Site Directed Antisera to the D-2 Polypeptide Subunit of Photosystem II

R. Geiger

Hoechst AG, Pharma-Synthese, Postfach 800320, D-6230 Frankfurt/M. 80, Bundesrepublik Deutschland

R. J. Berzborn, B. Depka, W. Oettmeier, and A. Trebst

Lehrstuhl Biochemie der Pflanzen, Ruhr-Universität Bochum, Postfach 102148, D-4630 Bochum 1, Bundesrepublik Deutschland

Z. Naturforsch. **42c,** 491–498 (1987); received November 18, 1986

Dedicated to Professor Helmut Simon on the occasion of his 60th birthday

Antibody, D-2 Polypeptide, Photosynthesis, Q_A Binding Protein, Thylakoid Membrane

Three synthetic oligopeptides were used for preparation of antibodies against the D-2 polypeptide of thylakoid membranes. Their sequence was chosen from a model of the folding of the amino acid sequence of the D-2 polypeptide subunit through the membrane that predicted these sequences to be exposed at the membrane surface. For the Merrifield solid-phase method on a fully automated synthesizer the N^{α}-amino group was protected by a fluorenyl-9-methylcarbonyl group. The oligopeptides were coupled to serum albumin by EDAC for immunizations in rabbits.

Antisera with high titer were obtained for the two oligopeptides that contained the amino acid sequence of the D-2 protein from amino acid 230 to 235 and from 235 to 241. The antisera reacted with the D-2 polypeptide, separated on SDS gel and agglutinated the thylakoid membrane.

It is known that certain photosystem II functions are impaired by short time trypsin treatment of the membrane. The antisera were used to show that under these conditions the D-2 polypeptide in the membrane is very sensitive. The trypsination yielded two cleavage products detected by the two antisera, a 20 kDa fragment blotted by antiserum against amino acids 230 to 235 and a 10 kDa fragment blotted by the antiserum against amino acids 235 to 241. As the polypeptide cleavage occurs between the two epitopes, the trypsin cut is therefore at arginine 234. This supports the prediction that the sequence containing this arginine is the most exposed part of the D-2 polypeptide on the membrane (matrix) surface. It is proposed that the high sensitivity of the D-2 polypeptide accounts for the known effect of membrane trypsination on Q_A accessibility in photosystem II.

Photosystem II of higher plants consists of six major integral polypeptide subunits of 47, 43, 34 (D-2), 32 (D-1) and 9 + 4 (cytochrome b_{559}) kDa molecular weight (for review see [1]). Although a "diffuse band" of a polypeptide called D-2 has been observed early [2–4], its participation in photosystem II function has only recently been considered (see [1]). Its DNA sequence had been determined and the deduced amino acid sequence showed high homology to the 32 kDa D-1 polypeptide subunit (Q_B or herbicide binding subunit) [5]. From the amino acid and membrane folding homology of the D-1 and D-2 polypeptides to the L and M subunits of the reaction center of bacterial photosynthesis it has been postulated that D-2 is part of the reaction center of PS II and carries the Q_A binding site [6–9]. Nevertheless, direct evidence for a D-2 role in photosystem II is scarce (see [10]). We have therefore prepared antibodies against synthetic oligopeptides as a tool for detailed functional studies.

Abbreviations: Boc, *t*-butyloxycarbonyl; BSA, bovine serum albumine; Bu^t, *t*-butyl; DCC, dicyclohexyl carbodiimide; DCMU, dichlorophenyldimethylurea; DMAP, 4-dimethylamino pyridine; EDAC, 1-ethyl-3-(-dimethylaminopropyl)carbodiimide; Fmoc, fluorenyl-9-methyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; PMSF, phenylmethylsulfonylfluoride; Q_A, primary plastoquinone acceptor of photosystem II.

Reprint requests to Prof. Dr. A. Trebst.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0400–0491 $ 01.30/0

Materials and Methods

Oligopeptide synthesis

Amino acid derivatives were prepared at the Hoechst laboratories. They met the necessary purity standards. Reagents and solvents for syntheses and analyses were purchased from Riedel de Haen, Seelze, and E. Merck, Darmstadt, the *p*-alkoxybenzyl alcohol resin for solid-phase synthesis [11] from Bachem, Bubendorf, Switzerland.

