Biochimica et Biophysica Acta 807 (1985) 64–73
Elsevier

BBA 41723

Properties of a peripheral 34 kDa protein in *Synechococcus vulcanus* Photosystem II particles. Its exchangeability with spinach 33 kDa protein in reconstitution of O_2 evolution

Hiroyuki Koike and Yorinao Inoue

Solar Energy Research Group, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01 (Japan)

(Received October 22nd, 1984)

Key words: Oxygen evolution; Photosystem II; (*Synechococcus*)

Photosystem II (PS II) particles retaining a high rate of O_2 evolution were prepared from a thermophilic cyanobacterium, *Synechococcus vulcanus* Copeland, and the composition and properties of their peripheral proteins were investigated. The following results were obtained. (1) The O_2-evolving PS II particles of *S. vulcanus* contained only one peripheral protein with a molecular mass of 34 000 which corresponded to the 33 kDa protein in higher plant PS II particles, but no other peripheral proteins corresponding to the 24 and 16 kDa proteins of higher plant PS II particles. (2) The cyanobacterial peripheral 34 kDa protein was removed from the particles by 1 M $CaCl_2$-washing concomitant with total inactivation of O_2 evolution, and the inactivated O_2 evolution was reconstituted to 75% of the original activity by rebinding of this protein back to the washed particles. (3) The cyanobacterial peripheral 34 kDa protein rebound to $CaCl_2$-washed spinach PS II particles and restored O_2 evolution to an appreciable extent (28%). (4) The spinach peripheral 33 kDa protein rebound to $CaCl_2$-washed PS II particles of *S. vulcanus* and partially restored O_2 evolution (60%). These results suggested that the peripheral 34 kDa protein of *S. vulcanus* possesses the determinants for both binding and activity reconstitution identical with those of the peripheral 33 kDa protein of spinach.

Introduction

Based on many disintegration and reconstitution experiments using spinach O_2-evolving PS II particles, it has been revealed that three peripheral proteins with molecular masses of 33, 24 and 16 kDa are involved in photosynthetic O_2 evolution [1–8]. Of these three proteins, the 24 and 16 kDa proteins are released from the particles by NaCl washing concomitant with partial loss of O_2 evolution. The lost activity can be well restored by Cl^- and/or Ca^{2+} present in the reaction medium [5,8], so that these two proteins are considered to play a regulatory rather than catalytic role in O_2 evolution. The 33 kDa protein, on the other hand, has been shown to be removed by $CaCl_2$-washing concomitant with total loss of O_2 evolution [7,9]. Even if the 33 kDa protein is removed, the $CaCl_2$-washed particles preserve Mn atoms being still associated with the particles, and the lost O_2 evolution can be significantly restored by rebinding of the 33 kDa protein [9]. Thus we assumed that the 33 kDa protein functions in maintaining the Mn atoms at a proper configuration in the O_2-evolving center. Similar particles were also prepared by Miyao and

Abbreviations: PS I and II, Photosystem I and II; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DCIP, 2,6-dichlorophenolindophenol; DMQ, 2,5-dimethylbenzoquinone; LDAO, lauryldimethylamine *N*-oxide.

Murata [10] by combination of NaCl wash and urea treatment, by which they showed that a high concentration of Cl^- is effective in keeping Mn associated with the particles in the absence of 33 kDa protein.

In spite of these data rapidly accumulating as to the O_2-evolving system of higher plants, the knowledge about the O_2-evolving system of other photosynthetic organisms is much limited. Even though highly active O_2-evolving PS II particles have recently been prepared from several cyanobacteria [11–14], only a little characterization as to the protein composition and the functional role of the proteins has so far been reported [13,14]. In the present communication, we report the protein composition of the PS II particles prepared from a thermophilic cyanobacterium, *Synechococcus vulcanus* Copeland, in comparison with that of spinach PS II particles and, in particular, the function of a peripheral 34 kDa protein studied by cross-exchange experiments with the spinach 33 kDa protein and spinach PS II particles.

Experimental

O_2-evolving PS II particles of *S. vulcanus* were prepared by the procedures as described by Stewart and Bendall [11] with slight modifications. Thylakoids of *S. vulcanus* were treated twice with 0.8% LDAO (LDAO/Chl = 4 : 1 (w/v)), and the solubilized thylakoids (supernatant of 100 000 × *g*, 1 h centrifugation) was layered on top of a sucrose gradient (5–17.5% (w/v)) containing 20% glycerol (v/v), 5 mM $MgCl_2$, 20 mM Hepes-NaOH (pH 7.0) and 0.05% LDAO (w/w). The gradient was centrifuged at 100 000 × *g* for 36–40 h and then the separated bands were collected either by use of a syringe or a fractionator as described in [15]. The PS II particles thus obtained showed a high activity of O_2 evolution (10 000–20 000 e^- μequiv. per mg Chl/h, ferricyanide photoreduction with H_2O as electron donor) at 50°C. Spinach PS II particles were obtained by the procedure of Berthold et al. [16] except that the second Triton treatment was omitted [7,17].

SDS-polyacrylamide gel electrophoresis was performed with the buffer system of Chua and Bennoun [18] containing 6 M urea as described in [7]. Two dimensional gel electrophoresis was performed by the procedure of O'Farrel [19] with isoelectric focusing electrophoresis (pH 3.5–10) in the first dimension and SDS polyacrylamide gradient gel electrophoresis (10–17.5%) in the second dimension. Samples for the 2-dimensional electrophoresis were prepared as described by Ikeuchi and Murakami [20].

