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Investigation of the neighbour relationships between photosystem II polypeptides in the two types of isolated reaction centres (D1/D2/cytb_{559} and CP47/D1/D2/cyt b_{559} complexes)

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The nearest neighbour relationships within the D1/D2/cyt b_{559} complex (PSIIRC) and the CP47/D1/D2/cyt b_{559} complex (RC–CP47) were investigated by using different length bifunctional crosslinking agents. The crosslinking products were identified by immunoblotting with polyclonal antibodies and by two-dimensional gel electrophoresis. Seven products (CP47/D2, D1/D2/α, D1/D2, D2/α, D1/α, α/α, α/β) have been revealed in both complexes. The crosslinking of both complexes does not increase their photostability. The photocrosslinking products (D1/α and D2/α) appeared under illumination of complexes with light of high intensity.

Photosystem II; Reaction center; Crosslinker; Nearest neighbour; Photobleaching; Photocrosslinking

1. INTRODUCTION

Photosystem II (PSII) in vivo is a complicated, highly structured assembly of geometrically arranged chlorophyll-(Chl) proteins and other proteins the main part of which are embedded in the membrane. PSII preparations, such as PSII core complex (RC–CP47–CP43), PSII core complex + CP29 (RC–CP47) complex, or PSII core complex depleted of CP43 (PSIIRC) and so on, have been isolated from the PSII assembly using different detergents and techniques [1–6].

Little is known about near-neighbour relationships among proteins and Chl-proteins in the PSII assembly. On the basis of fractionation studies Bassi and Dainese [2] proposed a scheme of Chl-protein arrangement in PSII. They suggested that CP43 may serve as a connector between RC and some Chl-*a*/*b*-proteins. However Peter and Thornber [1] demonstrated the possibility of isolating PSII core type complex depleted of CP43 but associated with Chl-*a*/*b*-proteins. Therefore a more effective approach for studying this problem may be the use of bifunctional crosslinking reagents alone or in combination with immunochemical techniques. Close contacts between CP47 and 33 kDa protein, CP47 and CP29, 33 and 23 kDa proteins, LHC and D1 protein have been revealed by using different crosslinking reagents in the PSII core complex or more complicated complexes [7–9]. Nevertheless, information about near-neighbour relationship proteins or Chl-proteins in the RC–CP47 complex or PSIIRC is not enough. Here we report on crosslinking experiments with PSIIRC (D1/D2/cyt b_{559} complex) and RC–CP47 complex (CP47/D1/D2/cyt b_{559} complex) using different types of bifunctional reagents, two-dimensional electrophoresis and immunochemical techniques.

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Abbreviations: PS, photosystem; RC, reaction center; CP, chlorophyll protein; cyt, cytochrome; Chl, chlorophyll; Pheo, pheophytin; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; NHS, *N*-hydroxysulfosuccinimide; DST, disuccinimidyl tartarate; DSP, dithiobis(succinimidylpropionate); EGS, ethyleneglycolbis(succinimidyl succinate); DBIMB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

2. MATERIALS AND METHODS

PSII-enriched membranes of spinach thylakoids were obtained according to [10] with modifications described in [11]. The D1/D2/cyt b_{559} complex and CP47/D1/D2/cyt b_{559} complex were isolated simultaneously from PSII membranes following essentially the procedure described by Chapman et al. [12] with modifications according to [13]. If complexes were not used immediately they were stored at 190 K after addition of glycerol to 10% (w/v).

A zero-length crosslinker, EDC, in combination with NHS, and *N*-hydroxysuccinimide esters of different chain lengths (DST 6.4 Å; DSP 12 Å; EGS 16.1 Å) were used (all reagents were purchased from Pierce). For crosslinking 10 μl of an aqueous solution of NHS (2 mg · ml^{-1}) followed by 10 μl of water solution of EDC (190 mg · ml^{-1}) or 4 μl of dimethylsulfoxide solution of each crosslinker (DST 17 mg · ml^{-1}; DSP 20 mg · ml^{-1}; EGS 23 mg · ml^{-1}) were added to 100 μl of a complex (to about 0.1 mg Chl · ml^{-1}). We used freshly prepared solutions of crosslinkers. After incubation for 20 min in the dark at room temperature 20–25 μl of 25% SDS were added to the sample. After 5 min treatment with SDS followed by centrifugation for 5 min, 12,000 × *g* the samples were loaded on the gel.

SDS-PAGE in the presence of 6 M urea was performed according to Gounaris et al. [26] using a 12–18% linear acrylamide gradient.

The immunoblot experiments were performed as described [15].

