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A PHOTOACTIVE PHOTOSYSTEM-II REACTION-CENTER COMPLEX LACKING A CHLOROPHYLL-BINDING 40 KILODALTON SUBUNIT FROM THE THERMOPHILIC CYANOBACTERIUM *SYNECHOCOCCUS* SP.

AKIHIKO YAMAGISHI and SAKAE KATOH

Department of Pure and Applied Sciences, College of Arts and Sciences, University of Tokyo, Komaba, Meguro-ku, Tokyo 153 (Japan)

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The Photosystem-II reaction-center complex of the thermophilic cyanobacterium *Synechococcus* sp. was resolved into two complemental chlorophyll-protein complexes, CP2b which contained a chlorophyll-binding 47 kDa polypeptide, two polypeptides in the 28–31 kDa region and a 9 kDa polypeptide, and CP2c which had only a chlorophyll-binding 40 kDa polypeptide. CP2b was found to be highly active in photoreduction of 2,6-dichlorophenolindophenol with diphenylcarbazide as an electron donor. The activity was insensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea and ioxynil but was half inactivated by the treatment of the complex at 50°C for 5 min, or on addition of 0.001% sodium dodecyl sulfate, indicating its dependence on the protein conformation. CP2c also showed a low activity of the dye photoreduction, which was insensitive to heat and enhanced at high concentrations of sodium dodecyl sulfate. The quantum yield of the photoreduction was estimated to be 0.12 for CP2b and 0.002 for CP2c. It is concluded that the 47 kDa polypeptide is the site of the Photosystem-II reaction center and the 40 kDa polypeptide is not required for the Photosystem-II-driven electron transport.

Introduction

The conversion of absorbed-light energy to chemical energy in photosynthesis takes place in the reaction center associated with specific chlorophyll-protein complexes. Satoh [1–3], among others, has highly purified and characterized the PS-II reaction-center complex from spinach, which consists of two chlorophyll-binding polypeptides of 47 and 43 kDa, a 30 kDa herbicide-binding polypeptide, a 6 kDa apoprotein of cytochrome *b*-559 and a 34 kDa polypeptide of unknown function.

Recently, we have isolated the PS-II reaction-center complex with a comparable polypeptide composition from the thermophilic cyanobacterium *Synechococcus* sp. [4]. SDS-polyacrylamide gel electrophoresis of the PS-II complex resolved a chorophyll-protein (CP2c), containing a 40 kDa polypeptide (equivalent to the 43 kDa polypeptide in higher plants), and three chlorophyll-protein complexes, containing a chlorophyll-binding 47 kDa polypeptide (CP2a, CP2b and CP2d). CP2b also had several colorless polypeptides. No photochemical activity of PS II was detected in any of isolated chlorophyll-protein complexes. At liquid nitrogen temperature, however, CP2a, CP2b and CP2d showed a fluorescence band at 694 nm, whereas CP2c emitted a band at

Abbreviations: PS II, Photosystem II; LDAO, lauryldimethylamine *N*-oxide; DCIP, 2,6-dichlorophenolindophenol; DPC, 1,5-diphenylcarbazide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Mes, 4-morpholineethanesulfonic acid; Hepes 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-glycine.

686 nm [4]. Separation of CP2c from the PS-II reaction-center complex with SDS resulted in a marked enhancement of the 686 nm band [5]. These observations strongly suggest that the 47 kDa subunit is the site of the primary photochemistry and the 40 kDa polypeptide carries the antenna of PS II.

Nakatani [6] also showed that the 47 kDa chlorophyll-protein (CP_{47}) separated from spinach PS II particles by SDS-gel electrophoresis emits a fluorescence band at 695 nm at liquid nitrogen temperature and shows small light-induced absorption changes which indicate the primary charge separation of PS II. Camm and Green [7] demonstrated a parallel distribution of the activity of DPC-dependent DCIP photoreduction and the 47 kDa polypeptide across a sucrose density gradient after centrifugation of octylglucoside extract of spinach chloroplasts.