1. Thin-layer chromatography

Pre-coated tlc plates "Merck" silica gel 60 were used; 1 µl of a 5% solution of the peptides was ap-

plied. The solvent systems were methyl ethyl ketone/pyridine/water/acetic acid 70:15:15:2 (A), *n*-butanol/acetic acid/water 3:1:1 (B) and *n*-butanol/acetic acid/pyridine/water 4:1:1:5 – upper phase (C).

2. High performance liquid chromatography (HPLC)

For preparative HPLC, Lobar^R "Fertigsäulen" C, filled with LiChoprep^R Si 60 (40–63 μm), E. Merck, Darmstadt, were used.

3. Amino acid analyses

After 24 h hydrolysis in 6 M HCl, at 120 °C, analyses were carried out with an amino acid analyzer LC 5001, Biotronik, Munich. The peptide content was calculated from the amount of amino acids, related to a defined amount of norleucine which was added before hydrolysis.

4. Synthesis

All of the oligopeptides were synthesized by the solid-phase method [12, 13]. Protected peptides were prepared on a semi-automatic SP 640 synthesizer (Fa. Labortec, Bubendorf, Switzerland) which had been remodeled into a fully automated synthesizer in the Hoechst laboratory, using a program written by D. Langner on an Apple computer IIe.

The program was adapted to the Fmoc strategy [14, 15]. N^{α}-amino groups are protected by the base-sensitive Fmoc residue, carboxyl and hydroxy groups in the side-chains are protected by Bu^t, amino groups are blocked with Boc, the guanido group of arginine with Mtr [16], a protecting group which is cleaved by 90% trifluoro acetic acid under only slightly stronger conditions than *t*-butyl protecting groups.

The C-terminal amino acid was linked to the hydroxyl group of the resin by the DCC/DMAP/HOBt procedure [17].

A reaction cycle comprises cleavage of Fmoc by 20% piperidine in dimethylformamide, washing with dimethylformamide, reacting twice with a 2.5-fold molar amount of the following Fmoc-amino acid, diisopropyl carbodiimide and 1-hydroxy-benzotriazole each in dimethylformamide and washing with dimethylformamide. The process is repeated. The completeness of the reaction can be followed by means of the Kaiser test [18]. The program has to be stopped for this purpose. The next cycle follows automatically.

At the end of the synthesis, the resin is additionally washed with *t*-butyl methyl ether and dried.

The peptide is cleaved from the resin by suspending it in a mixture of dimercaptoethane/kresol/methylene chloride (1:0, 5:12) and adding after 30 min. the 3-fold amount of 95% trifluoroacetic acid for 90 min. The resin is filtered off and washed twice with trifluoroacetic acid. The solvents are distilled off in vacuo. The product is in most cases insoluble in ether. It is repeatedly treated with *t*-butyl methyl ether and dried in vacuo.

Further treatment depends on the properties of the thus isolated crude product. It is described below in detail.

Oligopeptide 1, corresponding to amino acids 230–235 of the D-2 polypeptide: *H–Ala–Asn–Thr–Phe–Arg–Ala–OH.*

Fmoc–Ala–Asn–Thr(Bu^t)–Phe–Arg(Mtr)–Ala–O–resin

The above described protocol is applied to 3.0 g Fmoc–Ala–O–resin (loading 0.3 mmol/g), adding the Fmoc-amino acids successively. Yield: 3.8 g resin-bound protected peptide.

Fmoc–Ala–Asn–Thr–Phe–Arg(Mtr)–Ala–OH

3.8 g of the resin, loaded with the protected peptide, is treated as described above to cleave the Fmoc-peptide. Yield 900 mg. Tlc in A and C shows two spots corresponding to the Fmoc-peptide with partially deblocked arginine. Two additional minute spots are visible.

Amino acid analysis: Asp(1) 1.01; Thr(1) 0.91; Ala(2) 2.00; Phe(1) 0.99; Arg(1) 0.99. Peptide content 92%.

Fmoc–Ala–Asn–Thr–Phe–Arg–Ala–OH

860 mg Fmoc-peptide is reacted with 0.4 ml trifluoromethane sulfonic acid in 10 ml trifluoroacetic acid for 30 min at room temperature. Precipitation of the product with 200 ml *t*-buthylmethyl ether yields 1.1 g. Peptide content 68%.