Photoinhibition of the isolated complexes was carried out according to [15] in the eluent buffer. An electron acceptor, DBMIB, was added to a final concentration of 0.2 mM. Illumination was performed for fixed periods of time at 20°C using white light with an intensity of 4,500 $\mu E \cdot m^{-2} \cdot s^{-1}$.

3. RESULTS AND DISCUSSION

In Fig. 1 we present the results of treatment of PSIIRC with crosslinkers of different length. The crosslinking products were detected by Western blotting with the anti-α subunit of cyt b_{559} antiserum as it was part of practically all of these products. When the complex was treated with NHS and EDC, a zero-length crosslinker which forms a covalent bond between carboxy- and amino groups, one distinct band corresponding to a dimer of cyt b_{559} α-subunit appeared (Fig. 1, lane 2). The band of the α-subunit cyt b_{559} monomer was diminished in comparison with the same one in the control sample or in the *N*-hydroxysuccinimide ester-treated PSIIRC. The result of SDS-PAGE (not shown) confirmed that after treatment of the complexes with different concentrations of EDC, large aggregates were produced and were sedimented during centrifugation before SDS-PAGE or remained on the top of both gels. Our results did not agree with data obtained by Enami et al. [9]: in this cited work the distinct crosslinked products with apparent molecular weights within the range of 60–90 kDa were formed in PSII-enriched membranes treated with EDC alone.

The efficiency of producing crosslinking products in PSIIRC treated with *N*-hydroxysuccinimide esters depends on the chain length. DSP, with middle chain length (12 Å) forms 6 crosslinking products in contrast with those of a shorter (DST 6.4 Å) or longer length (EGS 16.1 Å): only three new bands appeared upon treatment of PSIIRC with DST or EGS (Fig. 1; lanes 3–5). This suggests that the distance between two amino groups involved in the crosslinking proteins is at most 12 Å.

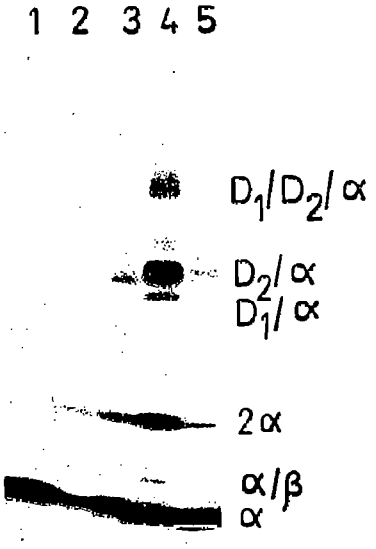

Fig. 1. Immunoblot with anti-α polyclonal antiserum of control PSIIRC (1) and PSIIRC treated with EDC and NHS (2), DST (3), DSP (4) and EGS (5).

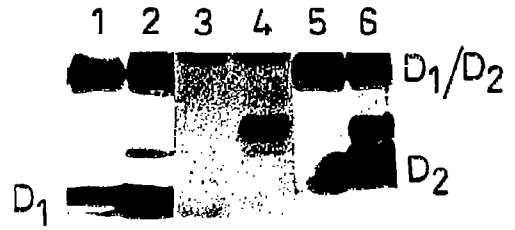

Fig. 2. Immunoblot with anti-D1 (1,2), anti-α (3,4) and anti-D2 (5,6) polyclonal antiserum of PSIIRC treated with DSP.

In order to determine proteins involved in crosslinking products we carried out immunoblot analysis and two-dimensional gel electrophoresis. The antisera raised against D1 and D2 proteins, CP47 apoprotein and the α-subunit of cyt b_{559} were used. All the antisera

