Association of the 33-kDa polypeptide with the 43-kDa component in photosystem II particles

Yasuhiro Isogai, Yasusi Yamamoto and Mitsuo Nishimura

Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan

Received 28 May 1985

The organization of polypeptides and Mn atoms in the oxygen-evolution system was studied by analyzing the effects of protease treatment on photosystem (PS) II particles from spinach chloroplasts. Hydrolysis of the 47- and 43-kDa polypeptides of the PS II core complex had similar profiles of dependence on trypsin concentration in the treatment of control PS II particles and NaCl-washed particles, which were devoid of the peripheral 24- and 18-kDa polypeptides. In $CaCl_2$-washed particles, which were depleted of the 33-kDa polypeptide besides the 24- and 18-kDa polypeptides, the 43-kDa polypeptide was much more sensitive to trypsin than the 47-kDa polypeptide. Chymotrypsin treatment gave similar results. These findings suggest that the 33-kDa polypeptide is associated to the 43-kDa polypeptide and shields it from tryptic attack. Changes of the amount of Mn in the PS II and salt-washed particles on protease treatment indicated a heterogeneity of binding sites of Mn in the PS II particles

Oxygen evolution *Salt-washing* *Protease treatment* *33-kDa polypeptide* *PS core II complex* *Mn*

1. INTRODUCTION

It has been generally accepted that Mn plays a central role in photosynthetic oxygen evolution. Recently, the peripheral polypeptides with molecular masses of 33, 24 and 18 kDa have been extensively studied as protein components that probably facilitate the functioning of Mn. Treatments of oxygen-evolving membrane preparations isolated from chloroplasts with highly concentrated salt liberated these polypeptides from the membranes and inactivated oxygen evolution, while Mn atoms were preserved associated with the membranes. The 24- and 18-kDa polypeptides were released by washing with concentrated NaCl [1,2]. The 33-kDa polypeptide, in addition to the 24- and 18-kDa polypeptides, was removed by $CaCl_2$ washing [3].

Abbreviations: PS, photosystem; Mes, 4-morpholineethanesulfonic acid; Chl, chlorophyll; LHCP, light-harvesting chlorophyll-protein complex

The 33-kDa polypeptide was found to be required for stabilization of Mn atoms in situ and the 24- and 18-kDa polypeptides appeared to play an auxiliary role in oxygen evolution [4–7,12]. These polypeptides and Mn may be associated with the reaction center complex of PS II (PS II core complex) and require a specific conformation for active oxygen evolution. The PS II core complex is composed of several intrinsic proteins including two Chl *a*-binding polypeptides with apparent molecular masses of 43 and 47 kDa [9]. Nakatani et al. [14] indicated that the 47-kDa component was the PS II reaction center polypeptide and the 43-kDa polypeptide served as an antenna Chl-protein, based on their fluorescence study. Here, we studied the effects of trypsin and chymotrypsin treatments on polypeptides and Mn in PS II particles, and investigated differences between the effects on the PS II particles with the 3 peripheral polypeptides and that on the salt-washed particles devoid of 2 or 3 of these polypeptides.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

2. MATERIALS AND METHODS

Oxygen-evolving PS II particles were prepared from spinach chloroplasts with digitonin and Triton X-100 as described in [8] and stored at −60°C. Before use, the preparation was washed with a medium containing 0.33 M sorbitol, 4 mM $MgCl_2$ and 10 mM Mes (pH 6.5) (medium A). For salt washing of PS II particles, which removed the membrane-bound polypeptides, the particles were suspended in a mixture of 0.33 M sorbitol and 10 mM Mes (pH 6.5) containing either 1 M NaCl or 1 M $CaCl_2$ at a Chl concentration of 0.5 mg/ml and incubated for 15 min at 4°C. The membrane fraction was collected by centrifugation at 35000 × *g* for 15 min. The pellet was washed once with medium A and resuspended in the same medium.

The PS II and salt-washed particles were treated with protease as follows. Samples were suspended in medium A with trypsin or chymotrypsin at 0.2 mg Chl/ml and incubated at 24°C for 15 min. The concentration of the proteases was varied from 10 μg/mg to 2 mg/mg Chl. After incubation, phenylmethylsulfonyl fluoride (PMSF) was added to a final concentration of 2 mM to stop the digestion. The digested particles were collected by centrifugation, washed once with medium A and finally suspended in medium A. Trypsin and PMSF were obtained from Boehringer Mannheim. Chymotrypsin used was Sigma type II.

