Volume 224, number 1, 71-74 FEB 05279 November 1987

Isolation of photosystem II reaction center complex by affinity chromatography with the peripheral 33-kDa polypeptide as ligand

Yasuhiro Isogai, Yasusi Yamamoto, Yasuyuki Yamamoto and Mitsuo Nishimura

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

Received 28 August 1987

We report a procedure for one-step isolation of the PS II reaction center complex from PS II particles by affinity chromatography with the peripheral 33-kDa polypeptide as ligand. The $CaCl_2$-washed PS II particles prepared from spinach chloroplasts were solubilized with *n*-octyl-β-D-glucopyranoside and subjected to affinity chromatography. The purified complex consisted of polypeptides with apparent molecular masses of 47, 43, 32, 30 and 8 kDa.

Oxygen evolution; Photosystem II; Reaction center; Affinity chromatography; 33 kDa polypeptide; Photosynthesis

1. INTRODUCTION

The photoinduced redox reaction in PS II is initiated by photochemical oxidation of the reaction center chlorophyll P680 and the coupled donation of an electron to the primary acceptor pheophytin. The electron is then transferred to acceptors Q_A and Q_B, of which the chemical species is plastoquinone. The oxidizing power generated by the photooxidation of P680 is transferred to a manganese complex, in which oxygen evolution occurs, via an intermediate electron carrier Z [1].

It is known that the PS II reaction center polypeptide(s) associated with P680 is in the reaction center 'core' complex consisting of six polypeptides: two chlorophyll-carrying polypeptides with apparent molecular masses of 47 and 43 kDa (CP47, CP43), two polypeptides of about 30 kDa (D1, D2) and two polypeptides of less than 10 kDa (cytochrome *b*-559) [2,3]. The PS II reaction center complexes that retain the capability of oxygen evolution have been isolated from spinach chloroplasts [4–6]. These preparations contained the six subunits of the PS II reaction center complex along with the peripheral 33-kDa polypeptide. The 33-kDa polypeptide has been shown to function in stabilizing the manganese bound to the membrane in PS II particles [7–9]. However, the 33-kDa polypeptide is not the exclusive element for retaining the manganese atoms [10] and does not form the oxygen-evolving site by itself. These facts show that the oxygen-evolving site exists on the reaction center complex. Recently, Nanba and Satoh [11] isolated a chlorophyll-protein complex which consisted of D1, D2 and cytochrome *b*-559, and showed that this complex contained the site of the primary charge separation in PS II. This preparation, however, lacked the plastoquinone molecule and the oxygen-evolution system. Thus, the precise

Correspondence address: Y. Isogai, Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan

Abbreviations: Chl, chlorophyll; DCIP, 2,6-dichloroindophenol; DPC, 1,5-diphenylcarbazide; Mes, 4-morpholineethanesulfonic acid; Mops, 3-*N*-morpholinepropanesulfonic acid; octylglucoside, *n*-octyl-β-D-glucopyranoside; PAGE, polyacrylamide gel electrophoresis; PS, photosystem

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

location of the oxygen-evolving site in PS II has not yet been elucidated.

Here, we report a rapid and simple procedure for isolation of the PS II reaction center complex from PS II particles by affinity chromatography with the peripheral 33-kDa polypeptide as ligand. This procedure may provide a powerful means of identifying the binding site of the 33-kDa polypeptide on the PS II reaction center complex and, possibly, the oxygen-evolving site.

2. MATERIALS AND METHODS

The peripheral 33-kDa polypeptide used as ligand in the affinity column was prepared according to Yamamoto et al. [12]. Affi-Gel 15 (Bio-Rad) was used for affinity chromatography, in which *N*-hydroxysuccinimide ester was linked to agarose gel with a spacer arm of 15 atoms. The 33-kDa polypeptide was incubated with Affi-Gel 15 (about 1 μmol polypeptide per 2.5 ml gel) with gentle shaking in a coupling buffer containing 0.1 M Mops-NaOH (pH 7.5) at 4°C for 24 h. The residual active groups in the gel were blocked by incubation with 0.1 M ethanolamine in coupling buffer at 4°C for 1 h. The gel was washed with 20 column volumes of the coupling buffer. The 33-kDa polypeptide was linked to the gel at a coupling efficiency of 60–70%.

