Binding of Lipids onto Polypeptides of the Thylakoid Membrane I. Galactolipids and Sulpholipid as Prosthetic Groups of Core Peptides of the Photosystem II Complex

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Photosystem II complexes were prepared from chloroplasts of wild type tobacco *Nicotiana tabacum* var. John William's Broadleaf and from two chlorophyll mutants derived from it, namely *N. tabacum* Su/su and *N. tabacum* Su/su var. Aurea. The hydrophobic peptides of these complexes were analyzed for bound lipid molecules by means of monospecific lipid antisera. A comparison of the peptide composition of the complexes of the three chloroplast types by means of polyacrylamide gel electrophoresis showed that the peptide composition was qualitatively identical. A major quantitative difference referred to a 66 kDa peptide which appeared to be much stronger in gels of photosystem II peptides originating from the yellow-green and the yellow tobacco variety. Furthermore, we were able to show that different SDS polyacrylamide gel electrophoresis runs of the same PS II preparation yielded differences in the band strength of this peptide. Comparative densitometric measurements showed that an increase in this 66 kDa peptide was always correlated with a decrease in the D_1 and D_2 peptides. Obviously, the 66 kDa peptide is the heterodimer of D_1 and D_2. Differences in the peptide composition of photosystem II preparations from the 3 tobacco species refer above all to peptides of the light-harvesting complex with molecular masses of 28 and 26 kDa.

After the transfer of the peptides from the polyacrylamide gel to nitrocellulose membranes, they were incubated with monospecific antisera to monogalactolipid, digalactolipid or sulfolipid. These experiments showed that the 66 kDa peptide reacted with antibodies to digalactolipid and with those to sulfolipid. The 66 kDa peptide reacts in the Western blot procedure also with an antiserum to a 66 kDa peptide prepared and characterized earlier and which was shown to inhibit electron transport reactions in the region of the reaction center of photosystem II. The monospecific antiserum to monogalactolipid reacts with the D_1 and D_2 peptide as well as with the chlorophyll-binding polypeptides of the masses 42 and 48 kDa, and also with the 26 and 28 kDa peptides of the light-harvesting complex as well as with the extrinsic peptides exhibiting the molecular masses, 33, 21–23 and 18 kDa. Whereas lipase treatment apparently destroys the lipids as antigenic determinants of the peptides on the nitrocellulose membrane, periodate treatment or treatment of the photosystem II preparations with organic solvents do not prevent the reaction of the 66 kDa peptide with the sulfolipid antiserum. These experiments show as the 66 kDa peptide appears to be the heterodimer of D_1 and D_2, that the galactolipids mono- and digalactosyldiglyceride as well as the sulfolipid are bound, much like prosthetic groups, onto the core peptides.

Introduction

Since it is known that the lipid environment of photosystem I and photosystem II and that of the cytochrome b_6/f complex in the thylakoid membrane is different, it is assumed that lipids exert certain tasks [1–12]. Our own studies have shown that antisera to glyco- and phospholipids can inhibit photosynthetic electron transport reactions in the region of photosystem II as well as in the region of photosystem I [8, 13–15]. These inhibitory effects were between 20 to 60% and depended on the activity condition of the lamellar system. In earlier work we have interpreted these inhibitory effects on electron transport reactions amongst others by assuming that the binding of antibodies induced a conformational change in certain electron transport components, thus leading to the observed inhibition of electron flux [8, 16]. This requires of course, that lipids as antigenic determinants are bound on these components. By means of the Western blot procedure we are able to show for the first time that the glycolipids, mono- and digalactosyldiglyceride as well as sulfoquinovosyl-

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diglyceride are bound onto various polypeptides of the photosystem II complex [17]. In the present publication we make a detailed analysis on the presence of the glycolipids bound onto peptides of the photosystem II complex. Furthermore, we compare glycolipid binding onto these peptides in photosystem II complexes from wild type tobacco, *N. tabacum* var. John William's Broadleaf, and two chlorophyll mutants, namely the yellow-green mutant *N. tabacum* Su/su and the yellow mutant *N. tabacum* Su/su var. Aurea. The mutants differ from the wild type by their chloroplast structure with the yellow-green mutant having lower grana stacks and more extended intergrana regions whereas the structure of the lamellar system of the yellow mutant consists just of membrane doublings and very extended intergrana lamellae [18, 19]. As membrane stacking was assumed to be correlated with the presence or absence of certain glycolipids we thought to include this aspect in the present analysis [20].

