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USE OF POLYCLONAL ANTIBODIES TO DETERMINE WHICH PEPTIDES OF A TRANSMEMBRANE COMPLEX HAVE AQUEOUS EXPOSURES

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The proteins that catalyze photosynthetic electron transport in the thylakoid membranes of chloroplasts exist as transmembrane protein complexes. One of these protein complexes is photosystem II (PS II). PS II can be isolated from thylakoids by detergent extraction such that its O_2-evolving activity remains (1), but little is known about the protein topography of this complex. In this report we describe the isolation of antibodies specific for antigenic sites on both the lumen and stroma sides of the thylakoid membranes. These two groups of antibodies are then used to identify those proteins exposed on the stroma and the lumen sides of the membrane.

MATERIAL AND METHODS

PS II complexes were isolated, purified, and injected into rabbits as described elsewhere (2). The polyclonal antisera were challenged with either right-side–out (RSO) or inside-out (ISO) thylakoid preparations. RSO thylakoids were prepared by washing control thylakoids in 200 mM sucrose containing 1 mM EDTA (pH 7.5) at 20°C three times. ISO thylakoids were prepared and assayed as described in (3). One ml aliquots of antiserum previously dialyzed against 200 mM sucrose containing 10 mM TES (pH 7.5) and 10 mM KCl were challenged with either RSO or ISO thylakoids equivalent to 30 mg chlorophyll. Nonspecifically bound antibodies were removed by washing, and specifically bound antibodies were removed by suspending the antibody-precipitated thylakoids in 100 mM glycine/HCl (pH 2.8) containing 200 mM sucrose. Membranes were removed by centrifugation and the supernatants were adjusted to pH 7.5 with 1 M Tris base (pH 9.2). These solutions contained antibodies specific for determinants exposed on either the stroma or lumen side of the thylakoid membrane. After electrophoretic transfer to nitrocellulose paper (NCP), LDS-PAGE–separated proteins with determinants specific for the stroma side or lumen side antibodies were identified by Western Blotting as described in (4).

RESULTS AND DISCUSSION

Comparison of lanes 1 and 8 of Fig. 1 shows that we have antibodies to virtually every PS II peptide. Peptides with M_r of 59, 57, 44, 40, 33, 32, 29, 28, 27, 26, 25, 23, 18, 17, and 12×10^3 d are visible. Lanes 3 and 4 show the proteins that have been labeled by antibodies that bound to the stroma surface of the RSO thylakoids. Major bands include those at 59, 28, 27, and 26×10^3 d. Faint bands are seen at 57, 33, 23, and 18 (lane 3) as well as a very faint band at 33×10^3 d (lane 4). Missing from the major bands are the Tris-removable peptides (33, 25, and 18×10^3 d) (5, 6) which reside on the lumen surface of the membrane (7). Lanes 5 and 6 show the proteins that have been labeled by antibodies which bound to the lumen surface of ISO thylakoids. Major bands of lane 5 include those at 59, 32 (diffuse), 28, 27, 26, 23, and 12×10^3 d. The 32 and 12×10^3 d peptides are included because the relative band strengths appear at least as strong as the bands visible with whole serum (lane 1). Faint bands are visible at 57, and 18×10^3 d. Bands that have been identified by stroma side antibodies as well as lumen side antibodies include those at 59, 57, 28, 27, 26, and 23×10^3 d. We conclude that these proteins are transmembranous. The 12 and 32×10^3 d proteins (lane 5) are clearly recognized by antibodies that

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Volume 49 January 1986 106–107