The reconstitution experiments were done according to the procedure described by Ono and Inoue [9] for spinach PS II particles. *S. vulcanus* PS II particles were suspended with a buffer solution containing 1 M $CaCl_2$/10% glycerol (v/v)/20 mM Hepes-NaOH (pH 7.0), incubated on ice for 30 min in the dark, and then the particles were separated from the extract by centrifugation at 300 000 × *g* for 1.5 h. The particles thus obtained (denoted as $CaCl_2$-washed particles) were resuspended in the same buffer ($CaCl_2$ concentration was lowered to 50 mM) after one wash in the same buffer. The $CaCl_2$-extract (supernatant) was concentrated in an Amicon Diaflo cell with a PM-10 membrane filter. Various amounts of the concentrated $CaCl_2$-extract were added to the suspension of $CaCl_2$-washed particles and the mixture was incubated in darkness on ice for 1 h to rebind the extracted proteins back to the particles. The reconstituted particles were collected, washed twice with the same buffer, resuspended in 25% glycerol (v/v)/20 mM Hepes-NaOH (pH 7.0)/10 mM $MgCl_2$, and then the O_2-evolving activity and the amount of proteins rebound were determined.

O_2-evolving activities were measured spectrophotometrically by ferricyanide or DCIP photoreduction with H_2O as electron donor as described in Refs. 7 and 15. The relative amount of 34 (or 33) kDa protein rebound was assayed by densitometry of an SDS polyacrylamide gel electrophoresis gel stained with 0.1% Coomasie brilliant blue R-250. Chlorophyll concentration was determined by the method of Arnon [21] for spinach particles in 80% acetone extract, and by the method of Mackinney [22] for *S. vulcanus* particles in 100% methanol extract.

Results and Discussion

Protein composition of S. vulcanus PS II particles

As reported previously [15], the conditions of LDAO treatment affected greatly the ratio of

solubilization of PS I and PS II from the thylakoids of the alga. The two step LDAO treatment used in the present study solubilized almost all PS II of *S. vulcanus* thylakoids, while only 5% of PS I, so that the supernatant after the LDAO treatment was rich in PS II by a factor of 20 as compared with the original thylakoids.

Sucrose density gradient centrifugation of the LDAO-extract thus obtained yielded four colored bands. Fig. 1 shows a typical fractionation profile of those bands. The first (top) band mainly contained detergent-solubilized carotenoids with a small amount of chlorophyll. The second and third bands contained phycocyanin and allophycocyanin, respectively, and PS I was enriched in the third band. The fourth (bottom) band contained a large amount of chlorophyll and was rich in PS II. The bottom band was collected and centrifuged at 300 000 × *g* for 5 h to pellet the PS II particles. The PS II particles thus obtained evolved O_2 at a high rate of 2430 and 780 μmol O_2/mg Chl per h at 48 and 24°C, respectively, indicating that the particles still retained the thermophilic characteristics. Contamination of P-700 in the PS II particles was less than 1/1500 on a chlorophyll basis.

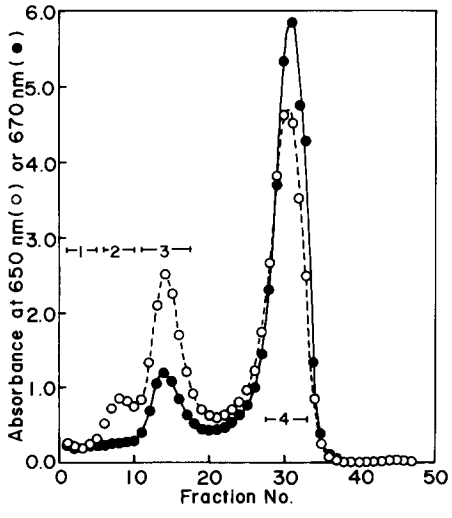

Fig. 1. Fractionation profile of sucrose density gradient centrifugation of LDAO-solubilized thylakoids of *S. vulcanus*. Solid and open circles are the absorbances at 670 and 650 nm for chlorophyll *a* and phycobilins, respectively.

The protein composition of the PS II particles was analyzed by SDS polyacrylamide gel electrophoresis. As shown by the densitogram in Fig. 2a, the particles contained about 12 proteins. By referring to the protein composition of the PS II reaction center complex of higher plants purified by Satoh [23,24], the four proteins with molecular masses of 47, 43, 32 and 30 kDa were assigned to the components of PS II core complex: the 47 and 43 kDa proteins are the two chlorophyll-binding proteins, the 30 kDa protein is the quinone (herbicide)-binding unstainable protein and the 32 kDa protein is the nonchlorophyll-containing intrinsic protein whose function remains unknown. The 65 kDa peak is probably a contribution by aggregation of 47 and 43 kDa proteins [25] judging from its behavior on SDS polyacrylamide gel electrophoresis: its height changes depending on pretreatment conditions. The large bands in 15–17 kDa region were colored (blue) and assigned to the subunits of phycocyanin and allophycocyanin (see later). The 34 kDa protein was assigned to a protein equivalent to the peripheral 33 kDa protein (Kuwabara-Murata's protein [26,27]) of spinach PS II particles as will be discussed later.