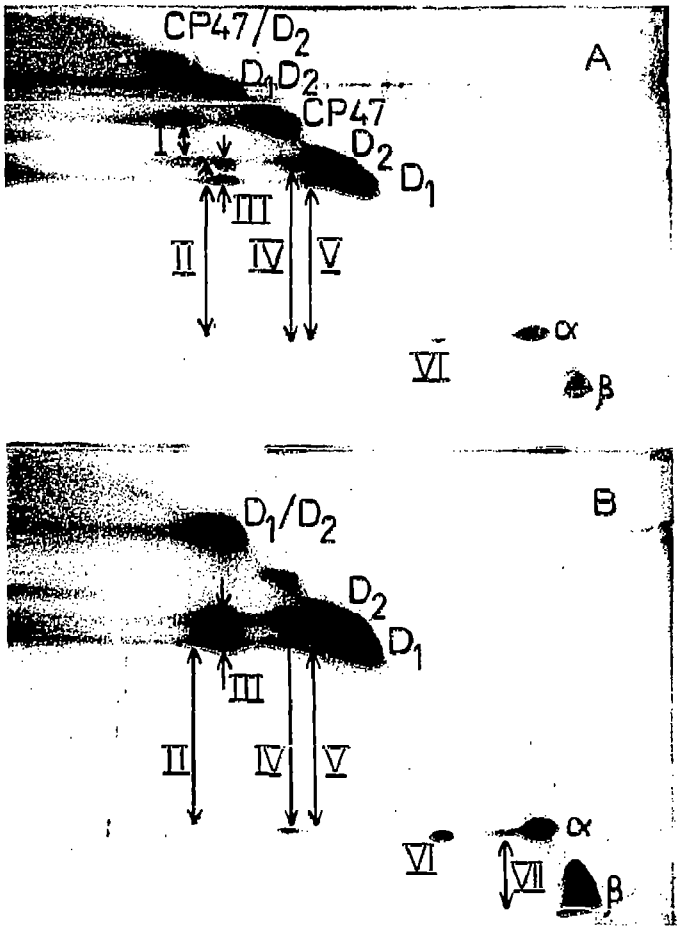

Fig. 3. Two-dimensional SDS-PAGE of RC-CP47 complex (A) and PSIIRC (B), both treated with DSP. Vertical arrows indicate crosslinked polypeptides (see section 3).

are essentially monospecific. Fig. 2 shows part of the immunoblots of PSIIRC which had been treated with DSP. On the basis of the same electrophoretic mobility of crosslinking products reacted with anti-D1 (lane D) and anti-α (lane 4) or with anti-D1 (lane 6) and anti-α (lane 4), it has been concluded that the upper crosslinking product (lane 4) belongs to the D2/α subunit of cyt b_{559}, and the lower one to the D1/α subunit of cyt b_{559}.

The results of two-dimensional gel electrophoresis are shown in Fig. 3. All non-crosslinked proteins or partially unbroken crosslinking products (spot CP47/D2, Fig. 3A) migrated along a diagonal path. When the crosslinked proteins were run after cleavage of the disulfide bond of DSP, several off-diagonal spots appeared. Seven crosslinked products have been revealed by this method in the RC–CP47 complex and PSIIRC. They are: CP47/D2 (product I), D1/D2/α (product II), D1/D2 (product III), D2/α (product IV), D1/α (product V), α/α (product VI) and α/β (product VII). Only products I and III could not be discovered using α-anti (Fig. 1).

The D1/D2 heterodimer is usually identified by immunoreaction with anti-D1 and anti-D2 [16]. In this work we present direct evidence that the D1/D2 heterodimer could appear after crosslinking, and that both heterodimers (being DSP crosslinked and existing in PSIIRC) have the same molecular mass.

There are three crosslinked products between D1 and D2 proteins and cyt b_{559} in PSIIRC and the RC–CP47 complex: D1/α, D2/α and D1/D2/α. The existence of the D1/α (or cyt b_{559}) product had been earlier predicted by Moskalenko and Kuznetsova [14] on the basis of studying the ability of the D1 protein to absorb the haem of cyt b_{559}. The appearance of dimer cyt b_{559} α-subunits in crosslinker-treated samples (Figs. 1 and 3) is interesting. The question about the amount of cyt b_{559} in PSII samples is as yet unclear. According to data [3,5] PSIIRC binds 1 haem of cyt b_{559}. Shuvalov et al. [17] and Dekker et al. [18] estimated the presence of 2 haems of cyt b_{559} in PSIIRC. Our data support the presence of two cyt b_{559} in PSIIRC or the RC–CP47 complex. The other possible explanation for the above results is dimerization of both complexes themselves in the presence of high salt concentration. However, these complexes migrated as monomers under mild (Derifat 160/DM, 4°C) electrophoretic conditions (not shown).

According to our data it is clear that DSP is a more effective crosslinker in comparison with DST and EGS for PSIIRC or the RC–CP47 complex. Eight crosslinked products have been found after treating both complexes with DSP. The question arises of whether it is possible, using treatment of the complexes with a crosslinker, to protect them from photobleaching or photoinduced cleavage of RC proteins. It has been recently established that after exposure of isolated PSIIRC to bright light in the presence of DBIMB a distinct pattern of D1 polypeptide fragments and corresponding autoproteolysis of the D1 protein is observed

