Identification of the site of iodide photooxidation in the photosystem II reaction center complex

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The highly purified PS II reaction center complex with 47, 43, 32, 30 and 9 kDa (and 4.5 kDa) polypeptides was subjected to iodo-labeling to identify the polypeptide(s) participating on the donor side of the photosystem. The 32 kDa component (D2) and the aggregate(s) of about 60 kDa were exclusively labeled in the dark, suggesting the presence of a dark-stable oxidizing component on this polypeptide. Illumination of the complex in the presence of 2,6-dichlorophenolindophenol and iodide resulted in the preferential iodination of the 30 kDa component (D1) and the aggregate(s), but not the 47 kDa polypeptide. This iodo-labeling was completely inhibited by the addition of an electron donor, 1,5-diphenylcarbazide. From these results, it is concluded that the site of photooxidation of iodide, and presumably the site of Z binding, is located on or near the herbicide-binding polypeptide, D1, in the PS II reaction center complex.

Photosystem II *Reaction center* *Iodo-labeling* *Secondary electron donor* *Herbicide-binding protein*

1. INTRODUCTION

The purified PS II reaction center complex, which lacks oxygen-evolving activity, consists of five (or six) polypeptides of apparent molecular mass of 47, 43, 32, 30 and 9 kDa (and 4.5 kDa) [1]. The polypeptides of 47 and 43 kDa bind chlorophyll *a* and β-carotene and those of 9 and 4.5 kDa are apoproteins of cytochrome b-559 [1]. The 30 kDa polypeptide (D1) has been identified as the site of herbicide binding and hence of Q_B binding [2]. However, the function of the 32 kDa polypeptide (D2) has not yet been elucidated.

The isolated PS II reaction center complex is composed of multiple electron carriers stabilizing the light-induced charge separation between donor chlorophyll P680 and acceptor pheophytin [1,3]. Reduced pheophytin transfers its electron to Q_A and oxidized P680 accepts an electron from the secondary electron donor, Z. Z^+ is attributed to EPR signal IIf while there is an additional component, which is attributed to D (signal IIs), also on the donor side.

Recently, a polypeptide in the region of D2 in SDS-polyacrylamide gel electrophoresis was shown to be the site of iodide photooxidation by the secondary electron donor of PS II in Tris-treated thylakoid membranes [4]. However, it was difficult in this experiment to identify conclusively the iodo-labeled polypeptide since thylakoid membranes contain many polypeptides around this M_r region. Here, the highly purified PS II reaction center complex with simpler polypeptide composition was subjected to iodo-labeling to identify the polypeptides participating on the donor side of PS II.

Abbreviations: PS, photosystem; DCIP, 2,6-dichlorophenolindophenol; CBB, Coomassie brilliant blue

2. MATERIALS AND METHODS

PS II reaction center complex was purified from

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digitonin extracts of spinach chloroplasts by two-step DEAE-Toyopearl chromatography following the method in [5]. Glycerol was added to the concentrated sample at 20% and the sample stored at 77 K until use.

The electron transfer reaction in the PS II reaction center complex from iodide to DCIP was driven at 25°C by saturating white light passed through heat absorption filters. The reaction mixture contained the PS II reaction center complex (100 μg chl/ml), 50 mM NaCl, 200 μM KI (about 0.1 mCi $^{125}I^-$) (ICN Biochemicals), 30 μM DCIP, 0.2% digitonin and 10 mM Tris-HCl (pH 7.2). After incubation, the iodo-labeled PS II reaction center complex was adsorbed on a small DEAE-Toyopearl column and eluted with 250 mM NaCl, 50 mM Tris-HCl (pH 7.2) and 0.2% digitonin to separate the complex from free iodide.

For non-specific iodo-labeling, the PS II reaction center complex was incubated with 250 mM NaCl, 50 mM Tris-HCl (pH 7.2), 0.2% digitonin and 25 μg/ml lactoperoxidase (Sigma, USA) in the presence of 50 μCi $^{125}I^-$. 1/20 vol. of 1 mM H_2O_2 was added to the solution 4 times every 1 min and the free iodide then removed as described above.

After solubilization of the complex with 2.5% SDS, 2.5% 2-mercaptoethanol and 3 M urea, the iodo-labeled PS II reaction center complex was resolved by SDS-polyacrylamide gel electrophoresis according to Laemmli [6] with slight modifications; 6 M urea and a 10–15% polyacrylamide gel gradient were included in the resolving gel. Polypeptides were stained with a solution containing 0.05% CBB, 50% methanol and 7% acetic acid (w/v/v). For fluorography, the gel was incubated with 2,5-diphenyloxazole in dimethyl sulfoxide [4]. Kodak XAR-5 film was exposed to the dried gel at − 80°C for 20–40 days.

For proteolytic digestion, the PS II reaction center complex was hydrolyzed by incubation with lysyl endopeptidase (0.1 mg/ml) (Wako, Osaka) in the presence of 4 M urea and 0.1% SDS at 30°C for 1 h.