A remarkable feature of the protein composition of the O_2-evolving PS II particles from *S. vulcanus* is that they did not contain any protein corresponding to the peripheral 24 kDa protein found in higher plant PS II particles. The densitogram in Fig. 2a shows a small peak in 25 kDa region, but this protein was shown to be an intrinsic protein as discussed later. Since the 24 kDa protein is well known to be released by various washing treatments [1–6], we examined the effect of Tris-, $CaCl_2$- and NaCl-washing expecting to find the protein in the extract. On washing the *S. vulcanus* PS II particles with 0.8 M Tris-HCl (pH 8.4), 10 proteins were found in the extract (Fig. 2c). The 34 kDa protein was completely extracted by this treatment, but no protein could be found in 24 kDa region. The 25 kDa peak in Fig. 2a could not be removed, but remained in the residual particles (Fig. 2b). 1 M $CaCl_2$-extract also showed a profile with no peak in 24 kDa region (Fig. 2e), which was more or less the same as Tris-extract except that $CaCl_2$-extract contained a larger amount of phycocyanin and allophycocyanin. 1.5 M NaCl-extract contained an appre-

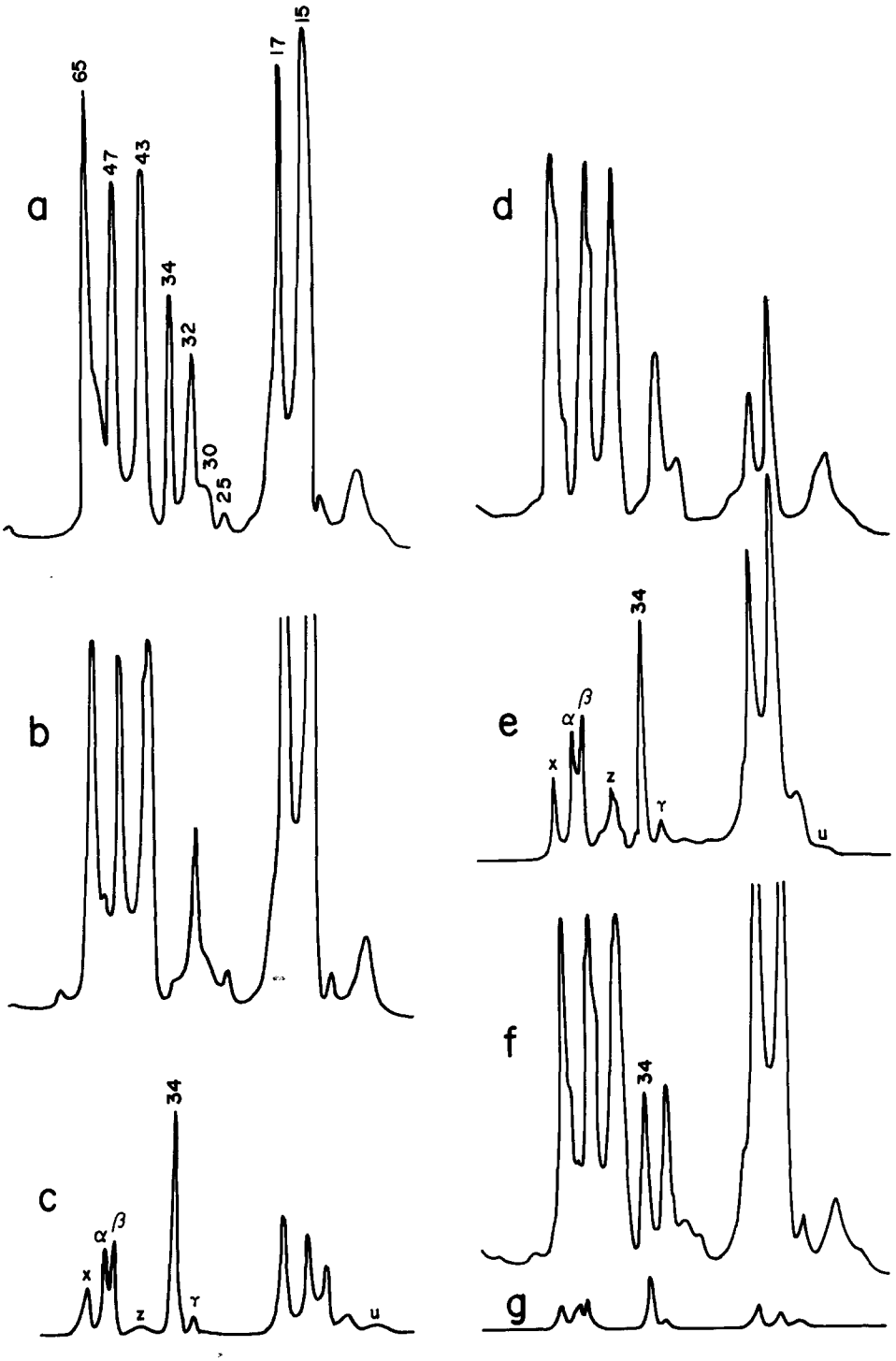

Fig. 2. SDS polyacrylamide gel electrophoresis profiles of *S. vulcanus* PS II particles before and after washing with various salt solutions (a) Control PS II particles; (b) 0.8 M Tris (pH 8.4)-washed particles; (c) Tris-extract; (d) 1 M $CaCl_2$-washed particles: (e) $CaCl_2$-extract; (f) 1.5 M NaCl-washed particles; (g) NaCl-extract.

ciable amount of 34 kDa protein but no protein could be found in 24 kDa region (Fig. 2g).

When the Tris-extract of *S. vulcanus* PS II particles was subjected to O'Farrel's 2-dimensional gel electrophoresis, the map depicted in Fig. 3A was obtained. Based on the isoelectric points determined on this map and also on the molecular masses determined on Fig. 2c profile, we could assign the two spots in 57–54 kDa region and a small spot in 33 kDa region to α, β and γ subunits, respectively, of the coupling factor [28,29], the four spots in 15–17 kDa region to α and β subunits of phycocyanin and allophycocyanin [30]. The 34 kDa protein showed an isoelectric point at pH 5.2, which is identical with the isoelectric point of spinach peripheral 33 kDa protein [26,31,32]. Besides these proteins, three unidentified proteins denoted as X, Y and Z were focused at an alkaline isoelectric point of pH 9, and another unidentified protein (U) at an acidic pH of 5. X, Z and U were resolved in Fig. 2c profile, while Y superimposed on the subunit of the coupling factor.