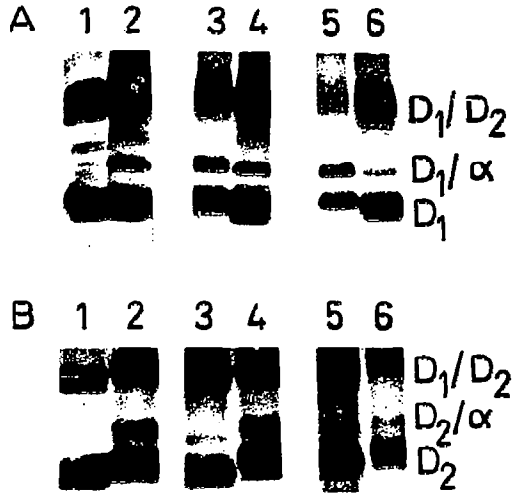

Fig. 4. Immunoblot with anti-D1 (A) and anti-D2 (B) polyclonal antiserum of control PSIIRC (lanes 1,3,5) or PSIIRC treated with DSP (lanes 2,4,6) under different times of illumination (lanes 1,2, 0 min; lanes 3,4, 5 min; lanes 5,6, 30 min) in the presence of DBIMB.

under aerobic and anaerobic conditions [15,19]. The crosslinked complexes did not increase their photostability as followed from the changes in their absorption spectra (not shown). This is probably due to a small amount of crosslinking products formed after treatment of the complexes with DSP (see Fig. 3) which cannot stabilize the spatial structure of the complexes. The appeared crosslinking products (D1/α and D2/α) degraded under the light (Fig. 4, lanes 2, 4 and 6). Thus the crosslinks between D1 and D2 proteins and cyt b_{559} α-subunit are not enough for protection from D1 (D2) proteolysis.

The photocrosslinked products appeared under the light (Fig. 4, lanes 1, 3 and 5). In contrast with DSP-crosslinked products the amount of photocrosslinked products did not decrease during the time of illumination. The nature of the photocrosslinking process is as yet unclear but undoubtedly it is generated by a post-primary charge separation process [20]. Probably this process is one of the first events in acceptor-side-mediated photodamage. We believe that tendency to form the D1/D2 heterodimer is also connected with the photocrosslinking process and is not associated with solubilization by Triton X-100, as was suggested by Marder et al. [16].

REFERENCES

[1] Peter, G.F. and Thornber, J.P. (1991) J. Biol. Chem. 266, 16745–16754.
[2] Bassi, R. and Dainese, P. (1992) Eur. J. Biochem. 204, 317–326.
[3] Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
[4] Moskalenko, A.A. and Kuznetsova, N.Yu. (1992) Biokhimiya 57, 226–235 (Russian).
[5] Van Leeuwen, P.J., Nieveen, M.C., Van de Meent, E.J., Dekker, J.P. and Van Gorkom, H.J. (1991) Photosynth. Res. 28, 149–153.

[6] Moskalenko, A.A. (1990) Biologich. Membr. 7, 736–741 (Russian).
[7] Enami, I., Satoh, K. and Katoh, S. (1987) FEBS Lett. 226, 161–165.
[8] Milner, P.A., Gogel, G. and Barber, J. (1987) Photosynth. Res. 13, 185–198.
[9] Enami, I., Mochizuki, Y., Kaneko, M., Kakuno, T., Horio, T., Satoh, K. and Katoh, S. (1990) in: Progress in Photosynthesis Research, vol. 1 (Baltscheffsky, M. ed.) pp. 319–322, Kluwer, Dordrecht.
[10] Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
[11] Moskalenko, A.A., Erokhin, Yu.E. and Kuznetsova, N.Yu. (1989) Photosynthetica 23, 324–332.
[12] Chapman, D.J., Gounaris, K. and Barber, J. (1988) Biochim. Biophys. Acta 933, 423–431.
[13] Moskalenko, A.A. and Kuznetsova, N.Yu. (1992) Biol. Membr. 9, 5–11 (Russian).
[14] Gounaris, K., Chapman, D.J. and Barber, J. (1988) FEBS Lett. 234, 374–378.
[15] Barbato, R., Friso, G., Giardi, M.T., Rigoni, F. and Giacometti, G.M. (1991) Biochemistry 30, 10220–10226.
[16] Marder, J.B., Chapman, D.J., Telfer, A., Nixon, P.J. and Barber, J. (1987) Plant Mol. Biol. 9, 325–333.
[17] Shuvalov, V.A., Heber, U. and Schreiber, U. (1989) FEBS Lett. 258, 27–31.
[18] Dekker, J.P., Bowlby, N.R. and Yocum, C.F. (1989) FEBS Lett. 254, 150–154.
[19] Barbato, R., Shipton, C.A., Giacometti, G.M. and Barber, J. (1991) FEBS Lett. 290, 162–166.
[20] Barber, J. and Andersson, B. (1992) Trends Biochem. 17, 61–66.