H–Ala–Asn–Thr–Phe–Arg–Ala–OH

1.1 g crude Fmoc-peptide is reacted with 25 ml dimethylformamide-diethylamine (4 + 1) for 20 min at room temperature. The solvent is distilled off in vacuo. The crude product is suspended twice in *t*-

butylmethyl ether, isolated by centrifugation and dried *in vacuo*. Yield 540 mg, peptide content 75%. Amino acid analysis correct. Several minor impurities are seen in tlc (A, B, C).

500 mg were dissolved in 5 ml *n*-butanol/acetic acid/water (3:1:1) and chromatographed over a column Merck C silica gel in the same solvent. Fractions of 17 ml each were collected according to tlc; 253 mg (34%) were obtained in pure form, 128 mg were slightly contaminated. The pure compound was uniform in tlc (A, B, C).

Amino acid analysis: Asp(1) 1.03; Thr(1) 0.91, Ala(2) 2.00; Phe(1) 1.01; Arg(1) 1.00. Peptide content 91%.

Oligopeptide 2, corresponding to amino acids 235 to 241 of the D-2 polypeptide: *H–Ala–Phe–Asn–Pro–Thr–Gln–Ala–OH.*

Fmoc–Ala–Phe–Asn–Pro–Thr(Bu^t)–Gln–Ala–O–resin

3.0 g Fmoc–Ala–O–resin (loading 0.2 mmol/g) is treated as described above, adding the Fmoc-amino acids successively. Yield 3.5 g.

H–Ala–Phe–Asn–Pro–Thr–Gln–Ala–OH

Cleavage of the Fmoc-peptide from the resin follows the general procedure. Treatment with a stronger acid is not required since the peptide does not contain Arg(Mtr). Cleavage of Fmoc follows again the description given above. Excess piperidine which is not eliminated by distillation is adsorbed at the weakly acidic ion exchanger Amberlite IRC-50 from aqueous solution. The freeze-dried peptide is already uniform in tlc (A, B, C). Yield 357 mg.

Amino acid analysis: Asp(1) 1.06; Thr(1) 0.94; Glu(1) 1.02; Pro(1) 1; Ala(2) 1.96; Phe(1) 1.00. Peptide content 89%.

Oligopeptide 3, corresponding to amino acids 326 to 332 of the D-2 polypeptide: *H–Ile–Arg–Ala–Gly–Trp–Ala–Ala–OH.*

Fmoc–Ile–Arg(Mtr)–Ala–Gly–Trp–Ala–Ala–OH

3.0 g Fmoc–Ala–O–resin (loading 0.3 mmol/g) is treated according to the above described protocol, adding the Fmoc-amino acids successively. Yield 3.65 g resin-bound protected peptide.

H–Ile–Arg–Ala–Gly–Trp–Ala–Ala–OH

Cleavage of the Fmoc-peptide from the resin, further deblocking and chromatographic purification as described for the before mentioned peptide.

Yield 180 mg, uniform in tlc (A, B, C). Side-fractions contained 125 mg contaminated material.

Amino acid analysis: Gly(1) 1.00; Ala(3) 1.01; Ile(1) 0.96; Arg(1) 0.98; Trp(1) 0.96 (determined by UV absorption). Peptide content 90%.

Coupling of the oligopeptides to serum albumin

The coupling of the synthetic oligopeptides to bovine serum albumin, essentially fatty acid free (Sigma) was performed either by use of glutaraldehyde (25% aqueous solution; Sigma) according to Streckert *et al.* [19] or by use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC; Sigma) according to Goodfriend *et al.* [20].

1. Glutaraldehyde coupling

To a solution of 10 mg (0.15 μmol) bovine serum albumin in 1 ml of 0.1 M sodium phosphate buffer (pH 7.5) a 30-fold M excess of the oligopeptide dissolved in 0.3 ml of the same medium was added. After addition of 100 μl of a 21 mM aqueous solution of glutaraldehyde the reaction mixture was stirred for 10 min. After 10 and 20 min another 100 μl of glutaraldehyde were added. The coupling reaction was completed within 30 min, the conjugate lyophilized and redissolved in 1.3 ml of H_2O.

2. EDAC coupling

To a solution of 10 mg (0.15 μmol) of bovine serum albumin and 20 mg of the oligopeptide in 0.5 ml H_2O 200 mg of EDAC in 0.25 ml H_2O were added. After 30 min the reaction mixture was dialyzed against H_2O for 24 hs.