Polypeptide composition in the PS II particles was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Gels with a 7–15% gradient of polyacrylamide containing 6 M urea were used for electrophoresis. The particles corresponding to 6 μg Chl were loaded on each gel slot. The relative amount of each polypeptide in the PS II particles was estimated from the peak heights of polypeptide bands in the densitogram. The Sigma MW-SDS-70-L kit was used for M_r standards. A PS II core complex preparation [9] was used for identification of PS II reaction center polypeptides of 43 and 47 kDa. The other procedures of electrophoresis have been described [10].

The amount of Mn was determined by atomic absorption according to [11] with the following modifications. Each sample equivalent to 0.2 mg Chl was dried at 90°C for 1 h and ashed at 500°C for 6–7 h in porcelain crucibles. 3–4 drops of concentrated HCl and 0.5 ml deionized water were added to the ashed samples and the solutions were dried at 90°C for 1 h. Thereafter, 1 ml deionized water was added to each sample, and the contents were carefully suspended with a glass rod covered with a silicone tube to avoid incomplete solubilization of Mn. Standard Mn solutions were treated in the same way. The contents of Mn were measured with a Hitachi model 170-10 atomic absorption spectrophotometer at 297.5 nm.

3. RESULTS AND DISCUSSION

The effects of trypsin on 3 types of PS II particles, i.e. control, NaCl-washed and $CaCl_2$-washed, are compared in fig.1. The peripheral polypeptides of 33 and 18 kDa were degraded significantly at low concentrations of trypsin. There seem to be 2 polypeptide bands in the 24–25 kDa range. In fig.1A, the band of smaller M_r, which was lost on salt washing (see fig.1B,C) and is therefore attributable to the water-soluble 24-kDa polypeptide, also disappeared at low trypsin concentration. For hydrolysis of the 43- and 47-kDa polypeptides, which are Chl *a*-binding apoproteins of PS II, a higher concentration of trypsin was required. In the control PS II particles, there was no apparent difference in the concentration dependence of proteolysis between the 43- and 47-kDa polypeptides.

The 33-, 24- and 18-kDa polypeptides or only the 24- and 18-kDa polypeptides were removed from the membranes by $CaCl_2$ or NaCl washing of the PS II particles (fig.1B,C). In the $CaCl_2$-washed particles depleted of the 33-, 24- and 18-kDa polypeptides, the 43-kDa protein of the PS II core complex was much more sensitive to trypsin digestion than the 47-kDa component (fig.1B). On the other hand, the proteolysis of the 43- and 47-kDa proteins by trypsin was not affected by NaCl washing which resulted in depletion of only the 24- and 18-kDa polypeptides before protease treatment (fig.1C). Similar results were obtained on treatment of the 3 types of PS II particles with another proteolytic enzyme, chymotrypsin (not shown). These findings indicate that the 33-kDa polypeptide is associated with the 43-kDa polypeptide on the surface of the membrane and shields it from the proteases. However, the possibility that the $CaCl_2$ treatment induces some structural change of the intrinsic proteins in PS II that makes