3. RESULTS AND DISCUSSION

In the experiments shown in fig.1, the PS II reaction center complex was incubated with iodine enzymatically produced by the action of lactoperoxidase in the presence of H_2O_2, in order to determine which polypeptide carries surface-exposed iodo-labeling sites, e.g., tyrosyl residues. The SDS-polyacrylamide gel electrophoretic profile of the purified complex exhibited 47, 43, 32, 30 and 9 kDa polypeptides together with aggregated bands of about 60 kDa (fig.1A). Fig.1B shows the enzymatically iodo-labeled polypeptides of the PS II reaction center complex. Each polypeptide band showed different affinity to iodine. The 47 kDa chlorophyll-binding polypeptide, D1 protein and cytochrome *b*-559 were highly labeled with iodine. The 43 kDa polypeptide was also labeled with iodine, but to a much smaller extent. On the other hand, the D2 protein was scarcely labeled under these conditions. These results cannot simply be explained by the hydropathy data of the individual polypeptide deduced from the nucleotide sequence of the chloroplast genome [7].

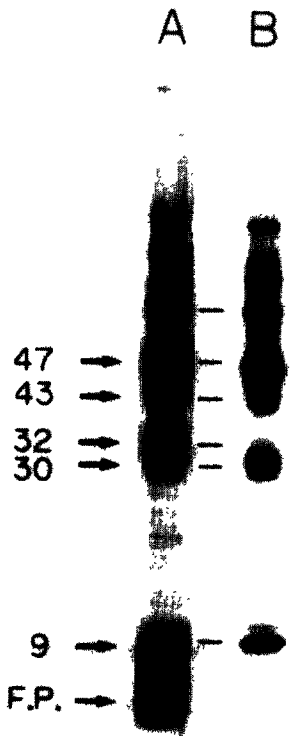

Fig.1. Labeling of polypeptides of the PS II reaction center complex with iodine enzymatically activated by lactoperoxidase with H_2O_2. (A) Polypeptides stained with CBB. (B) Fluorography of polypeptides labeled with iodine.

To identify the polypeptides which participate

on the oxidizing side of PS II, the PS II reaction center complex was iodo-labeled in the absence of lactoperoxidase as shown in fig.2. When the PS II reaction center complex was incubated with iodide in the presence of DCIP for 10–20 min, D2 and one of the aggregated polypeptides of about 60 kDa were exclusively labeled without illumination (fig.2B). It is unlikely that this labeling is due to non-specific iodination by interaction of the polypeptide(s) with iodine which is contaminated in iodide solution since D2 was the least labeled component with iodine enzymatically activated (fig.1B). Therefore, it is suggested that a dark-stable oxidizing component which oxidizes iodide in the dark is located on or near the D2 protein in the PS II complex.

The PS II reaction center complex drives electron flow from iodide to DCIP during illumination [2]. Thus, illumination of the reaction center complex results in the formation of iodine on the donor side, and this in turn reacts with tyrosyl residues near the photooxidation site. In the experiment shown in fig.2C, actinic illumination resulted in a considerable degree of iodo-labeling of the polypeptide around 30 kDa and also the aggregated band of about 60 kDa. Remarkably, the 47, 43 and 9 kDa polypeptides, which exhibited high affinity for iodine in non-specific labeling (fig.1B), were not labeled in both the light and dark. A sharp band with a slightly higher electrophoretic mobility than that of the 43 kDa polypeptide was also labeled by illumination in this experiment. This band is not a constituent polypeptide of the PS II complex and is ascribed to a small contaminant [1].

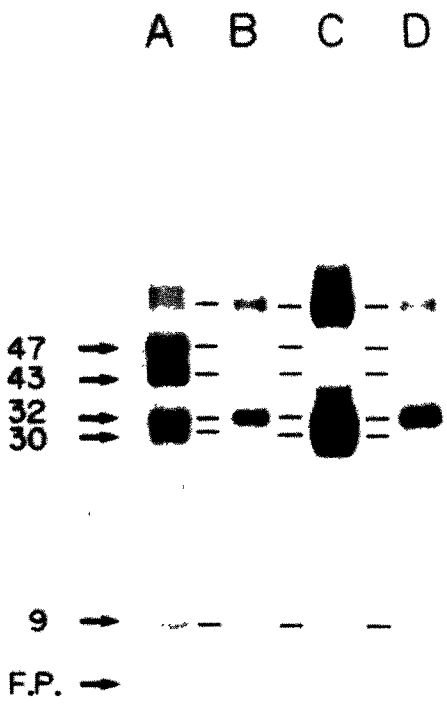

Fig.2. Labeling of polypeptides of the PS II reaction center complex with iodine in the presence of DCIP. (A) Polypeptides stained with CBB. Fluorography of polypeptides labeled with iodine (B) in the dark for 10 min, (C) in the light for 10 min and (D) in the light for 10 min in the presence of 0.15 mM diphenylcarbazide.