Preparation of antisera

Rabbits were immunized according to the published schedule [21]; for the primary multisidal injections 500 μg BSA labeled with the respective oligopeptides, as a paste with Freunds compl. adjuvant were used, for the i.v. booster injections another 500 μg. Antisera were tested in ring tests according to [21] and in western blots [22]. Spinach thylakoid membranes were dissolved in 3% LiDS + $NaHCO_3$ + saccharose at room temperature, sepa-

rated on polyacrylamide gel (11–15%) at 4 °C for 16 h. The gels were blotted for 3 h and 0.6 Å at 10 °C on nitrocellulose in 25 mM tris, 192 mM glycine and 20% methanol. After saturation with 3% gelatine in tris buffer pH 7.5 the first antibody was allowed to react over night at room temperature in 1% gelatine. After washing in tris and 0.05% tween 20 the second antibody (horseradish peroxidase conjugated) was allowed to react in 1% gelatine for 1 h, dilution 1:3000, and developed with HPR colour development and 0.005% H_2O_2.

Thylakoid membrane preparation and photosynthetic activity

Spinach thylakoids were prepared according to standard methods [23] in 0.4 M NaCl and 20 mM tris buffer pH 8.0 and chloroplasts broken in 20 mM tris buffer pH 8.0 + 0.15 M NaCl. Their photosynthetic activity (DCMU sensitive) was 380 μ atoms 0/mg chlorophyll/h with methylviologen as acceptor.

For trypsination according to [24] spinach thylakoid membranes with 300 μg chlorophyll in 3 ml were incubated with 100 μg trypsin (Boehringer) in 30 mM MES buffer pH 6.5 and 6 mM $MgCl_2$ at room temperature for the time indicated. The reaction was stopped with 1.5 mg aprotinin (Trasylol^R, Bayer) and 2 mM PMSF. The photosynthetic activity after two minutes trypsination (now DCMU insensitive) was 300 μ atoms 0/mg chlorophyll/h with ferricyanide as acceptor.

Results

The three oligopeptide sequences, synthesized and used for antibody preparation were chosen according to the amino acid sequence of the D-2 polypeptide – as derived from the DNA sequence of the chloroplast coded gene [5, 26–28]. Our recent folding model [9] of the D-2 subunit predicted sequences exposed on either the matrix or lumen side of the thylakoid membrane. According to this prediction [9] the sequence of amino acids 230 to 245 is the most exposed on the matrix side, as it is a sequence after the transmembrane helix IV ending at amino acid 225 and before a parallel helix that starts at amino acid 250. Two sequences for oligopeptide synthesis were chosen on either side of arginine 234 – a possible trypsin cut site. The third oligopeptide corresponds to a sequence from the carboxyl end that – according to the prediction – should extend into the

Table I. Synopsis of antibodies against synthetic oligopeptides of partial sequences of the D-2 subunit of photosystem II.

oligopeptides	1				2		3			
sequence of the synthesized oligopeptides	Ala_{230}–Asn–Thr–Phe–Arg–Ala_{235}				Ala_{235}–Phe–Asn–Pro–Thr–Gln–Ala_{241}		Ile_{326}–Arg–Ala–Gly–Trp–Ala–Ala			
linking to BSA via	glutaraldehyde		EDAC		glutaraldehyde	EDAC	glutaraldehyde		EDAC	
rabbit no.	260	261	264	265	269	268	262	263	266	267
antibodies against the carrier BSA	+++	+	+++	+	+++	++	+	+	+	+
antibodies against the oligopeptide coupled to BSA (ring test)	±	±	++	+	+	++	±	+	+	+
number of bands with thylakoid polypeptides after LiDS-PAGE and western blot	0	0	1	2	0	1	0	0	6	4
titer in western blot with the 32 kDa region	–	–	1/250	1/1000	–	1/4000	–	–	–	–
titer in agglutination of suspension of isolated thylakoid systems	–	–	–	1/60	–	1/120	–	–	–	–