Materials and Methods

Plant material and photosystem II preparations

Photosystem II preparations were prepared from wild type tobacco *N. tabacum* var. JWB, the yellow-green mutant *N. t.* Su/su and the yellow mutant *N. t.* Su/su var. Aurea. The plants were grown in a climatized growth chamber with a light/dark cycle of 16/8 h at a day temperature of 27 °C, a night temperature of 22 °C and constant 60% relative humidity. Photosystem II preparations from the three tobacco varieties were prepared according to Berthold *et al.* [21]. In the isolation procedure of photosystem II from the yellow mutant, due to the low yield, the second Tris treatment described there [21] had to be omitted.

SDS polyacrylamide gel electrophoresis

The polypeptides of the respective photosystem II preparations were modified in the SDS polyacrylamide gel electrophoresis according to the methods of Weber and Osborn [22] and Laemmli [23] and analyzed in a 12.5% separation gel and 3.8% collection gel. The 9 cm high separation gel was covered during 1 h with a layer of water-saturated isobutanol, then overlaid with water and permitted to polymerize during 18 h. The water was removed and the separation gel covered with the collection gel. For shaping the sample pockets the comb was inserted air bubble-free into the gel. After 1 h of polymerization the samples were introduced and the electrophoresis started. Electrophoresis was carried out at 4 °C and a current of 0.12 mA/cm^2 in an electrophoresis buffer containing 0.32 M Tris, 0.25 M glycine, 4 M urea and 8.7 mM sodium dodecyl sulfate. Subsequently, the gels were stained for 12 h with Coomassie Blue G-250 and then destained. The staining solution contained 0.1% Coomassie Blue G-250 in an acetic acid/methanol/water mixture having the volume portions 7/25/68. For destaining, the solvent mixture without dye was used. In order to prepare the gel electrophoresis, 30 µl sample buffer containing 0.2 M Tris, pH 6.8, 9 M urea, 0.14 M SDS and 4% mercaptoethanol, were added to the respective photosystem II preparation corresponding to 30 µg chlorophyll and incubated for 2 h. The samples were supplemented with a drop of 60% sucrose and filled into the gel pockets.

Composition of the 12.5% separation gel

16 ml of a 30% aqueous acrylamide and 0.8% bisacrylamide solution, 4.8 ml 3 M Tris, pH 8.8, 0.384 ml 10% aqueous SDS solution, 19 µl TEMED (= N,N, N′N′-tetramethylethylendiamine), 192 µl 10% ammonium persulfate and 10.4 g urea are filled up with water to give a total volume of 38.4 ml.

Composition of the 3.8% collection gel

1.27 ml 30% acrylamide and 0.8% bisacrylamide solution, 1.27 ml 1 M Tris, pH 6.8, 0.1 ml 10% SDS, 1.5 ml 60% sucrose, 7 µl TEMED, 64 µl 10% ammonium persulfate and 2.7 g urea are filled up with water to give a total volume of 10 ml.

Transfer of the peptides and marking with antibodies

The developed SDS polyacrylamide gels containing the peptides were washed with transfer buffer (10 mM Tris, pH 8.8, 2 mM EDTA, 50 mM NaCl and 0.1 M dithiotreitole (DTE)) and the peptides transferred by diffusion at room temperature during 20–30 h or at 4 °C during 50–60 h to the cellulose nitrate membranes (Schleicher & Schüll,