When the same Tris-extract was co-electrophoresed with the Tris-extract of spinach PS II particles, the map depicted in Fig. 3B was obtained. The Tris-extract of spinach PS II particles contained three proteins (33, 24 and 16 kDa) which yielded spots at pH 5.1, 6.5 and 9.3, respectively. As mentioned above, the 34 kDa protein of *S. vulcanus* yielded a spot at an isoelectric point (pH 5.2) almost identical with that of spinach 33 kDa protein. Judging from the release pattern on washing with various salt solutions (Fig. 2) and also from the similarities in molecular mass and isoelectric point, we can conclude that the 34 kDa protein of *S. vulcanus* is a protein equivalent to the 33 kDa peripheral protein of spinach. No protein, however, could be found in the area near the 24 or 16 kDa proteins. By comparing the two maps, Fig. 3A and B, we conclude that *S. vulcanus* PS II particles contain a protein corresponding to the spinach 33 kDa peripheral protein, but no proteins corresponding to the spinach 24 and 16 kDa peripheral proteins. This is consistent with the previous suggestion by Bowes et al. for the PS II preparation from a thermophilic cyanobacterium, *Phormidium laminosum* [14].

These results may not always rule out the possibility that the two peripheral proteins corresponding to spinach 24 and 16 kDa proteins do not exist in the cells of this alga, since they could have been removed out of the PS II particles

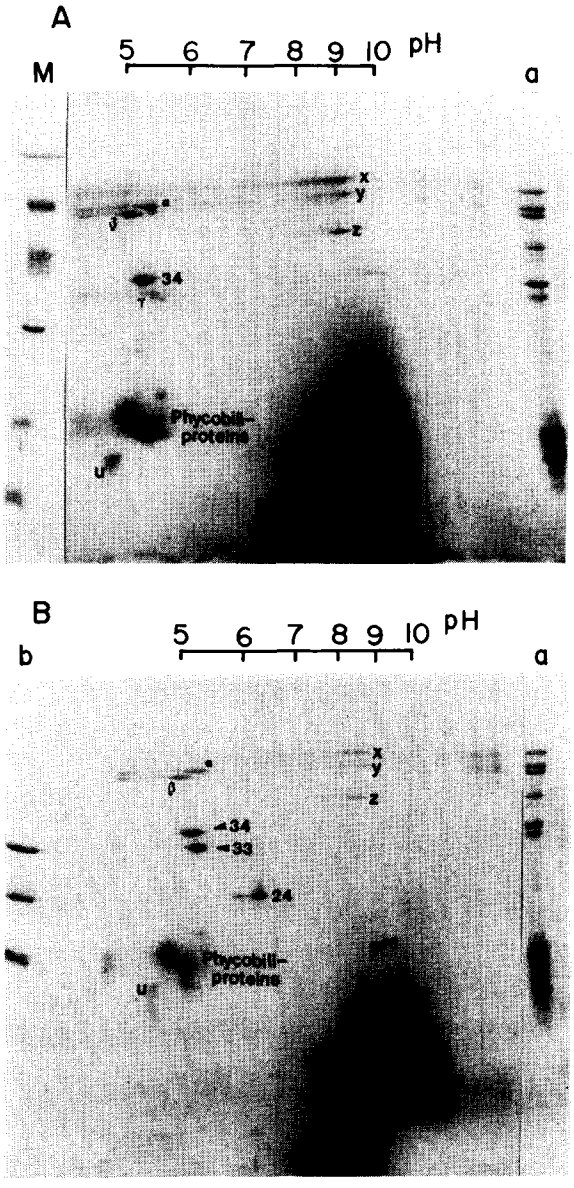

Fig. 3. 2-Dimensional electrophoretogram of Tris-extract of *S. vulcanus* PS II particles (A) and that of the same sample coelectrophoresed with Tris-extract of spinach PS II particles (B). Samples were subjected to isoelectric focusing (pH 3.5–10) in the first dimension and then to SDS polyacrylamide gradient gel electrophoresis (10–17.5%) in the second dimension. (M) Molecular-weight markers; (a) Tris-extract of *S. vulcanus* PS II particles; (b) Tris-extract of spinach PS II particles. These samples were electrophoresed only in the second dimension.

during the LDAO treatments. We attempted to detect these proteins, particularly the 24 kDa protein, in the LDAO-extract by means of both rebinding to the PS II particles (SDS polyacrylamide gel electrophoresis) and enhancement of O_2-evolving activity which would accompany the rebinding. However, no indication supporting the existence of these proteins has so far been obtained.