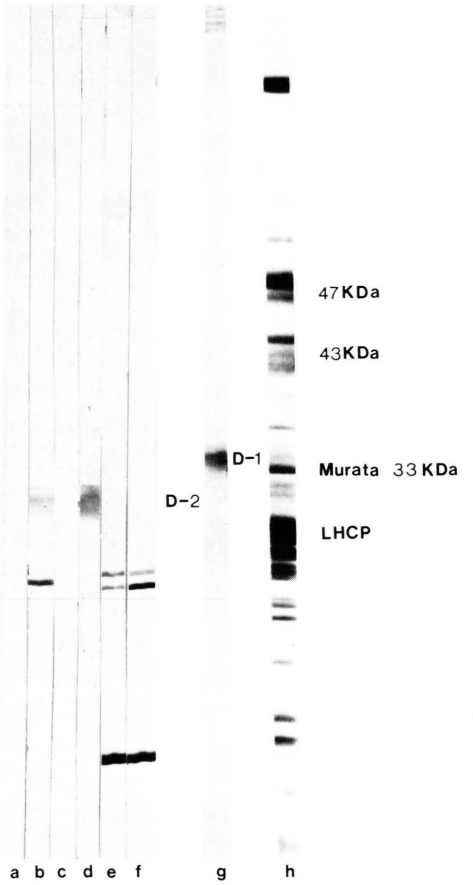

Fig. 1. Immune blot of thylakoid membrane polypeptides separated on a LiDS gel with antisera against synthetic oligopeptides.
Lane a: antiserum 1 against amino acids 230–235 of D-2 coupled to BSA by glutaraldehyde (rabbit 260); Lane b: as a, but coupled with EDAC (rabbit 265); Lane c: antiserum 2 against amino acids 235–241 of D-2 coupled to BSA with glutaraldehyde (rabbit 269); Lane d: as c, but coupled with EDAC (rabbit 268); Lane e: antiserum 3 against amino acids 326–332 of D-2 coupled to BSA with EDAC (rabbit 267); Lane f: as e, but rabbit 266; Lane g: antiserum against the D-1 polypeptide (prepared by U. Johanningmeier against a fusion protein consisting of part of the D-1 protein (amino acids 167–353) and galactosidase expressed in *E. coli* (rabbit 270); Lane h: coomassie blue stain of the LiDS gel electrophoresis pattern of thylakoid membrane polypeptides.

lumen space of the thylakoid membrane. All three sequences end with an alanine at the C-terminal in order to use the same Ala-O-resin for synthesis.

After synthesis the oligopeptides were coupled to bovine serum albumine by either glutaraldehyde or EDAC. With each preparation thus obtained, one or two rabbits were immunized (rabbits numbered 260 to 269). The sera obtained were tested in ring tests [21] and western blots [22] against thylakoid membrane proteins after LiDS gel electrophoresis. Table I and Fig. 1 show the specifity and titers of the antisera obtained, i.e. the band(s) stained after western blot of thylakoid membrane polypeptides. No appreciable amount of antibodies against the oligopeptides were formed in those rabbits immunized with glutaraldehyde coupled probes. Positive were, however, rabbits immunized with the EDAC coupled probes. The titer of the antibody serum against the oligopeptide 1 (amino acids 230–235) was strong in rabbit 265, showed a major band against a polypeptide of the thylakoid protein pattern in the region of 32 kDa and an additional lower band (that disappears also in the trypsination experiments – see below) (Fig. 1, lane b). The titer of the antibody serum against oligopeptide 2 (amino acids 235–241) was particularly strong in rabbit 268. The band in the western blot was diffuse, but identical to the major band stained with the antiserum against oligopeptide 1 (Fig. 1, lane d). This band in the western blot is poorly stained by coomassie blue (Fig. 1). For comparison a blot with an antibody against a part sequence of the D-1 polypeptide is included (Fig. 1, lane g).

The antibodies from rabbits 266 and 267 against the third oligopeptide (amino acids 326–332) gave an entirely different western blot pattern. There were three major bands (Fig. 1, lane e and f), but no band in the position where the D-2 polypeptide is to be expected and where the antisera against the other two oligopeptides reacted. The bands at low molecular weight indicated a reaction of antibodies with functionally unknown polypeptides of the thylakoid membrane. These bands are also not identical to the trypsin cleavage products of the D-2 polypeptide – as discussed below. The blot pattern brought about by the antisera with the polypeptides of an enriched photosystem II preparation was the same as that with thylakoid membrane polypeptides (and is therefore not documented).

Antisera against oligopeptides 1 and 2 were tested in precipitation test with the intact thylakoid membrane (Table II). Although the titers were not very high, it was clear that serum 268 did agglutinate the membrane (Table II). Therefore antibodies in this serum reached the antigene. It indicates that the

Table II. Agglutination of suspensions of isolated spinach thylakoid membranes by antisera against synthetic oligopeptides of partial sequences in the D-2 polypeptide.