Dassel, Cellulosenitrat-Membranen BA 85) according to the procedure by Rennart *et al.* [24]. The membranes with the peptides were washed 3 times with a buffer containing 5.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.1 M NaCl, 2.6 mM KCl and 2.5% fish gelatine. As the glycolipid antisera also contain antibodies to methylated bovine serum albumin, saturation of the free cellulose nitrate membrane regions was not done in the present analysis with bovine serum albumin but with 2.5% fish gelatine. For the reaction with lipid antibodies, the membranes were gently shaken for 1 h with the glycolipid antisera. For this purpose the digalactolipid and sulfolipid antisera were diluted 1/25 with the above buffer, containing instead of fish gelatine 0.5% ovalbumin. The dilution of the monogalactolipid antiserum was 1/100. Thereafter, we incubated with a 1/100 diluted peroxidase-conjugated anti-rabbit IgG (pig) during 30 min in the dark (preparation: DAKO Diagnostika GmbH, Hamburg; peroxidase-conjugated swine immunoglobuline to rabbit immunoglobulines). All further steps were also carried out in the dark. In order to make the antigen-antibody complexes visible the cellulose nitrate membrane strip was given into 5 ml of developer solution containing Tris 50 mM, pH 7.35, and 3 mg 4-chloro-1-naphthol dissolved in 1 ml methanol. In order to start the reaction 1 µl 30% H_2O_2 was added and the nitrate cellulose strip was gently shaken for a few minutes in this mixture. The membrane strips were washed with water and stored between filter-paper in the dark.

Pretreatment of the photosystem II preparations with organic solvents, periodate or with lipase

a. Extraction with organic solvents: A sedimented photosystem II preparation was suspended in fourfold its quantity of methanol and was shaken for 10 min at room temperature, then the preparation was centrifuged for 5 min at 6000 × *g* and the pellet washed twice in 20 mM Hepes, pH 6.5, containing 15 mM NaCl, 5 mM $MgCl_2$ and 400 mM sucrose. The sediment remaining after the second wash was suspended in the same Hepes buffer as before and used for SDS gel electrophoresis. Photosystem II preparations were treated according to this procedure also with acetone and chloroform.

b. Periodate treatment: For sugar oxidation a photosystem II sediment was suspended in the above (under a.) described Hepes buffer, containing 5% sodium periodate and gently shaken for 12 h at room temperature. Thereafter, the preparation was, as described above, used for SDS gel electrophoresis.

c. Lipase treatment of the polypeptides: Cellulose nitrate membranes holding the transferred photosystem II peptides were shaken at room temperature for 30 min in a buffer containing 5.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.15 M NaCl, 2.6 mM KCl and supplemented with 1/100 dilution of lipase from *Rhiziopis arrhizus* (Boehringer Mannheim, 50,000 units/ml). Thereafter, saturation of the free cellulose nitrate membrane regions was carried out with a 3% fish gelatine solution followed by the incubation with the glycolipid antisera.

Antisera

The preparation of monospecific antisera to the monogalactolipid, digalactolipid and to the sulfolipid as well as that to the 66 kDa peptide has been described in earlier publications [25–28]. The monospecificity of these antisera has been established by means of the passive heme agglutination test and the ELISA test. Demonstration of monospecificity by the Dot blot procedure is given in the following: 60 ng of mono-, digalacto- or sulfolipid in an emulsion which contained 1% desoxycholate were dropped onto cellulose nitrate membrane (Schleicher & Schüll, Ba 85) and incubated with the respective lipid antisera. The serum dilutions are given in Fig. 1. The demonstration of the oc-

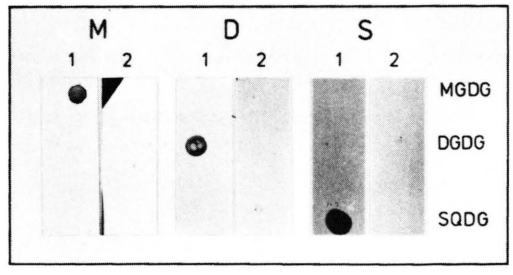

Fig. 1. Demonstration of monospecificity of antisera to glycolipids in the dot blot assay. *Antigen:* MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; SQDG, sulfoquinovosyldiglyceride. The concentration of the lipids used is in all cases 60 ng. *Antisera:* M_1, antiserum to MGDG (serum dilution 1:100). D_1, antiserum to DGDG (serum dilution 1:25); S_1, antiserum to SQDG (serum dilution 1:25), M_2, D_2 and S_2, control serum (serum dilutions as indicated for the respective antisera).