Exchangeability between S. vulcanus 34 kDa protein and spinach 33 kDa protein in reconstitution of O_2 evolution

Ono and Inoue [7,9] reported that 1 M $CaCl_2$-washing of spinach PS II particles results in extraction of 33, 24 and 16 kDa proteins concomitant with complete loss of O_2 evolution, while leaving all Mn atoms associated with the particles, and that the inhibited O_2 evolution can be restored by rebinding of the 33 kDa protein on readdition of $CaCl_2$-extract to the washed particles. When the *S. vulcanus* PS II particles were washed with 1 M $CaCl_2$, the O_2-evolving activity with ferricyanide as electron acceptor was completely inhibited as in spinach PS II particles: the activities measured at the optimum temperature (48°C) before and after the $CaCl_2$-wash were 11 500 and 370 e^- μequiv./mg Chl per h, respectively. When the $CaCl_2$-extract was concentrated by ultrafiltration and readded to the washed *S. vulcanus* PS II particles, the 34 kDa protein rebound to the particles concomitant with significant restoration of O_2 evolution. As shown by the electrophoretograms in Fig. 4A, however, not only the 34 kDa protein, but also several other proteins rebound to the washed particles: phycobiliproteins in 15–18 kDa region, coupling factor as indicated by α, β and γ subunits, an unidentified protein X previously mentioned, and a protein with an approximate molecular mass of 100 000, which is similar in molecular mass to the so-called anchor protein reported by Redlinger and Gantt [33]. The content of the 100 kDa protein in PS II particles varied greatly depending on

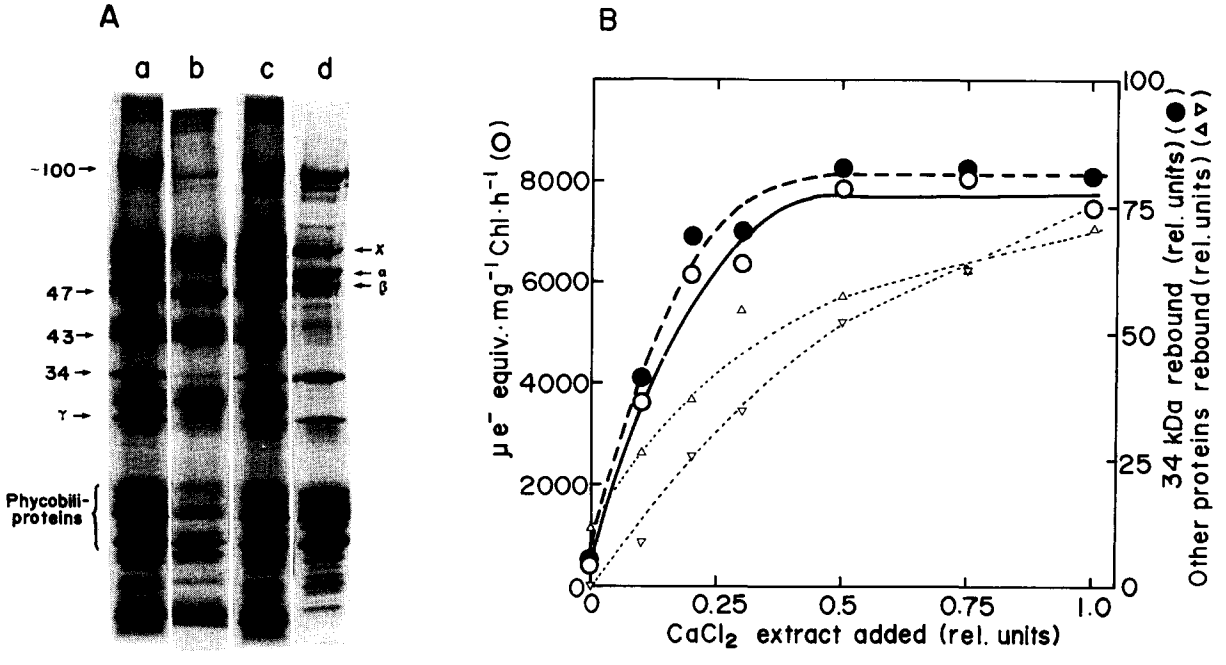

Fig. 4. Reconstitution between *S. vulcanus* 34 kDa protein and $CaCl_2$-washed *S. vulcanus* PS II particles. (A) SDS polyacrylamide gel electrophoresis profiles before and after reconstitution. (a) Control PS II particles; (b) $CaCl_2$-washed particles; (c) $CaCl_2$-washed and then reconstituted particles; (d) $CaCl_2$-extract. (B) Activity restoration titrated against the amount of $CaCl_2$-extract readded. O_2 evolution was measured at 48°C by ferricyanide photoreduction with H_2O as electron donor. Solid and open circles are the amounts of 34 kDa protein rebound and the activity restored, respectively. Normal and inverted triangles are the rebound amounts of the 100 kDa protein and coupling factor, respectively.

the sample, almost absent in the samples used for Figs. 2 and 6 experiments but present at a considerable amount in the samples for this and Fig. 5A, lane d experiments.

In order to confirm that the activity restoration is caused by rebinding of the 34 kDa protein but not by rebinding of the other proteins, a titration experiment shown in Fig. 4B was done, in which the extent of activity restoration and the amounts of the proteins rebound were plotted against the amount of $CaCl_2$-extract readded. As shown by the solid curve with open circles, the activity increased with increasing the readded amount of $CaCl_2$-extract to reach a saturation level. The rebound amounts of all the proteins capable of rebinding to the particles also increased with increasing the amount of $CaCl_2$-extract, but the amount of the 34 kDa protein (broken curve with solid circles) showed the best correlation with activity restoration: the rebound amount of the 34 kDa protein increased to reach saturation almost in parallel with the activity, while those of the coupling factor (▽) and the 100 kDa protein (△) continued increasing after the activity restoration reached saturation. The maximal extent of activity restoration in this reconstitution system was 8000 e^- μequiv./mg Chl per h (at 48°C), which was 75–80% of the activity of the orginal PS II particles. These results indicate that 1 M $CaCl_2$-washing can be applied to the O_2-evolving system of cyanobacteria and confirm that the 34 kDa protein of *S. vulcanus* functions similarly to the 33 kDa protein of spinach.