Agglutination was performed by mixing 4 µl of serum with 10 µl of thylakoid suspension from spinach, 0.1 mg chl/ml, and judging the reaction at 160 × in a microscope after gently rotating the drop for 20 sec at room temperature. − 0 denotes the preimmune sera, − 2 the antisera after the boosterinjection; dilution was with 100 mM NaCl, 5 mM $MgCl_2$.
Agglutination was direct, *i.e.* no enhancing goat anti rabbit gammaglobulin was added.

serum dilution	1:4	1:8	1:16	1:32	1:62
final dilution	1:15	1:30	1:60	1:120	1:240
serum (rabbit no.)					
264−0	+	−	−	−	−
264−2	+	−	−	−	−
265−0	+	±	−	−	−
265−2	++	++	±	−	−
268−0	+	±	−	−	−
268−2	++++	+++	++	+	−
266−0	+	−		−	−
266−2	+			−	−
267−0	+	−	−	−	−
267−2	+	−	−	−	−

antigenic epitope of the D-2 polypeptide, *i.e.* amino acids 235−241 is accessible from the matrix side.

This accessibility is also indicated by the high sensitivity of the D-2 polypeptide in thylakoid membrane vesicles to trypsin. As known, two minutes trypsination at pH 6.5 inactivates the Q_B site of PS II and exposes Q_A, but even ten minutes trypsination do not inhibit either the primary photochemistry and Q_A reduction nor oxygen evolution [8, 24, 25]. Western blots with antisera against oligopeptides 1 and 2 revealed two bands after mild trypsination of the membrane (Fig. 2). After two minutes trypsin treatment of the membrane a band at about 10 kDa is observed when the antibody against the sequence 235 to 241 is used (Fig. 2, column 3). A double band at about 20 kDa is observed in the same trypsinated membrane preparation when the antibody against the sequence 230−235 is used (Fig. 2, column 1). Therefore trypsin cuts the D-2 polypeptide in the membrane between the two epitopes, *i.e.* at arginine 234.

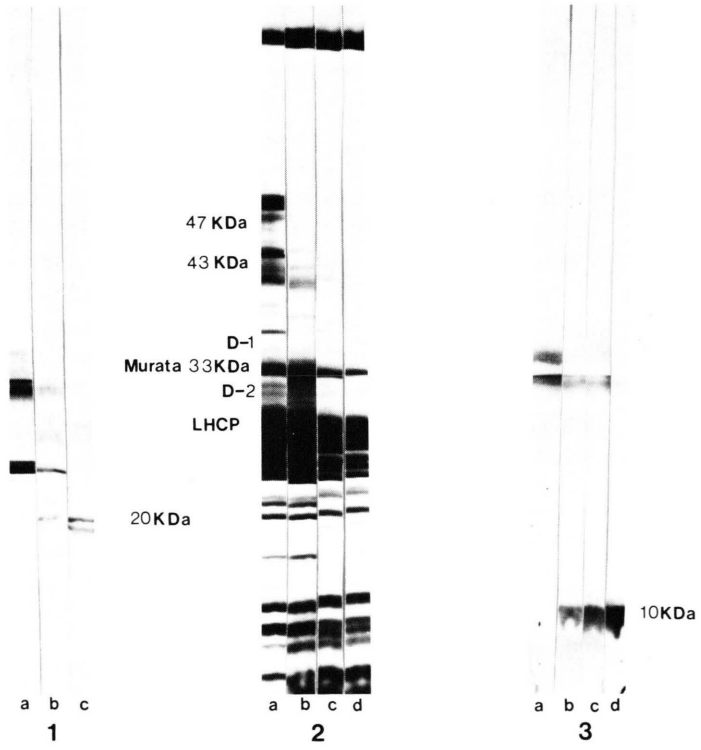

Fig. 2. Immune blot of trypsinated thylakoid membrane polypeptides separated on a LiDS gel with site directed antisera to the D-2 polypeptide.
Column 1: blot with antiserum 1 against amino acids 230−235; Column 2: coomassie blue stain; Column 3: blot with antiserum 2 against amino acids 235−241; Lane a: control thylakoid membrane; Lane b: 2 min trypsination of the membrane at pH 6.5; Lane c: 5 min trypsination; Lane d: 10 min trypsination.