currence of the antigen-antibody reaction was carried out according to the Western blot procedure, as described above. As seen in Fig. 1 the lipid antisera react only with the homologous lipids. Cross reactions do not occur. In order to verify the sensitivity of this test on lipid antibodies on cellulose nitrate membranes, glycolipids were applied in a concentration series of 0.5, 5, 15, 30 and 60 ng. The incubation with the homologous antisera showed, that from a concentration of 5 ng onward a positive reaction was observed (Fig. 2). With increasing amounts of the lipid antigen, the intensity of the color reaction increases also. For the test in the Western blot procedure IgG molecules were concentrated from the sera by precipitation with ammonium sulfate.

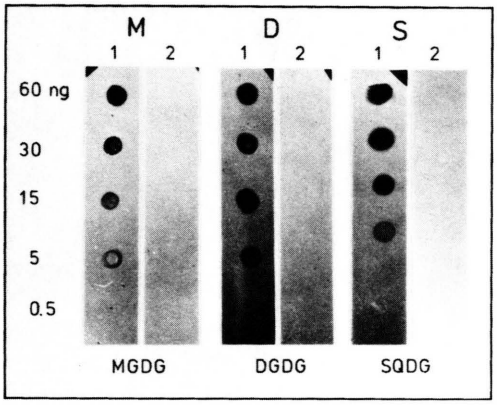

Fig. 2. Reaction of the glycolipid antisera in dependence on the concentration of lipids in the dot blot procedure. *Antigen:* MGDG, monogalactosyldigyceride; DGDG, digalactosyldiglyceride; SQDG, sulfoquinovosyldiglyceride. The concentration of the lipids used range from 0.5–60 ng. *Antisera:* M_1, antiserum to MGDG (serum dilution 1:100); D_1, antiserum to DGDG (serum dilution 1:25); S_1, antiserum to SQDG (serum dilution 1:25), M_2, D_2, S_2 are control sera (serum dilutions as indicated for the respective antisera).

Chlorophyll and protein determinations

Quantitative protein determinations were carried out with the Folin-phenol reagent according to Lowry *et al.* [29]. The marker used was bovine serum albumin. Chlorophyll determinations were carried out in 10% aqueous methanol according to Schmid [30]. Densitometric measurements of stained SDS gels were carried out with the device Chromoscan 3 from Joyce Loeble at 626 nm and a slit width of 0.3 mm.

Results

In order to address the question whether glycolipids are bound onto peptides of the photosystem II complex, analyses by SDS gel electrophoresis were carried out with photosystem II preparations of wild type tobacco *N. tabacum* var. John William's Broadleaf, of the yellow-green tobacco mutant Su/su and of the yellow mutant Su/su var. Aurea. The qualitative peptide composition is except for a 66 kDa peptide in the three preparations the same (Fig. 3). The preparations consist of the reaction center peptides D_1 and D_2 (32 and 34 kDa), of the two chlorophyll-binding peptides with the molecular masses of 43 and 48 kDa, the 26 and 28 kDa peptides of the light-harvesting complex, of the apoprotein of cytochrome b_{559} with the molecular masses of 10 and 4 kDa and of the peptides of the oxygen-evolving complex (OEC) having molecular masses of 33, 23 and 16 kDa. Quantitative differences exist with respect to the peptides of the light-harvesting complex, which occur in the highest concentration in preparations from the wild type. In photosystem II preparations of the yellow-green tobacco mutant Su/su and especially in those of the yellow aurea mutant a 66 kDa peptide occurs. The concentration of this

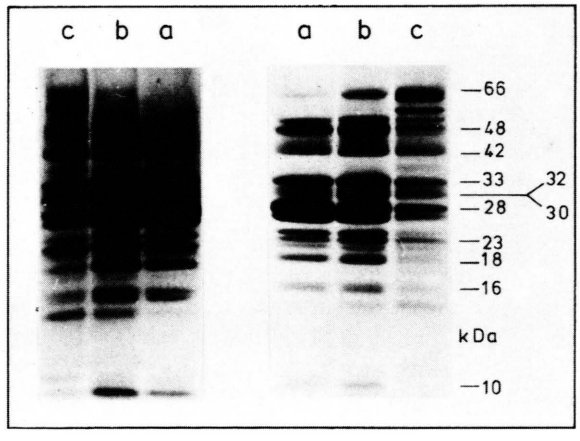

Fig. 3. Comparison of the polypeptides of photosystem II from the wild type *Nicotiana tabacum* var. John William's Broadleaf a) and of the mutants *N. t.* Su/su b) and *N. t.* Su/su var. Aurea c) in SDS polyacrylamide gels; staining with amido black.