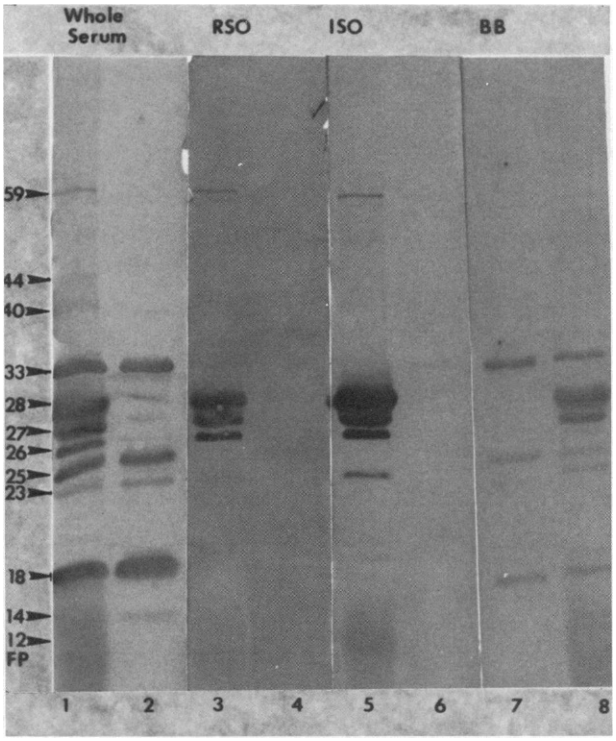

FIGURE 1 Western Blots labeled with whole serum (lanes 1, 2), RSO antibodies (lanes 3, 4), ISO antibodies (lanes 5, 6), and stained with Buffalo Black (lanes 7, 8). Lanes 1, 3, 5, and 8 have the complete protein profile of the PS II complex. Lanes 2, 4, 6, and 7 have only the extrinsic polypeptides, which were removed by Tris treatment (5). Please refer to the color figure section at the back of this book.

bound to the lumen surface of the thylakoid. They were not identified by those antibodies that bound to the stroma side of the membrane. The positive results indicate that these proteins are exposed on the lumen side of the protein complex. The negative results should not be interpreted. If the 12 × 10^3 d protein is cytochrome b-559, then its exposure at the lumen surface is consistent with its postulated roles in PS II activity and water oxidation (8). The data in Fig. 1 do not allow us to conclude that the 12 × 10^3 d protein is transmembranous. We believe that the diffuse 32 × 10^3 d protein (lane 5) may be the herbicide-binding protein (HBP-32). If this is the case, this report offers the first experimental evidence that this protein is exposed at the lumen surface. Protease studies have shown that HBP-32 is also exposed on the stroma surface of the PS II complex (9) and so we conclude that this protein is also transmembranous.

Since three peripheral proteins (33, 25, and 18 × 10^3 d) are known to be bound to PS II on the lumen side, we expected strong identification of these bands when separated PS II proteins were challenged with lumen-side antibodies. Unfortunately these bands are only faintly visible, and their presence is similar in both the blots using stroma side (lanes 3, 4) and lumen side (lanes 5, 6) antibodies. Most of these extrinsic proteins and their specifically bound antibodies were probably removed during the low-pH wash. Fig. 2 summarizes the results of this report. This model shows only the proteins for which we have antibodies. Other proteins are part of the PS II complex, but they have been omitted for clarity.

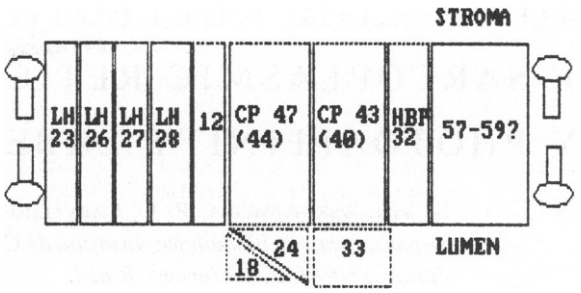

FIGURE 2 A model showing which proteins of PS II have stroma or lumen exposure, as determined with antibodies. Solid lines indicate that the protein was clearly identified with antibodies at that surface. Dashed lines indicate that antibody identification at that surface did not occur. We have not considered data from other workers that may positively assign some of these proteins to one exposure or the other.

This work was supported by a grant (82-CRCR-1-1125) from the Competitive Grants Office of the U. S. Department of Agriculture to S. P. Berg.

Received for publication 4 May 1985.

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