In order to further confirm the functional role of *S. vulcanus* 34 kDa protein, we investigated the exchangeability between this protein and the 33 kDa protein of spinach. We first tried a reconstitution experiment between $CaCl_2$-extract of *S. vulcanus* PS II particles and $CaCl_2$-washed spinach PS II particles. Fig. 5A shows the SDS poly-

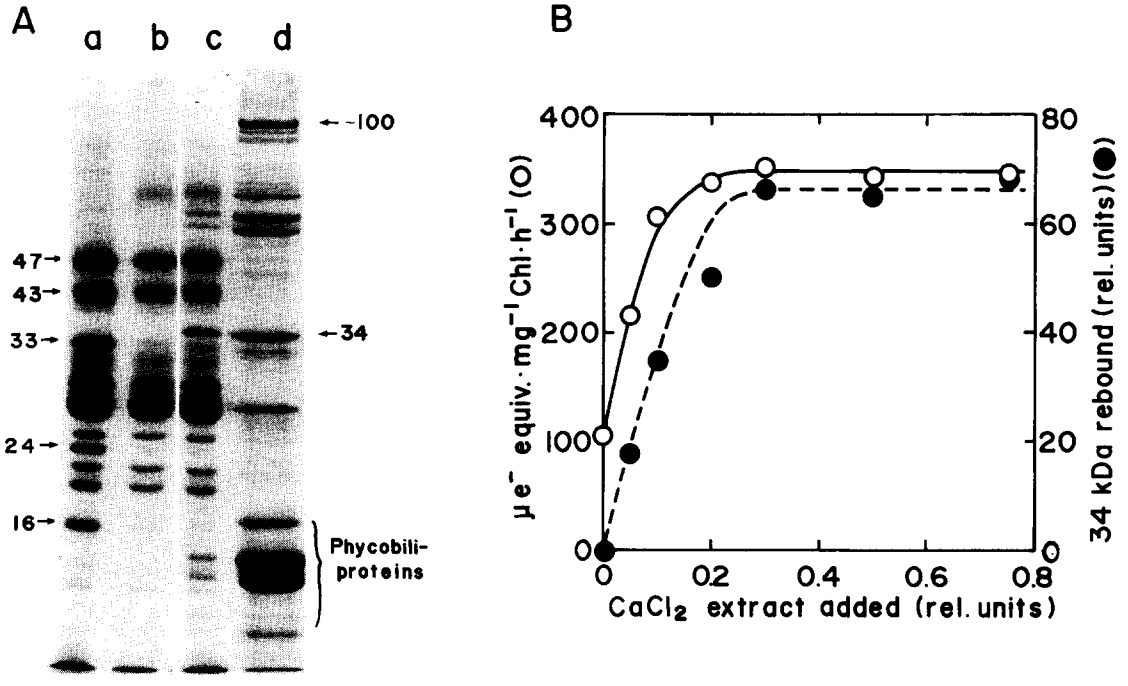

Fig. 5. Reconstitution between *S. vulcanus* 34 kDa protein and $CaCl_2$-washed spinach PS II particles. (A) SDS polyacrylamide gel electrophoresis profiles before and after reconstitution. (a) control spinach PS II particles; (b) $CaCl_2$-washed spinach PS II particles; (c) spinach PS II particles reconstituted with *S. vulcanus* 34 kDa protein; (d) $CaCl_2$-extract of *S. vulcanus* PS II particles. (B) Activity reconstitution titrated against the amount of *S. vulcanus* $CaCl_2$ extract added. O_2 evolution was measured at 23°C by DCIP photoreduction with H_2O as electron donor. Solid and open circles are the amounts of 34 kDa protein rebound and the activity restored, respectively.

acrylamide gel electrophoresis analysis of the protein compositoin of the spinach PS II particles before and after reconstitution. By $CaCl_2$-washing, the 33 kDa protein as well as 24 and 16 kDa proteins was completely removed from the spinach PS II particles (Fig. 5A, lanes a and b). On reconstitution, the 34 kDa protein of *S. vulcanus* rebound to the spinach PS II particles, and the profile of the reconstituted particles showed the protein at a position with a mobility slightly lower than that of spinach 33 kDa protein (Fig. 5A, lane c). It is of note that among a variety of proteins found in *S. vulcanus* $CaCl_2$-extract, the 34 kDa protein is the only main protein which rebound to the spinach PS II particles (Fig. 5A, lanes c and d): the amount of other proteins rebound, e.g., coupling factor around 47 kDa and phycobilins around 16 kDa, was far smaller. This suggests that the 34 kDa protein of *S. vulcanus* possesses the binding determinants similar to those of spinach 33 kDa protein.

Fig. 5B shows the activity restoration in this reconstitution system. The O_2 evolution as measured by DCIP photoreduction with H_2O as donor was 1230 and 100 e^- μequiv. per mg Chl/h before and after $CaCl_2$ washing, respectively, and the inhibition extent was about 92%. This inhibition extent was not as high as that measured in terms of O_2 evolution with DMQ as electron acceptor *, but we had to use spectrophotometric measurements because of limitation in the amount of $CaCl_2$-extract available from *S. vulcanus* PS II particles. The activity increased with increasing the amount of $CaCl_2$-extract added and reached a saturation level. This course was in good correlation with the amount of 34 kDa protein rebound to spinach PS II particles. The maximal extent of activity restoration was 350 e^- μequiv. per mg Chl/h. The extent of reconstitution (28%) was about half of the activity reconstitution previously reported for spinach PS II particles [9] *. The results indicate that the 34 kDa protein of *S. vulcanus* is effective in reconstituting the O_2-evolving activity of spinach PS II particles.