peptide may come out different in different runs of SDS polyacrylamide gel electrophoresis of the same photosystem II preparation (Fig. 3). Also in photosystem II preparations from the wild type this peptide band may occur occasionally. The peptide composition of the photosystem II complexes of *N. tabacum* preparations agrees with those described for higher plant preparations in the literature [31–34]. In the tobacco aurea mutant *N. tabacum* Su/su var. Aurea a 56 kDa peptide is observed in addition.

We first studied the provenance of the 66 kDa peptide. It appeared improbable that the peptide was a contamination from photosystem I, since, as mentioned above, in a given preparation the peptide might occur in different electrophoresis runs or be absent. The assumption, that the peptide was an aggregation product, was tested by densitometric measurements of the bands of different electrophoresis runs in photosystem II preparations of the Su/su mutant which showed or did not show this peptide band. A comparison of these densitograms is shown in Fig. 4a and b. It is seen that the occurrence of the 66 kDa peptide is correlated with a decrease of the D_1 and D_2 peptides. For the exact analysis the areas of the D_1 and D_2 peak in the densitogram were related to the total densitogram area (Fig. 4a). In Fig. 4b the band areas of D_1 and D_2 and that of the 66 kDa peptide were compared to the total area of all recorded bands. In both cases these areas represent 13% of the total densitogram area. This means that a direct correlation exists between the 66 kDa peptide and the D_1 and D_2 peptides in these SDS polyacrylamide gel electrophoresis runs of the Su/su preparation. Thus, it appears that the 66 kDa peptide is the heterodimer of D_1/D_2. On the other hand the densitogram also shows, that the occurrence of the 66 kDa peptide is also correlated with a reduction of the 23 and 42 kDa peptides (Fig. 4a and b). A calculation of the areas according to the above described method also seems to lead to the conclusion, that a relationship exists between the 23 and 42 kDa peptides on the one side and the 66 kDa peptide on the other.

For the test of binding glycolipids onto the peptides of the photosystem II complexes of the three tobacco varieties, the peptides, separated by SDS polyacrylamide gel electrophoresis, were transferred by diffusion to cellulose nitrate membranes

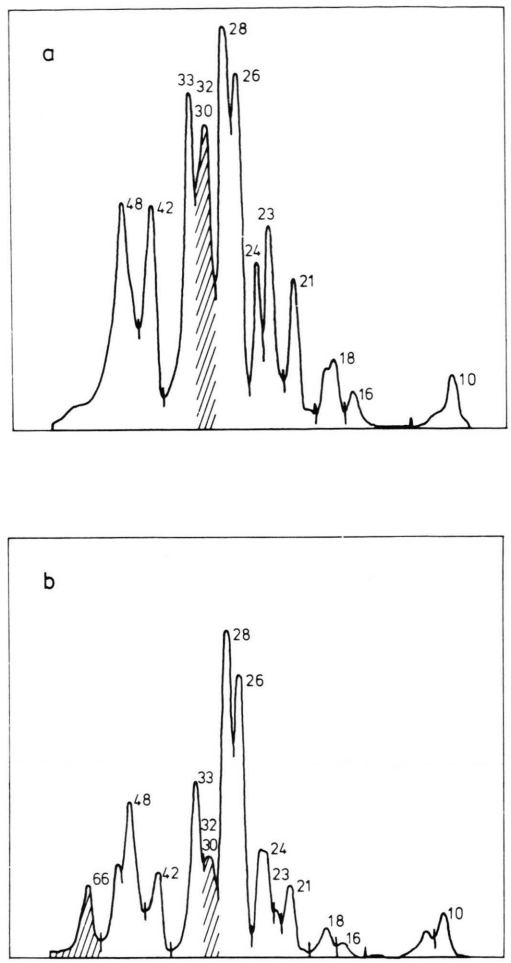

Fig. 4. Densitograms of the polypeptide patterns of photosystem II particle preparations of the tobacco mutant *N.t.* Su/su. a. Preparation without 66 kDa peptide. b. Preparation with 66 kDa peptide.

and incubated with the monospecific lipid antisera.