PS II particles were prepared from spinach chloroplasts according to Berthold et al. [13], with a modification in which the second Triton X-100 treatment was omitted. Three peripheral polypeptides of 33, 24 and 18 kDa were removed from the particles by $CaCl_2$ washing essentially according to Ono and Inoue [10]. The PS II particles were suspended in a medium containing 20 mM Mes-NaOH (pH 6.0), 10 mM NaCl and 1500 mM $CaCl_2$ (about 0.2 mg Chl/ml), and centrifuged at 35 000 × *g* for 30 min. The resultant pellet was washed with 20 mM Mes-NaOH (pH 6.0), 10 mM NaCl, 5 mM $CaCl_2$ (MNC buffer). The $CaCl_2$-washed PS II particles were solubilized with MNC buffer containing 2% octylglucoside at a chlorophyll concentration of 1 mg/ml. The suspension of the solubilized particles was centrifuged at 35 000 × *g* for 10 min to remove insoluble materials. The supernatant (4 ml) was loaded onto the 33-kDa polypeptide affinity column (1.5 cm diameter × 1.5 cm) preequilibrated with MNC buffer containing 2% octylglucoside. The column was washed with MNC buffer containing 2% octylglucoside until the eluant became colorless (about 20 column volumes were needed). Then a green-colored component bound onto the column was eluted with a gradient of $CaCl_2$ concentration (5–1500 mM) in 20 mM Mes-NaOH (pH 6.0), 10 mM NaCl and 0.5% octylglucoside. The whole chromatographic procedure was performed at 5–10°C.

SDS-PAGE was performed as in [14] with the modification that the resolving gel contained 12.5% acrylamide and 6 M urea. Absorption spectra were measured with a Shimadzu UV-240 spectrophotometer. The rate of photoreduction of DCIP was measured spectrophotometrically at 580 nm (12.9 $mM^{-1} \cdot cm^{-1}$) in MNC buffer containing 30% glycerol and 60 μM DCIP with a Hitachi 356 spectrophotometer. DPC was used as an artificial electron donor at 0.5 mM. Photoreduction of pheophytin was measured with a Hitachi 356 spectrophotometer in the dual-wavelength mode (450 minus 500 nm) in 50 mM Tris-HCl (pH 7.5) in the presence of 5 μM methyl viologen, 2–5 mg/ml sodium dithionite and 30% glycerol [15]. The sample (5–10 μg Chl/ml) was excited by strong actinic light passed through a red cut-off filter (VR-66, Toshiba) with a cross-illumination system. The photomultiplier was protected from the actinic light by a blue filter (Corning 4-96).

3. RESULTS

A chlorophyll-containing protein complex was obtained by the procedure described in section 2. The complex eluted in a single peak in the concentration range 0.5–0.7 M $CaCl_2$ (fig.1). The absorption spectrum of the preparation was characteristic of the PS II reaction center complexes previously reported [4,16] (fig.2). The Chl *a*/*b* ratio of the preparation obtained by this procedure was always higher than 8.0. The yield of chlorophyll was about 10% with respect to the PS II particles.

The preparation obtained here retained no activity of DCIP photoreduction without an artificial electron donor. However, photoreduction of DCIP with DPC (40–50 μmol DCIP $\cdot$ mg^{-1} Chl $\cdot$ h^{-1}) and of pheophytin (not shown) was observed, which showed that the purified chloro-