In this communication, we report the isolation from *Synechococcus* thylakoid membranes of a PS-II reaction-center complex, which lacks the 40 kDa polypeptide but is highly active in DCIP photoreduction with DPC as an electron donor. The pH dependence, the sensitivity to heat and SDS as well as quantum yield of the DCIP photoreduction indicate that the activity is mediated by the PS-II reaction center. A chlorophyll-protein containing only the 40 kDa polypeptide was also isolated. A low activity associated with the chlorophyll-protein was ascribed to photochemistry of chlorophyll molecules not related to the PS-II reaction center.

Materials and Methods

Synechococcus sp. was grown at 55°C and the thylakoid membranes were prepared as described previously [4,8,9]. The procedures for preparation of the PS-II reaction-center complex described in Ref. 4 were considerably modified. The thylakoid membranes suspended in 50 mM Tris-HCl (pH 7.5) containing 10 mM NaCl (1 mg chlorophyll *a*/ml) were treated with 0.4% octylglucoside for 20 min at 0°C and pelleted by centrifugation at 250 000 × *g* for 20 min. The treatment removed extraneous proteins (mostly allophycocyanin) but no chlorophyll from the membranes. The washed membranes were suspended in the above Tris buffer containing 0.8% octylglucoside and left at 25°C for 30 min. The green supernatants obtained after centrifugation at 250 000 × *g* for 20 min were subjected to digitonin-polyacrylamide gel electrophoresis. The procedures for the electrophoresis and for the extraction of the PS-II complex from gels were as described previously [4].

To prepare the reaction center complex lacking the 40 kDa polypeptide, the membranes were incubated with 0.3% LDAO in the Tris buffer for 30 min at 0°C and centrifuged at 230 000 × *g* for 40 min. The supernatant, after addition of glycerol to a final concentration of 10%, was directly applied to a 9.2% polyacrylamide gel as described previously [4], except that 0.05% SDS was present in the reservoir buffer but not in the gel.

Polypeptide composition of chlorophyll-protein complexes were examined by SDS-polyacrylamide gel electrophoresis by the method of Laemmli [10] with some modifications [4]. Samples were treated with 2.5% SDS, 8 M urea and 5% 2-mercaptoethanol for 30 min at room temperature and run on 10–15% gradient gels containing 0.1% SDS and 6 M urea for 17 h. Gels were stained with Coomassie brilliant blue R250.

Photoreduction of DCIP was measured spectrophotometrically at 590 nm in a medium containing 50 mM Mes-NaOH (pH 6.0), 50 μM DCIP, 1 mM DPC and chlorophyll-protein complexes (2–10 μg chlorophyll/ml). The actinic illumination of 2.3 kW/m^2 was provided from a 300 W xenon lamp through a water layer (6 cm thick), a Toshiba VR-65 and a Hoya HA-50 filters. To measure the quantum yield of photoreduction, samples were irradiated with 680 nm light from a 100 W halogen lamp. An interference filter and a suitable combination of neutral density filters were used in addition to the above filter system. Light intensity was determined with a calibrated thermopile. All measurements were carried out at 25°C.

Results

The treatment of *Synechococcus* thylakoid membranes with 0.3% LDAO extracted considerable amounts of chlorophyll *a* in a state which could not be precipitated by centrifugation. Fig. 1 shows a typical densitometric tracing of chlorophyll bands resolved from the LDAO-extract after 3 h of electrophoresis on polyacrylamide gel with the re-

servoir buffer containing SDS. Three chlorophyll bands labelled A, B, and C were stained for protein and a band near the gel front contained phycobilins and free chlorophyll and carotenoids.