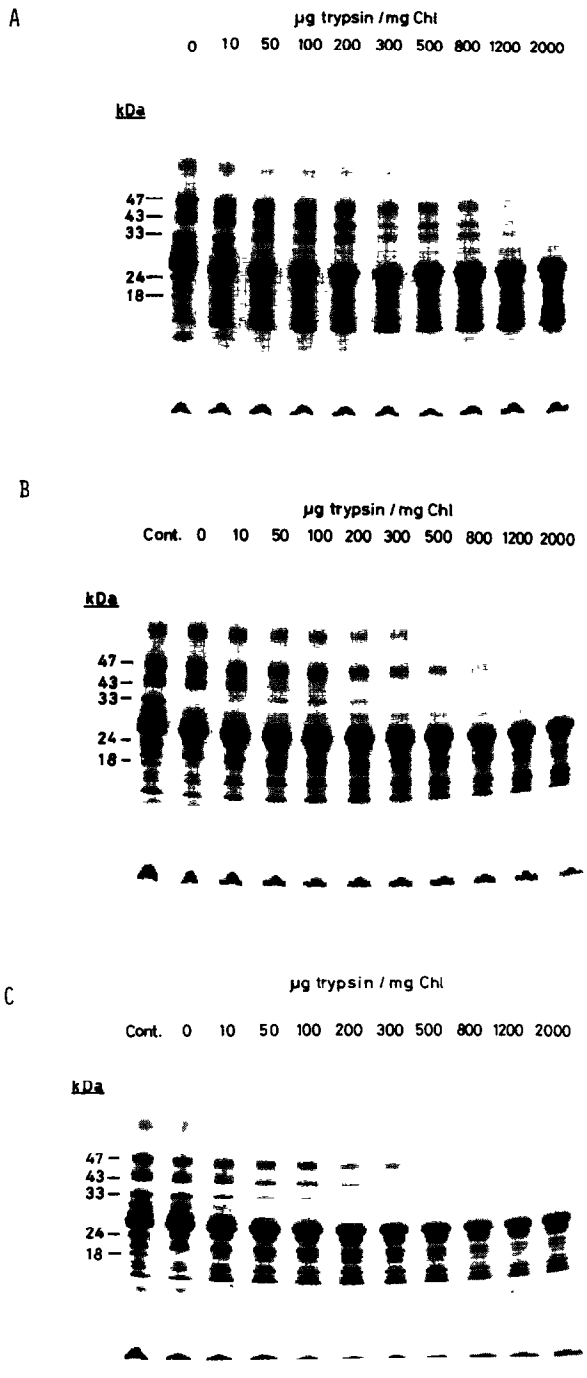

Fig.1. Effects of trypsin on the polypeptide pattern for control PS II particles (A), 1 M $CaCl_2$-washed particles (B) and 1 M NaCl-washed particles (C), shown in SDS-PAGE. Experimental procedures are described in section 2.

the 43-kDa polypeptide sensitive to the proteases cannot be ruled out. The same difference in susceptibility to trypsin between the 43- and 47-kDa polypeptides was also shown with the purified reaction center complex of PS II [9].

There was another effect of salt washing on hydrolysis of the polypeptides in PS II particles with trypsin. A band with an apparent molecular mass of 29 kDa and the upper part of a band in the 26–28-kDa range disappeared at low trypsin concentrations in the control PS II particles (fig.1A), while these unidentified polypeptides were rather resistant to trypsin in the $CaCl_2$-washed and NaCl-washed particles (fig.1B,C). In the treatment of the control PS II particles with chymotrypsin, these polypeptide bands remained after treatment with the enzyme at low concentrations. The salt washing did not affect hydrolysis of the polypeptides in the 26–30 kDa range in the chymotrypsin treatment. A possible explanation for these phenomena may be a conformational change(s) of PS II due to the salt washing, but further studies are required for identification of these polypeptides and understanding of their behavior in the protease treatments.

The correlation between liberation of the 33-kDa polypeptide by treatment with $CaCl_2$ and hydrolysis of the intrinsic proteins by trypsin was investigated in more detail. By washing of the PS II particles with 0.3 M $CaCl_2$, the 24- and 18-kDa polypeptides were completely liberated from the particles, whereas almost all the 33-kDa polypeptide remained associated with the membranes (not shown). The 33-kDa polypeptide was gradually removed from the particles by increasing the $CaCl_2$ concentration from 0.3 to 1 M in the salt washing before trypsin treatment. Upon treatment of $CaCl_2$-washed particles with trypsin, the degree of degradation of the 43-kDa protein depended on the amount of the 33-kDa polypeptide remaining on the membranes. A linear relationship between them was observed (fig.2). It seems that digestion of the 47-kDa polypeptide also depended on the amount of 33-kDa protein removed from the membranes. The degree of hydrolysis of the 47-kDa polypeptide and the amount of the membrane-bound 33-kDa polypeptide, however, did not show a linear correlation and these polypeptides might be interacting indirectly, probably through the 43-kDa polypeptide. Proteolysis of the 26–27-kDa

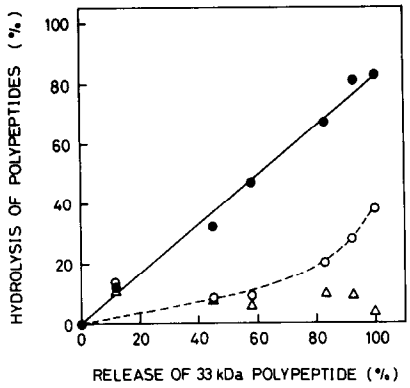

Fig.2. Dependence of hydrolysis of intrinsic polypeptides in PS II particles by trypsin on the amount of 33-kDa polypeptide liberated from the membranes by washing with $CaCl_2$. (●—●) 43-kDa polypeptide, (○---○) 47-kDa polypeptide and (△) 27–27-kDa polypeptide, probably corresponding to LHCP. Concentrations of $CaCl_2$ used to remove the 33-kDa polypeptide in various degrees before trypsin treatment were 0.4, 0.5, 0.6, 0.7, 0.8 and 1.0 M. Trypsin treatment was carried out for 15 min at 24°C and pH 6.5 with a trypsin concentration of 0.2 mg/mg Chl (0.2 mg Chl/ml). The amount of 33-kDa polypeptide released is relative to the control PS II particles. The degree of hydrolysis is expressed as a percentage of that of the unwashed particles treated with trypsin under the same conditions.