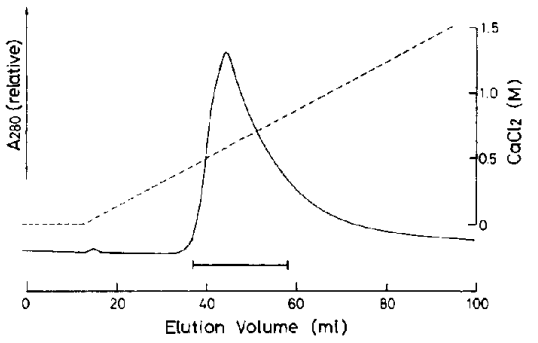

Fig.1. Elution profile of the PS II reaction center complex from the affinity column of the 33-kDa polypeptide. $CaCl_2$-washed PS II particles were solubilized with 2% octylglucoside in MNC buffer and loaded onto the affinity column. The profile after washing of the column with MNC buffer containing 2% octylglucoside was monitored at 280 nm and is shown in the figure. The chlorophyll-protein complex bound to the column was eluted with a $CaCl_2$ gradient (broken line) in 20 mM Mes-NaOH (pH 6.5), 10 mM NaCl and 0.5% octylglucoside. The fraction in the region indicated by the short horizontal bar was collected. The detailed procedure is described in section 2.

phyll-protein complex preserved the photochemical activity of the PS II reaction center.

The polypeptide composition of the purified complex is shown in fig.3. The complex apparently consisted of 5 polypeptides with molecular masses of about 47, 43, 32, 30 and 8 kDa. (A polypeptide band in the molecular mass region lower than 8 kDa was not resolved in the SDS-PAGE profile.) Polypeptide bands in the molecular mass region

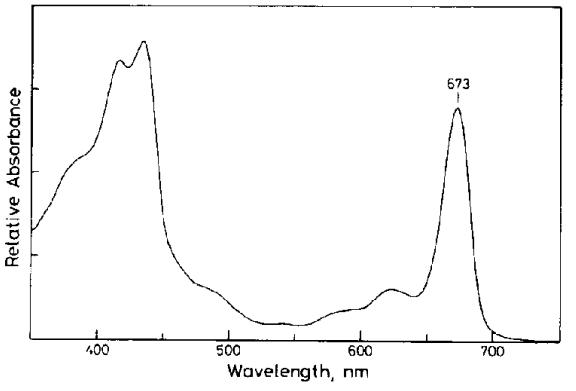

Fig.2. Absorption spectrum of the purified chlorophyll-protein complex at room temperature.

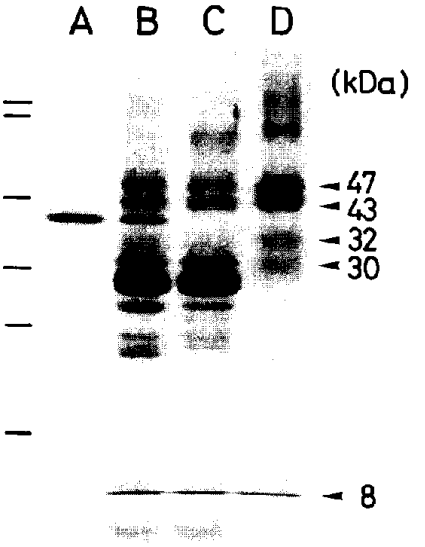

Fig.3. SDS-PAGE patterns of the 33-kDa polypeptide used for affinity chromatography (lane A), PS II particles (lane B), $CaCl_2$-washed PS II particles (lane C) and the purified complex (lane D). Bars on the left indicate the positions of standard protein markers: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

higher than 50 kDa in the SDS-PAGE profile of the purified sample were insignificant in the starting material. These bands were present in various amounts in different preparations. Thus, these bands seem to correspond to aggregates of the lower molecular mass components as recently reported by Satoh et al. [17].

The reaction center complex was not purified by the affinity column that has no linked 33-kDa polypeptide but with all active groups blocked by ethanolamine. This fact shows that the reaction center complex was obtained by the specific interaction with the 33-kDa polypeptide in the affinity column and not by non-specific interaction with the gel matrix or the spacer for ligand.