As seen from Fig. 5, 6, and 7 antisera to glycolipids react with certain peptides. Thus, the antiserum to monogalactolipid marks the two hydrophobic reaction center peptides D_1 and D_2 as well as the two chlorophyll-binding peptides with the molecular masses 43 and 48 kDa, the 26 and 28 kDa peptides of the light-harvesting complex and the three extrinsic peptides of the oxygen-evolving complex, having the molecular masses 18 [21–23] and 33 kDa. Among all photosystem II peptides of the wild type and the chlorophyll mu-

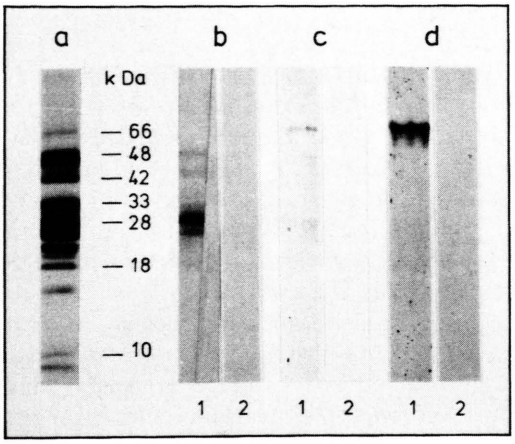

Fig. 5. Demonstration of binding of monogalactosyldiglyceride, digalactosyldiglyceride and sulfoquinovosyldiglyceride onto peptides of the reaction center of photosystem II from the wild type of *Nicotiana tabacum* var. John William's Broadleaf in the Western blot procedure. a. Polypeptides of photosystem II in the SDS polyacrylamide gel electrophoresis (amido black staining). b. Nitrocellulose membrane with polypeptides of photosystem II after the reaction with 1) the antiserum to monogalactolipid, 2) the control serum (serum dilution 1:100). c. Nitrocellulose membrane with polypeptides of photosystem II after reaction with 1) the antiserum to digalactolipid, 2) the control serum (serum dilution 1:25). d. Nitrocellulose membrane with polypeptides of photosystem II after reaction with 1) the antiserum to sulfolipid. 2) the control serum (serum dilution 1:25).

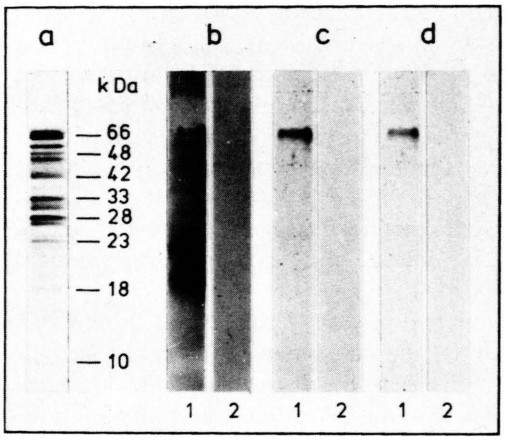

Fig. 7. Demonstration of binding of monogalactosyldiglyceride, digalactosyldiglyceride and sulfoquinovosyldiglyceride onto peptides of the reaction center of photosystem II from the aurea mutant *Nicotiana tabacum* Su/su var. Aurea in the Western blot procedure. a. Polypeptides of photosystem II in the SDS polyacrylamide gel. b. Nitrocellulose membrane with photosystem II peptides after reaction with 1) the antiserum to monogalactolipid, 2) the control serum (serum dilution 1:100). c. Nitrocellulose membrane with photosystem II peptides after reaction with 1) the antiserum to digalactolipid 2) the control serum (serum dilution 1:25). d. Nitrocellulose membrane with photosystem II peptides after reaction with 1) the antiserum to sulfolipid, 2) the control serum (serum dilution 1:25).