Fig. 6 shows the results of another reconstitution experiment with different combination, in which the spinach $CaCl_2$-extract was added to the $CaCl_2$-washed *S. vulcanus* PS II particles. As shown by the electrophoretogram (Fig. 6A, lanes a and b), *S. vulcanus* PS II particles contained an intrinsic 32 kDa protein. Since the mobility of this protein was almost the same as that of spinach 33 kDa protein, it was difficult to detect the rebinding of 33 kDa protein on the densitogram of reconstituted particles. However, when the reconstituted particles were again extracted with 0.8 M Tris-HCl (pH 8.4), we could clearly observe the 33 kDa protein in the extract (Fig. 6A, lane d), since the 32 kDa protein was resistant to both $CaCl_2$- and Tris-washing. It is of note that the Tris-extract contained two more proteins in 16–18 kDa region, but they were assigned to phycobiliproteins which partly remained in the particles after $CaCl_2$-washing and were partly extracted by Tris-washing of the reconstituted particles. It is also of note that neither the 24 nor the 16 kDa proteins of spinach rebound to *S. vulcanus* PS II particles. Thus, by Tris-washing of the reconstituted particles, we could confirm the rebinding of spinach 33 kDa protein to *S. vulcanus* PS II particles and also could estimate the amount of this protein rebound to the particles.

The activity reconstitution in *S. vulcanus* PS II particles versus spinach 33 kDa protein system is shown in Fig. 6B. In this experiment, the activity was measured at 23°C, since the reconstitution system involved a nonthermophilic protein, the 33 kDa protein of spinach. The *S. vulcanus* PS II particles used for this experiment showed an O_2-evolving activity of 18 200 e^- μequiv. per mg Chl/h as measured by ferricyanide photoreduction at 48°C, which was inactivated to 870 e^- μequiv. per mg Chl/h (4.8%) by $CaCl_2$ washing. On lowering the temperature to 23°C, the activity of non-treated particles was lowered to 4640 e^- μequiv. per mg Chl/h, while the residual activity observed after $CaCl_2$-washing did not show temperature dependence, so that the apparent inhibition extent by the $CaCl_2$-washing at 23°C was as low as 82%. At present, we cannot give any reasonable interpretation for this phenomenon. As shown by the two

* The extent of inhibition by $CaCl_2$-washing and that of restoration by reconstitution usually depend on the way of activity measurement. When measured by O_2 evolution with DMQ as electron acceptor, the extents of inhibition and restoration were 98% and 28%, respectively, but when measured by DCIP photoreduction with H_2O as electron donor, they were 93% and 59%, respectively [9].

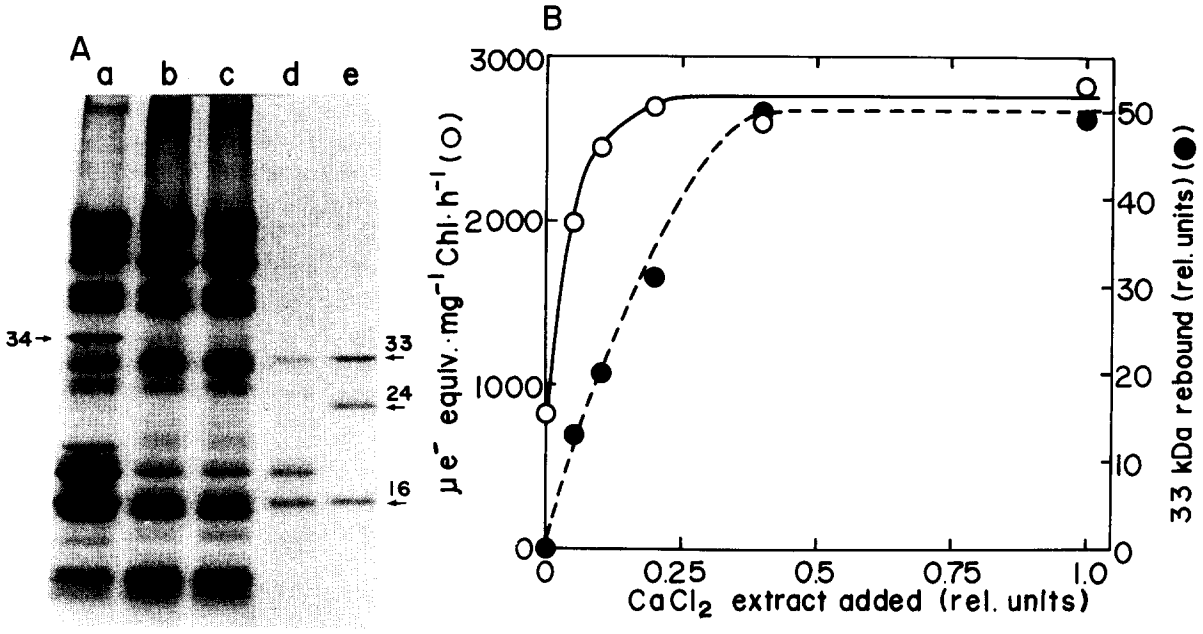

Fig. 6. Reconstitution between spinach 33 kDa protein and $CaCl_2$-washed *S. vulcanus* PS II particles. (A) SDS polyacrylamide gel electrophoresis profiles before and after reconstitution. (a) Control *S. vulcanus* PS II particles; (b) $CaCl_2$-washed *S. vulcanus* PS II particles; (c) *S. vulcanus* PS II particles reconstituted with spinach 33 kDa protein; (d) 0.8 M Tris-extract of the reconstituted particles; (e) $CaCl_2$-extract of spinach PS II particles. (B) Activity restoration titrated against the amount of spinach $CaCl_2$-extract added. O_2 evolution was measured at 23°C as ferricyanide photoreduction with H_2O as electron donor. Solid and open circles are the amounts of 33 kDa protein rebound and the activity restored, respectively.