Chlorophyll-protein complexes resolved were extracted from gels and their polypeptide compositions were examined by SDS-polyacrylamide gel electrophoresis in the presence of PS-II reaction-center complex was shown in the upper trace. The PS-II complex contains two chlorophyll-binding polypeptides of 47 and 40 kDa and two polypeptides in the 28–31 kDa region. In contrast to the previous preparation [4], a 66 kDa polypeptide was present only in a negligible amount. Instead, a polypeptide of about 9 kDa, which may be attributed to cytochrome *b*-559 [1], was clearly resolved in the present preparation. Other weak bands were contaminations. The polypeptide composition of the PS-II reaction-center complex from the cyanobacterium is very similar to that of the PS-II complex isolated from spinach [3].

Bands B and C were found to contain two complemental chlorophyll-proteins of the PS-II reaction-center complex. Polypeptides of 47, 28–31 and 9 kDa were resolved from band B (middle trace). Thus the chlorophyll-protein complex present in band B represents the reaction-center complex from which the 40 kDa polypeptide is completely removed. However, comparison of peak heights of the 28–31 and 9 kDa polypeptide bands with that of the 47 kDa polypeptide band indicates that the smaller polypeptides were also partially released by the SDS treatment. The 40 kDa polypeptide separated from the PS-II complex was

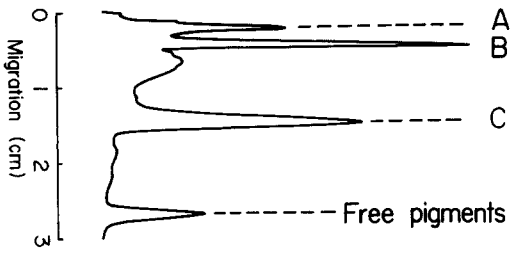

Fig. 1. Electrophoretic separation of chlorophyll-protein complexes from the LDAO extract of *Synechococcus* thylakoid membranes. Experimental conditions, see text. 0.05% SDS was present in the reservoir buffer but not in the gel. After 3 h of electrophoresis at 4°C (0.06 mA/mm^2), the gel was scanned at 675 nm without staining.

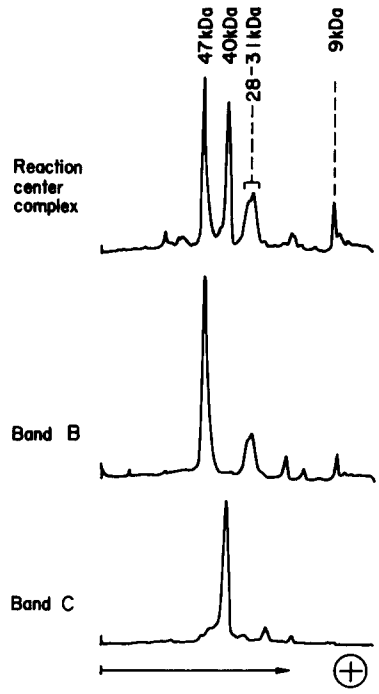

Fig. 2. Polypeptide compositions of the PS II reaction-center complexes and chlorophyll-protein complexes in band B and C. Samples were incubated with 2.5% SDS, 8 M urea, 5% 2-mercaptoethanol for 30 min at room temperature and run on 10–15% gradient polyacrylamide gels containing 0.1% SDS and 6 M urea for 17 h at room temperature. The gels were stained with Coomassie brilliant blue R250 and scanned at 560 nm.

recovered in band C in a chlorophyll-bound state (bottom trace). Band A contained polypeptides of both PS-I and PS-II reaction-center complexes (data not presented), and was not further studied. The results indicate that LDAO preferentially extracted PS II from the cyanobacterial membranes. Stewart and Bendall [11] have previously extracted PS-II particles from a thermophilic cyanobacterium with LDAO.