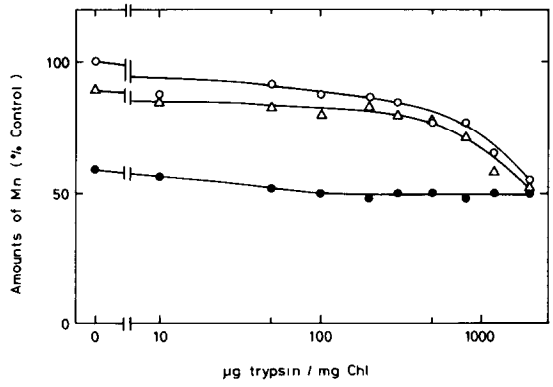

Fig.3. Effects of trypsin on the amount of Mn. Control PS II particles (○), 1 M $CaCl_2$-washed particles (●) and 1 M NaCl-washed particles (△). The values are relative to the amount of Mn in each original PS II preparation before salt washing.

polypeptide, probably corresponding to LHCP [15], was not apparently affected by pre-washing with $CaCl_2$. These results confirm that the 33-kDa polypeptide is associated exclusively with the 43-kDa polypeptide.

The PS II particles used here contained 3.6–4.0 Mn atoms per 200 Chl molecules. About half the Mn in the control PS II particles was released by treatment with trypsin at high concentration (fig.3). The amount of Mn in the NaCl-washed particles was decreased on trypsin treatment with nearly the same dependence on trypsin concentration as that in control particles. As the reference (0 μg trypsin/mg Chl), the 3 types of PS II particles were treated similarly but with no protease in the incubation medium. It has been reported that, when the 33-kDa polypeptide was removed by treatment with concentrated $CaCl_2$ or urea, half the amount of Mn in the PS II particles became loosely associated with the membrane and was gradually released in a medium with a low salt concentration [5,12]. After $CaCl_2$ washing, 40–50% of the total Mn was released during incubation at 24°C for 15 min without protease. The remaining fraction of Mn, about half of the original content in the PS II particles, was resistant to trypsin treatment. Treatment of the 3 types of PS II particles with chymotrypsin resulted in effects similar to those obtained with trypsin concerning the amount of Mn (not shown). These results suggest that 2 of the 4 Mn atoms per PS II complex, being resistant to the protease, may be deeply embedded in the PS II core complex and the other 2, easily released from the particles during incubation after $CaCl_2$ washing, seem to exist near the surface of the membrane. The latter 2 atoms might correspond to the Mn atoms believed to interact with the 33-kDa polypeptide in the oxygen-evolution system [12,13].

ACKNOWLEDGEMENTS

The authors are grateful to Professor K. Satoh, Department of Biology, Faculty of Science, Okayama University, for providing us with a PS II core complex preparation. Thanks are also due to Dr K. Tabata for useful discussion and Mr Y. Onda for technical assistance.

REFERENCES

[1] Åkerlund, H.-E., Jansson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 681, 1–10.
[2] Kuwabara, T. and Murata, Y. (1983) Plant Cell Physiol. 24, 741–747.
[3] Ono, T. and Inoue, Y. (1983) FEBS Lett. 164, 255–260.
[4] Ono, T. and Inoue, Y. (1984) FEBS Lett. 166, 381–384.
[5] Ono, T. and Inoue, Y. (1984) FEBS Lett. 168, 281–286.
[6] Miyao, M. and Murata, Y. (1984) FEBS Lett. 170, 350–354.
[7] Miyao, M. and Murata, N. (1985) FEBS Lett. 180, 303–308.
[8] Yamamoto, Y., Tabata, K., Isogai, Y., Nishimura, M., Matsuura, K. and Ito, S. (1984) Biochim. Biophys. Acta 767, 493–500.
[9] Satoh, K., Nakatani, H.Y., Steinback, K.E. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 142–150.
[10] Yamamoto, Y., Shimada, S. and Nishimura, M. (1983) FEBS Lett. 151, 49–53.
[11] Yamamoto, Y. and Nishimura, M. (1983) Biochim. Biophys. Acta 724, 294–297.
[12] Kuwabara, T., Miyao, M., Murata, T. and Murata, N. (1985) Biochim. Biophys. Acta 806, 283–289.
[13] Yamamoto, Y., Shinkai, H., Isogai, Y. and Nishimura, M. (1984) FEBS Lett. 175, 429–432.
[14] Nakatani, H.Y., Ke, B., Dolan, E. and Arntzen, C.J. (1984) Biochim. Biophys. Acta 765, 347–352.
[15] Lam, E., Baltimore, B., Ortiz, W., Chollar, S., Melis, A. and Malkin, R. (1983) Biochim. Biophys. Acta 724, 201–211.