4. DISCUSSION

The present work provides a new procedure for the rapid and simple isolation of the PS II reaction

center complex. Components of the purified complex, i.e. the 47-, 43-, 32-, 30- and 8-kDa proteins, can be assigned to CP47, CP43, D1, D2 and cytochrome *b*-559 [2,3]. The polypeptide composition was identical with the preparation reported in [16]. Recently, the PS II reaction center complexes, which retained the 33-kDa polypeptide and oxygen-evolution activity, were prepared from thylakoids using digitonin [4] and from PS II particles using octylglucoside [5,6]. We have shown that the 33-kDa polypeptide can be used for the isolation of the reaction center complex, which was photochemically active but preserved no 33-kDa polypeptide or oxygen-evolution activity, in affinity chromatography. A drawback of this technique is the removal of the peripheral polypeptides, which are involved in oxygen evolution, from the starting material before the chromatography. This makes it difficult to purify the complex with the structural integrity of the oxygen-evolving site. A similar disadvantage also existed in the isolation of bacterial reaction centers by cytochrome *c* affinity chromatography [18].

The present result indicates that the binding site of the 33-kDa polypeptide and, possibly, the site of oxygen evolution exist on the purified reaction center complex. However, the polypeptide specific for the binding site was not identified here. Protease treatment of the PS II particles suggested that the 33-kDa polypeptide associated with the 43-kDa component of the reaction center complex [19]. However, this result does not exclude the possibility of an association of the 33-kDa polypeptide with a component other than the 43-kDa protein of the reaction center complex because the binding site of the 33-kDa polypeptide on a component other than the 43-kDa protein, if any, would not always be sensitive to trypsin or chymotrypsin used in the experiments. Thus, the precise location of the binding site of the 33-kDa polypeptide or the site of oxygen evolution in PS II has yet to be determined.

A more detailed analysis of the localization of the oxygen-evolving site is now in progress with the use of peripheral-polypeptide affinity chromatography.

ACKNOWLEDGEMENTS

The authors would like to thank Ms F. Kubota for preparation of the 33-kDa polypeptide and Mr Y. Onda for technical assistance. This study was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

REFERENCES

[1] Bouges-Bocquet, B. (1980) Biochim. Biophys. Acta 594, 85–103.
[2] Satoh, K. (1985) Photochem. Photobiol. 42, 845–853.
[3] Widger, W.R., Cramer, W.A., Hermodson, M. and Herrmann, R.G. (1985) FEBS Lett. 191, 186–190.
[4] Tang, X.-S. and Satoh, K. (1985) FEBS Lett. 179, 60–64.
[5] Ikeuchi, M., Yuasa, M. and Inoue, N. (1985) FEBS Lett. 185, 316–322.
[6] Ghanotakis, D.F., Demetriou, D.M. and Yocum, C.F. (1987) Biochim. Biophys. Acta 891, 15–21.
[7] Miyao, M. and Murata, N. (1984) FEBS Lett. 170, 350–354.
[8] Ono, T. and Inoue, Y. (1984) FEBS Lett. 168, 281–286.
[9] Yamamoto, Y., Shinkai, H., Isogai, Y. and Nishimura, M. (1984) FEBS Lett. 175, 429–432.
[10] Ono, T. and Inoue, Y. (1983) FEBS Lett. 164, 255–260.
[11] Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
[12] Yamamoto, Y., Hermodson, M.A. and Krogmann, D.W. (1986) FEBS Lett. 195, 155–158.
[13] Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
[14] Chua, N.H. (1980) Methods Enzymol. 69, 434–446.
[15] Klimov, V.V., Klevanik, A.V., Shuvalov, V.A. and Krasnovsky, A.A. (1977) FEBS Lett. 82, 183–186.
[16] Satoh, K., Nakatani, H.Y., Steinback, K.E. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 142–150.
[17] Satoh, K., Fujii, Y., Aoshima, T. and Tado, T. (1987) FEBS Lett. 216, 7–10.
[18] Brudvig, G.W., Worland, S.T. and Sauer, K. (1983) Proc. Natl. Acad. Sci. USA 80, 683–686.
[19] Isogai, Y., Yamamoto, Y. and Nishimura, M. (1985) FEBS Lett. 187, 240–244.