Because of similarity in the polypeptide composition to CP2b isolated in the previous work [4] and because of the absence of other suitable terminology for the reaction center complex that lacks the 40 kDa chlorophyll-binding subunit, the chlorophyll-protein in band B is called CP2b. Then, the chlorophyll-protein in band C is CP2c [4]. CP2b and CP2c prepared in the present work showed fluorescence characteristics similar to those of the previous preparations [4].

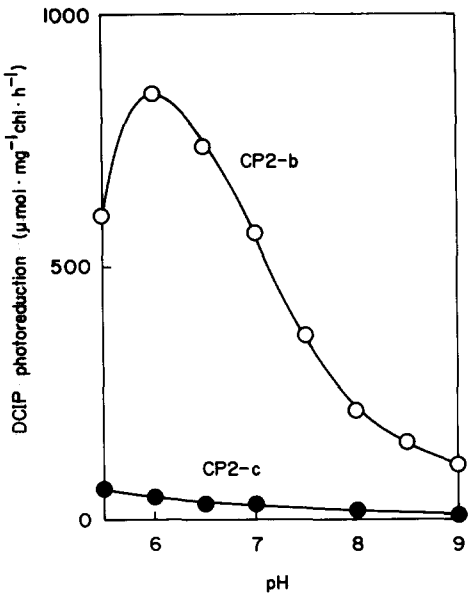

Fig. 3. pH dependence of DCIP photoreduction in CP2b and CP2c. Buffers used were Mes-NaoH at pH 5.5–6.5, Hepes-NaOH at pH 7.0–8.0 and Tricine-NaOH at pH 8.5 and 9.0.

tron donor is considered as a specific reaction of PS II [12]. The intact PS-II reaction-center complex mediates the photoreduction at rates of 500–800 μmol/mg Chl per h. CP2b prepared in the present work was found to show a high activity of DCIP photoreduction. The reduction rates ranged between 500 and 900 μmol/mg Chl per h and thus are comparable to that in the intact PS-II reaction-center complex on the chlorophyll basis.

Fig. 3 shows that the activity was maximal at about pH 6.0 and decreased with increasing pH. An acidic optimum pH is a characteristic of electron transport of PS-II in isolated thylakoid membranes [8]. CP2c was found to show a low but significant activity of DCIP photoreduction in the presence of DPC. The activity of this chlorophyll-protein was less pH-dependent, showing a monotonous decrease with increasing pH.

The DCIP photoreduction in the PS – II reaction-center complex was strongly inhibited in the presence of 20 μM DCMU, suggesting that one of the polypeptides in the 28–31 kDa region is the DCMU-binding protein [3]. The activity of CP2b, which has the two polypeptides in somewhat reduced amounts, was, however, totally insensitive to DCMU at concentrations up to 100 μM. Presumably, the SDS-treatment would have caused a structural modification of the herbicide-binding protein. The PS-II reaction-center complex isolated by Satoh is largely insensitive to several herbicides but still contains a herbicide-binding protein [3]. DCMU had no effect on the activity in CP2c, either.

The activity of CP2b was suppressed by ioxynil, a phenol-type herbicide which is assumed to have a binding site different from that for DCMU [13]. However, the maximum inhibition amounted at most 35% of the control activity and was attained only at the herbicide concentration as high as 50 μM. As ioxynil affects electron transport in intact systems at much lower concentrations [13,14], the inhibition may be ascribed to unspecific secondary actions of the herbicide. DCIP photoreduction of CP2c was also somewhat reduced by such a high concentration of ioxynil.