curves in Fig. 6B, the extent of activity restoration increased with increasing the amount of $CaCl_2$-extract of spinach PS II particles. However, the activity restoration was not always in good correlation with the amount of 33 kDa protein rebound: the activity reached saturation, while the rebinding amount was still increasing. This would be due to either an incomplete matching, an unbalanced ability of the cyanobacterial protein in activity restoration and rebinding in the spinach system, or partial denaturation of the protein which would have caused preferential loss of activity-restoring ability, while retaining the rebinding ability. In spite of this incomplete correlation, the results depicted in Fig. 6 show that spinach 33 kDa protein is effective in restoring the O_2 evolution of $CaCl_2$-washed *S. vulcanus* PS II particles. The reconstituted particles did not show any activity at 48°C. Probably, the 33 kDa protein will loose its function or will be released from the particles at such a high temperature.

Concluding remarks

The present study indicated that the 33 kDa protein of spinach and the 34 kDa protein of *S. vulcanus* are cross-reactive: *S. vulcanus* 34 kDa protein is capable of binding to spinach PS II particles, and vice versa, as well as to its own original counterpart, and every combination of rebinding results in significant restoration of O_2 evolution. These results suggest that the determinants for both binding and activity restoration are common between the two peripheral proteins. Probably the structure and function of these proteins have been conserved during the evolutionary process from Cyanophyta to Tracheophyta.

Acknowledgments

We are grateful to Drs. T. Ono and M. Ikeuchi for their valuable discussions and kind advices. This work was supported by a research grant on

Solar Energy Conversion by Means of Photosynthesis given by Science and Technology Agency of Japan (STA) to The Institute of Physical and Chemical Research (RIKEN), and partly by Grants-in-Aid (58340037, 58380029) from the Ministry of Education, Science and Culture (MESC).

References

1 Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 533–539
2 Kuwabara, T. and Murata, N. (1983) Plant Cell Physiol. 24, 741–747
3 Åkerlund, H.-E. and Jansson, C. (1981) FEBS Lett. 124, 229–232
4 Åkerlund, H.-E., Jansson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 68, 1–10
5 Miyao, M. and Murata, N. (1984) FEBS Lett. 168, 118–120
6 Yamamoto, Y., Doi, M., Tamura, N. and Nishimura, M. (1981) FEBS Lett. 133, 265–268
7 Ono, T. and Inoue, Y. (1983) FEBS Lett. 163, 255–260
8 Ghanotakis, D.F., Jopper, J.N., Babcock, G.T. and Yocum, C.F. (1984) FEBS Lett. 170, 169–173
9 Ono, T. and Inoue, Y. (1983) FEBS Lett. 166, 381–384
10 Miyao, M. and Murata, N. (1984) FEBS Lett. 170, 350–354
11 Stewart, A.C. and Bendall, D.S. (1979) FEBS Lett. 107, 308–312
12 England, R.R. and Evans, E.H. (1981) FEBS Lett. 134, 175–177
13 Ke, B., Inoué, H., Babcock, G.T., Fang, Z. and Dolan, E. (1982) Biochim. Biophys. Acta 682, 297–306
14 Bowes, J.M., Stewart, A.C. and Bendall, D.S. (1983) Biochim. Biophys. Acta 725, 210–219
15 Koike, H. and Inoue, Y. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 257–263, Academic Press Japan, Tokyo
16 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234
17 Yuasa, M., Koike, H. and Inoue, Y. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 103–112, Academic Press Japan, Tokyo
18 Chua, N.-H. and Bennoun, P. (1975) Proc. Natl. Acad. Sci. USA 72, 2175–2179
19 O'Farrel, P.H. (1975) J. Biol. Chem. 250, 4007–4021
20 Ikeuchi, M. and Murakami, S. (1982) Plant Cell Physiol. 23, 575–583
21 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
22 Mackinney, G. (1941) J. Biol. Chem. 140, 315–322
23 Satoh, K. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 27–38, Academic Press Japan, Tokyo
24 Satoh, K., Nakatani, H.Y., Steinback, K.E., Watson, J. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 142–150
25 Tang, X.-S. and Satoh, K. (1984) Plant Cell Physiol. 25, 935–946
26 Kuwabara, T. and Murata, N. (1979) Biochim. Biophys. Acta 581, 228–236
27 Kuwabara, T. and Murata, N. (1982) Biochim. Biophys. Acta 680, 210–215
28 Kwanyen, P. and Wildman, S.G. (1978) Biochim. Biophys. Acta 502, 269–275
29 Roscoe, T.J. and Ellis, R.J. (1982) in Methods in Chloroplast Molecular Biology (Edelman, M., Hallick, R.B. and Chua, N.-H. eds.), pp. 1015–1028, Elsevier Biomedical Press, Amsterdam
30 Glazer, A.N. and Cohen-Bazire, G. (1971) Proc. Natl. Acad. Sci. USA 68, 1398–1401
31 Yamamoto, Y., Shimada, S. and Nishimura, M. (1983), FEBS Lett. 151, 49–53
32 Kuwabara, T. and Murata, N. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 223–228, Academic Press Japan, Tokyo.
33 Redlinger, T. and Gantt, E. (1981) Plant Physiol. 68, 1375–1378