The light-induced iodo-labeling of the polypeptides around 30 and 60 kDa was completely inhibited by the addition of diphenylcarbazide (fig.2D) since it competitively replaces iodide as an electron donor [4]. On the other hand, the extent of iodo-labeling of the D2 protein in the dark was not decreased by the addition of diphenylcarbazide. These results strongly support the interpretation that light-induced iodo-labeling is due to photooxidation of iodide by the secondary electron donor of PS II. Iodo-labeling was not inhibited by DCMU (not shown), inconsistent with the fact that the DCIP photoreduction activity is insensitive to the inhibitor in this preparation [1].

To compare quantitatively the radioactivities of incorporated iodine between the samples in the dark and light, the radioactivity distributions were determined after slicing the gel into strips. Illumination increased the extent of radioactivities incorporated into the polypeptides more than 5-fold as shown in fig.3. Though the separation between D1 and D2 was not sufficient in this experiment, calculation by subtracting the radioactivity in the dark from that in the light showed that the illumination preferentially increased the extent of radioactivity in D1, but not in D2. Moreover, the extent of radioactivity of D1 increased in proportion to illumination time (not shown). Therefore, it is concluded that D1 is a specific polypeptide labeled with iodine activated by photooxidation at the PS II reaction center. This result indicates that the site of photooxidation of iodide is located on or near the D1 protein of the PS II complex.

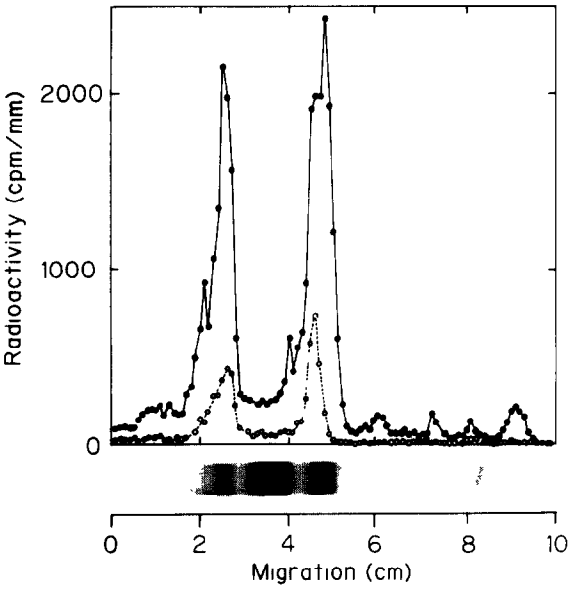

Fig.3. Radioactivity distributions of iodo-labeled polypeptides of the PS II reaction center complex. Conditions for iodo-labeling were the same as in fig.2. After SDS-polyacrylamide gel electrophoresis, the gel was sliced into strips of 1 mm width and the radioactivity then counted. (– – –) Iodo-labeled in the dark, (——) iodo-labeled in the light.

Since D1 has been identified as a herbicide-binding protein from azido[^{14}C]atrazine-labeling experiments [2], the above result indicates that this polypeptide participates on both donor and acceptor sides in PS II. To reconfirm that the iodo-labeled polypeptide by illumination is a herbicide-binding protein, the PS II reaction center complex was treated with lysyl endopeptidase which specifically hydrolyzes the carboxyl group side of lysine [8], since the herbicide-binding protein is known to be unique in that it does not contain any lysine residues [9]. SDS-polyacrylamide gel electrophoresis of the enzyme-treated sample revealed that all polypeptides, except for D1, were digested with this proteinase (not shown), reconfirming that D1 is a herbicide-binding protein.

Iodo-labeling of the band(s) around 60 kDa in this experiment in the dark (fig.2B) and light (fig.2C) supports the interpretation that this band(s) is a dimer or heterodimer of D1 and D2 proteins [10]. This band(s) has recently been shown to be present in a preparation consisting of D1, D2 and cytochrome *b*-559 [10], to cross-react with antibody against D1 polypeptide (Camm, E.L., personal communication) and not to cross-react with antibodies against 47, 43 and cytochrome *b*-559 [10].

There is controversy about the site of charge separation in PS II; some evidence suggests that the 47 kDa polypeptide is the site of primary photochemistry [11–15] whereas other data suggest that D1 and D2 constitute the reaction center complex [7, 10, 16]. Our result that D1 participates on both donor and acceptor sides supports the proposal that the site of primary charge separation is located on D1 and/or D2.

It is interesting to note that D2 was exclusively labeled with iodine in the dark though this polypeptide was the least labeled with iodine enzymatically activated (fig.1B). This result suggests that D2 contains the oxidant which is stable in the dark and can activate iodide. The species responsible for signal IIs might be one of the candidates for this component.

4. CONCLUSIONS

(i) The site of photooxidation of iodide in the PS II reaction center complex is located on or near the herbicide-binding protein, D1.

(ii) A dark-stable oxidizing component which activates iodide in the dark is supposedly present on D2.

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