Discussion

Although a second diffuse polypeptide band among the thylakoid membrane proteins – in addition to the D-1 or Q_B and herbicide binding protein – is long known, a role for this D-2 polypeptide in photosystem II has been proposed only recently [6–10]. Following the DNA sequencing of the psbD gene [5, 26–28] its striking amino acid homology to the D-1 polypeptide suggested it to carry also a plastoquinone binding site [5]. From the homology in the hydropathy plots and of conserved amino acids in homologous positions in the D-1/D-2 subunits of PS II to that of the sequence of the L and M subunits of the reaction center of purple bacteria, where a X-ray structure is available for showing great details [29], it has been proposed [6–9] that the D-1/D-2 subunits carry the reaction center of photosystem II with the D-2 subunit being the Q_A binding protein – contradicting the assignment of the reaction center of PS II to the 47 kDa subunit (see [1]). A recent reaction center preparation by Nanba and Satoh [10] supports the new view. Antibodies against the D-2 subunit should be very useful for establishing the role proposed.

We have chosen amino acid sequences for the preparation of site directed antisera from our folding prediction [9] that indicates areas of the polypeptide exposed to either side of the membrane. We succeeded with two antisera directed against sequences on the matrix side. The antisera obtained react with the same band on the LiDS gel of thylakoid membrane polypeptides. The position on the gel for the D-2 polypeptide is in accordance with the literature [2–4], *i.e.* a band below that of the D-1 polypeptide and the peripheral (Murata) 33 kDa polypeptide, but above those of the light harvesting complex subunits (in gels without urea). We failed so far to obtain a monospecific antiserum against the sequence of the carboxyl end of the D-2 polypeptide.

The strong antibody against the oligopeptide sequence of amino acids 235 to 241 of the D-2 protein agglutinated the thylakoid membrane indicating accessibility of the site from the matrix side. Also the high trypsin sensitivity of the D-2 polypeptide – reported here for the first time – is in support of an accessibility of this sequence of the D-2 polypeptide as predicted [9] from its folding through the membrane. Already after two minutes trypsination of the membrane two degradation products appear, as visualized with the two antisera. The antiserum against the sequence 235 to 241 revealed a cleavage product of about 10 kDa, the other against the sequence 231 to 235 one at about 20 kDa. The first indicates the carboxyl end, the other the N-terminal end. The trypsin cut is exactly between the two epitopes (Ala 235 in the first sequence is coupled to the BSA for antibody preparation and therefore probably does not contribute to the epitope). As the two synthetic oligopeptides overlap, it follows that the trypsin cut is precisely at arginine 234 – the amino acid the most exposed to the matrix side [9]. 10 kDa is about the size of the carboxyl end expected as of amino acids 235 to 355. The 20 kDa size is somewhat smaller than expected for the N-terminus and therefore another trypsin cut might be indicated.

This trypsin cut at arginine 234 is very similar to the first cut of the D-1 polypeptide by trypsin [30]. Indeed, there is an arginine at the expected homologous position according to the folding prediction of D-1 (see [5, 9]). As is long known, mild trypsination of the membrane does not disturb electron flow from water to Q_A, but does inactivate the Q_B and herbicide binding site [24, 25, 31–33]. This had been attributed to degradation of the D-1 polypeptide [24, 25, 32, 33]. However, the Q_A accessibility to ferricyanide, also brought about by mild trypsination [25], is explained more easily by the trypsin cut of the D-2 polypeptide as reported here, as this polypeptide is proposed to carry the Q_A site according to the model [9].

According to the prediction the two D-1/D-2 polypeptides carry the reaction center of PS II [6–10]. This activity is kept even after the trypsin cut of the D-2 polypeptide at arginine 234. This indicates that the cut as such does not yet disturb the conformation of the polypeptide fragments *in* the membrane and their proper function. Only after SDS solubilization will the cleavage products separate. This is true also for the obvious trypsin digestion of the 47 and 43 kDa polypeptides of PS II as well [8] (seen also in Fig. 2). These functional considerations will be more fully explored in a later paper.

Acknowledgements

Work at Bochum was supported by the Deutsche Forschungsgemeinschaft (SFB 168) and by Fonds der chemischen Industrie. We are indebted to Dr. Streckert, Medizinische Mikrobiologie und Virologie, Ruhr-Universität, for his advice in the oligopeptide coupling procedure.