In spite of its insensitivity of the herbicides, the following data indicate that DCIP photoreduction in CP2b is a reaction mediated by the reaction center of PS-II. When the complex was treated at various temperatures for 5 min, the activity decreased at temperatures above 30°C and was half inactivated at about 50°C (fig. 4). In contrast, PS-II-driven electron transport in thylakoid membranes [8] or in PS-II-enriched particles [15] are stable at 50°C. Thus, the heat-stability seems to be

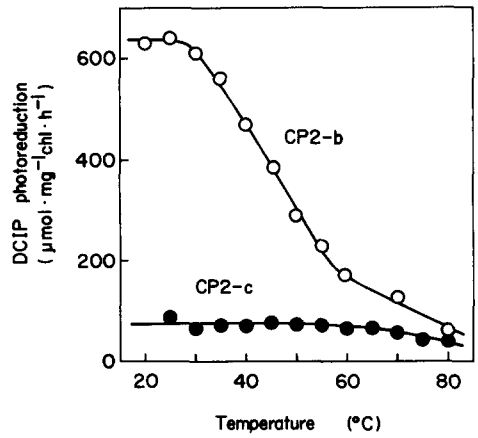

Fig. 4. Effects of heat treatment on DCIP photoreduction in CP2b and CP2c. Chlorophyll-protein complexes were incubated at indicated temperatures for 5 min, and DCIP photoreduction was measured at 25°C.

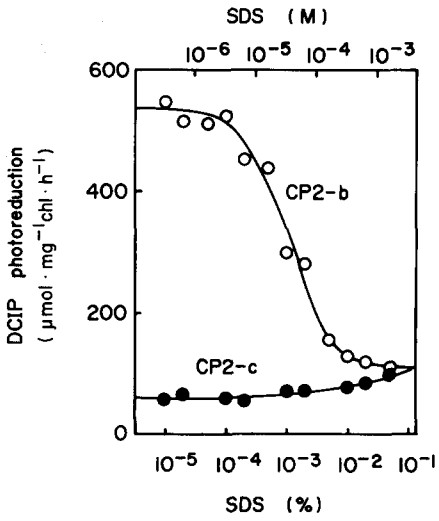

Fig. 5. Effects of SDS on DCIP photoreduction in CP2b and CP2c. SDS was added to rhe reaction medium at indicated concentrations.

reduced by the binding of SDS with the chlorophyll-protein complex. At any event, the results indicate that the DCIP photoreduction relies upon the intact conformation of the complex. The activity of CP2b was therefore assayed at 25°C.

The dependence of the activity on the functional structure of CP2b was also indicated from its sensitivity to SDS (Fig. 5). The activity decreased markedly when assayed in the presence of low concentrations of SDS, although high concentrations of the detergent failed to inhibit the activity completely. The half inactivation was observed at 0.001% SDS. This finding is rather surprising because CP2b was isolated by electrophoresis with the reservoir buffer containing 0.05% SDS. However, the complex was extracted from gels with, and after centrifugation, suspended in the Tris buffer containing no SDS. We suggest, therefore, that the inhibition induced by SDS is at least partly reversible.

Figs. 4 and 5 also show that the activity of CP2c was strongly resistant to heat and SDS. Even an appreciable enhancement of the activity was observed at high concentrations of SDS (Fig. 5). The results strongly suggest that DCIP photoreduction in CP2c is due to an unphysiological photochemistry of antenna chlorophyll molecules. A small fraction of the activity of CP2b which survived the treatments at temperatures above 60°C, or with SDS at concentrations higher than 0.01%, can be explained by this type of chlorophyll photochemistry.

Finally, we measured the quantum yield of DCIP photoreduction. The PS-II reaction-center complexes gave high quantum yields ranging between 0.7 and 0.9. This indicates that the complex isolated by the present procedures retains the intact functional organization of the PS-II reaction center. Absorbed photons are less efficiently utilized in CP2b than in the intact PS-II reaction-center complex. The quantum yield of the DCIP photoreduction in this complex was 0.12. This value is, however, much larger than that of DCIP photoreduction in CP2c, which was estimated as 0.002. Heating CP2b at 60°C for 5 min decreased the quantum yield to 0.006. The results are consistent with the view that the DCIp photoreduction in CP2b employs the photochemical machinery of photosynthesis, whereas the activity in CP2c, or in heated CP2b, is sensitized by chlorophyll molecules not related to the reaction center.

