to be reasonable to conclude that the Tyr-161 is the only residue which participates in the photochemical iodination in D1-protein.

In the previous iodination experiment [6], it was shown that the D2-protein in the PS II core complex is iodinated even in the dark. The iodination was suggested to be due to iodide oxidation by dark-stable EPR species (signal IIs) [6] and the suggestion was supported by recent kinetic evidence [20]. A recent selective deuteration experiment [21], together with the analysis using site-directed mutagenesis [22,23], indicated that a Tyr residue on D2-protein (Tyr-160 in the deduced sequenc eof *Synechocystis* protein, which corresponds to Tyr-161 of spinach protein) is the chemical entity responsible for the dark-stable EPR signal II. This Tyr residue is present in the transmembrane helices C on the lumenal side of D2-protein, in a symmetrical way to the iodinated Tyr residue in D1-protein mentioned above. It is noted, furthermore, that the sequence surrounding this Tyr in D1-protein is highly conserved in D2-protein as shown in Fig. 9, and thus it is very reasonable to conclude that the iodinated Tyr residue discussed in the present study, by itself, is the EPR signal IIf(vf) species and the secondary electron donor of PS II (Z), as already presumed, based on the sequence symmetry and EPR signal shape [20,22].

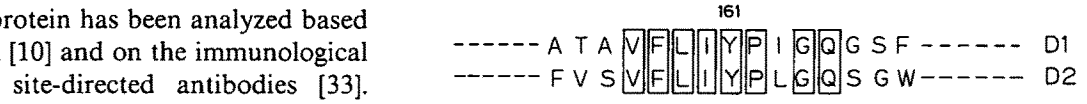

Fig. 9. Comparison of part of the deduced primary structure of D1- and D2-proteins which includes the Tyr-161 in question [2,35,36].

This conclusion becomes more realistic when we consider the symmetrical organization on a two-fold symmetry axis of the proteins and components in photochemical reaction centers. It is well documented that in purple bacterial reaction centers the L and M subunits are organized symmetrically in the complex [13,14] and that there exists homology between these two proteins and D1 and D2-proteins of PS II in the primary structure [2,35,36]. The cofactors of purple bacterial reaction centers are also shown to be organized symmetrically in the complex [13,14], and those on the reducing side of PS II are expected to be organized in a similar way; both photosystems have two quinone acceptors, Q_A and Q_B, and also two pheophytin molecules, one of which is disconnected from the main electron-transport chain. This also extends to the primary donor, special pair, which consists of two Bchl (or Chl) molecules. The present analysis further extended this symmetrical organization to the secondary donors, Z and D. In this case, however, Z is directly connected to and D is disconnected from the electron-transport chain; similar to the two pheophytin molecules on the reducing side.

It is interesting that another type of reaction center, PS I, is also composed of two similar large subunits, which are psIA1 and psIA2 gene products [37]. Cys residues which are assumed to bind iron-sulfur clusters are conserved in both subunits and it is therefore proposed that a PS I reaction center is symmeetrically organized [38]. It may be possible to speculate, therefore, that the symmetrical organization is a general characteristic of photosynthetic reaction centers.

Addendum

After submission of this manuscript, a report by Ikeuchi and Inoue [39] was published which also suggests that the 3 kDa peptide fragment of D1-protein obtained after CNBr treatment is photochemically iodinated.

Acknowledgements

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