[1] K. Satoh, Photochem. Photobiol. **42,** 845–853 (1985).
[2] N. H. Chua and N. W. Gillham, J. Cell Biol. **74,** 441–452 (1977).
[3] R. J. Ellis, Ann. Rev. Plant Physiol. **32,** 111–137 (1981).
[4] P. Delepelaire, Photobiochem. Photobiophys. **6,** 279–292 (1983).
[5] J.-D. Rochaix, M. Dron, M. Rahire, and P. Malnoe, Plant Molec. Biol. **3,** 363–370 (1984).
[6] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, J. J. Molec. Biol. **180,** 385–398 (1984).
[7] J. Deisenhofer, H. Michel, and R. Huber, Trends Biochem. Sci. **10,** 243–248 (1985).
[8] A. Trebst and B. Depka, in: Springer Series in Chemical Physics **42**: Antennas and Reaction Centers of Photosynthetic Bacteria – Structure, Interactions and Dynamics (M. E. Michel-Beyerle, ed.), pp. 215–224, Springer Verlag, Heidelberg 1985.
[9] A. Trebst, Z. Naturforsch. **41c,** 240–245 (1986).
[10] O. Nanba and K. Satoh, Proc. Natl. Acad. Sci. USA, in press (1986).
[11] S. S. Wang, J. Amer. Chem. Soc. **95,** 1328–1333 (1973).
[12] R. B. Merrifield, Fed. Proc. **21,** 412 (1962).
[13] R. B. Merrifield, J. Amer. Chem. Soc. **85,** 2149–2154 (1963).
[14] L. A. Carpino and G. Y. Han, J. Amer. Chem. Soc. **92,** 5748–5749 (1970).
[15] E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, and R. B. Williams, J. Amer. Chem. Soc. **97,** 6584–6585 (1975).
[16] M. Fujino, M. Wakimasu, and C. Kitada, Chem. Pharm. Bull. (Japan) **29,** 2825–2831 (1981).
[17] J. W. van Nispen, J. P. Poldesdijk, and H. M. Greven, Rec. Trav. Chim. Pays-Bas. **104,** 99–100 (1985).
[18] E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, Anal. Biochem. **34,** 595–598 (1970).
[19] H. J. Streckert, B. Grunert, and H. Werchau, J. Cell Biochem. **30,** 41–49 (1986).
[20] T. L. Goodfriend, L. Levine, and G. D. Fasman, Science **144,** 1344–1346 (1964).
[21] R. J. Berzborn, in: Methods in Enzymol. **69,** (A. San Pietro, ed.), pp. 492–502, Academic Press, New York 1980.
[22] H. Towbin, T. Staehelin, and J. Gordon, Proc. Natl. Acad. Sci. USA **76,** 4350–4354 (1979).
[23] H. H. Robinson and C. F. Yocum, Biochim. Biophys. Acta **590,** 97–106 (1980).
[24] M. Völker, T. Ono, Y. Inoue, and G. Renger, Biochim. Biophys. Acta **806,** 25–34 (1985).
[25] G. Renger, Biochim. Biophys. Acta **440,** 287–300 (1976).
[26] J. Alt, J. Morris, P. Westhoff, and R. G. Herrmann, Current Genetics **8,** 597–606 (1984).
[27] K. Holschuh, W. Bottomley, and P. R. Whitfeld, Nucl. Acids. Res. **12,** 8819–8834 (1984).
[28] O. F. Rasmussen, G. Bookjans, B. M. Stumman, and K. W. Henningsen, Plant Molec. Biol. **3,** 191–199 (1984).
[29] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, Nature **318,** 618–624 (1985).
[30] J. B. Marder, P. Goloubinoff, and M. Edelman, J. Biol. Chem. **259,** 3900–3908 (1984).
[31] G. Regitz and I. Ohad, J. Biol. Chem. **251,** 247–252 (1976).
[32] A. Trebst, Z. Naturforsch. **34c,** 986–991 (1979).
[33] K. E. Steinback, K. Pfister, and C. J. Arntzen, in: Biochemical Responses Induced by Herbicides. ASC Symposium Series 181 (D. E. Moreland, J. B. StJohn and F. D. Hess, eds.), pp. 37–55, American Chemical Society, Washington 1982.

Verantwortlich für den Inhalt: A. KLEMM
Satz und Druck: Allgäuer Zeitungsverlag GmbH, Kempten