Discussion

The present work reports the isolation of two photoactive chlorophyll-protein complexes from the *Synechococcus* membranes. In contrast, no photochemical activity was detected in comparable complexes prepared in the previous work [4]. The difference can be ascribed to modifications of the preparation and assay procedures made in the present work. In the previous preparation procedures [4], the PS-II reaction-center complex is separated from digitonin-solubilized membranes by means of sucrose density gradient centrifugation and gel electrophoresis in the presence of digitonin, and then the chlorophyll-protein complexes are resolved from the reaction center complex by SDS-gel electrophoresis after treatment with SDS. The whole procedure takes at least four days to complete [4]. In the present work, the time needed to isolate the chlorophyll-protein complexes was reduced to two days by extracting them directly from the membranes. In addition, the exposure of the LDAO-extract to SDS was minimized by omitting the SDS-treatment prior to the electrophoresis and by adding SDS only to the

reservoir buffer but not to the gel. The high DCIP photoreducing activity found in CP2b can be ascribed to the milder, time-saving methods employed here. The disintegration of CP2b to CP2a and CP2d (chlorophyll-proteins containing only the 47 kDa subunit) [4] was not detected in the present work.

Another important change made in the present work was more than 5-times increase in intensity of the actinic illumination used for the measurement of DCIP photoreduction. This is particularly important for the assay of a low activity in CP2c. Due to its diminished antennal size and its lowered quantum yield, the activity of CP2b also requires the strong actinic light to saturate.

The pH dependence, sensitivity to heat and SDS and quantum yield of DCIP photoreduction in CP2b indicate that the activity is mediated by the PS-II reaction center. The insensitivity of CP2b to DCMU and ioxynil may be ascribed to the action of SDS because treatments with milder detergents diminished the sensitivity to the herbicides [3,7]. In particular the insensitivity of CP2b to ioxynil may be related to the loss of the 40 kDa polypeptide [13]. We could not correlate the loss of the herbicide-sensitivity to the release of polypeptides in the 28–31 kDa region from the complex.

The quantum yield of the photoreduction in CP2b, which is considerable lower than that in the intact complex, also suggests a modification of the photochemical machinery caused by the detergent. The high quantum yield in the intact reaction center complex relies upon its structurally and functionally well-organized arrangement of antenna pigments and the primary and secondary electron carriers. Thus the primary charge separation takes place with a high quantum efficiency and the separated charges are stabilized by means of the secondary electron flow. If, for instance, a secondary electron carrier is partially depleted or inactivated by the SDS treatment, a portion of absorbed photons would be wasted by back reaction between oxidized and reduced products. The loss of the herbicide-sensitivity or the partial release of the 28–31 kDa polypeptides suggest such a modification of the secondary electron transfer. Comparative studies on the functional components in the two complexes are in progress.

There are several indications that the PS-II reaction center is associated with the 47 kDa polypeptides [4–7]. It was previously shown that the PS-II reaction-center complex from *Synechococcus* contains two, and only two, chorophyll-proteins having the 47 or 40 kDa apoprotein [4]. Thus the isolation of the photoactive PS II complex, which completely lacks 40 kDa polypeptide, provides a conclusive evidence that the 47 kDa polypeptide is the site of the primary photochemistry of PS II. It follows that the 40 kDa polypeptide is not needed for the PS II-driven electron transport.

CP2c, i.e. an isolated chlorophyll-protein containing the 40 kDa polypeptide shows, however, a low but significant activity of DCIP photoreduction. Its heat stability, pH independence and low quantum yield strongly argue against the view that a PS-II reaction center is present on this chlorophyll-protein. Especially, a marked enhancement of the activity observed in the presence of high concentrations of SDS indicates that the photoreduction is sensitized by chlorophyll molecules not related to the reaction center. Thus, the finding cautions that DPS-dependent photoreduction of DCIP cannot always be regarded as a specific reaction of the PS-II reaction center.

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