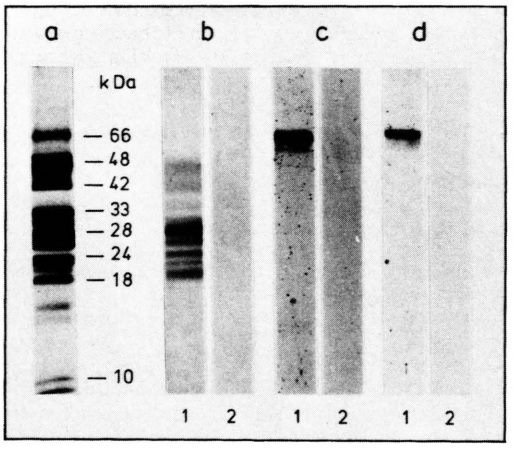

Fig. 6. Demonstration of binding of monogalactosyldiglyceride, digalactosyldiglyceride and sulfoquinovosyldiglyceride onto peptides of the reaction center of photosystem II from the chlorophyll mutant *Nicotiana tabacum* Su/su in the Western blot procedure. a. Polypeptides of photosystem II in the SDS polyacrylamide gel. b. Nitrocellulose membrane with photosystem II peptides after reaction with 1) the antiserum to monogalactolipid, 2) the control serum (serum dilution 1:100). c. Nitrocellulose membrane with photosystem II polypeptides after reaction with 1) the antiserum to digalactolipid, 2) the control serum (serum dilution 1:25). d. Nitrocellulosmembrane with photosystem II peptides after reaction with 1) the antiserum to sulfolipid 2) the control serum (serum dilution 1:25).

tant Su/su the two peptides of the light-harvesting complex are marked strongest. With lower intensity the two hydrophobic peptides D_1 and D_2 react, followed by the chlorophyll binding 42 and 48 kDa peptides and the extrinsic 33 kDa peptide. In the case of the yellow tobacco mutant Su/su var. Aurea all peptides in the region of 66 kDa to 18 kDa were strongly marked. However, peptide bands are diffuse, thus not permitting precise localizations.

In comparison to this the sulfolipid and digalactolipid antiserum only reacts with the 66 kDa peptide. All other peptides are not labelled (Fig. 5–7). The intensity of this label depends on the quantitative occurrence of the peptide. Obviously, if in preparations of the wild type and the yellow-green mutant no 66 kDa peptide is detected no labelling by the digalactolipid and the sulfolipid antisera is observed in this molecular mass region.

In the preparations of the yellow mutant Su/su var. Aurea in which the 66 kDa peptide always occurs to a more or lesser extent in all analyses the peptide was intensively labelled by both glycolipid antisera. Control experiments under identical conditions with the corresponding control sera did not lead to a reaction (Fig. 5–7). The described labelling by glycolipid antisera only occurred if the antisera were used in relatively high concentration. Whereas protein and peptide antisera in Western blot analysis still yield positive reactions at dilutions up to $1/1000$, the antiserum to digalactolipid and to the sulfolipid were used in the present study in a $1/25$ dilution and that of the monogalactolipid in a dilution of $1/100$.

In the following we had to verify whether the glycolipids, functioning as antigenic determinants, are just weakly adsorbed (*e.g.* during the isolation procedure) onto the respective polypeptides or whether they are chemically *i.e.* covalently bound onto these. For this purpose the isolated photosystem II preparations of the mutant Su/su were washed with various unpolar and also polar organic solvents such as chloroform, acetone and methanol. Thereafter, the analysis of the peptides in the SDS polyacrylamide gel electrophoresis was carried out. After the transfer of the peptides to cellulose nitrate membranes they were incubated with the respective glycolipid antiserum. The antiserum to sulfolipid and the one to galactolipids reacts with the 66 kDa peptide. A comparison with the not washed preparation showed, that the intensity of the color reaction in peptides, washed with organic solvents is weaker. This shows that at least part of the sulfolipid and digalactolipid molecules are tightly bound and are not washed away with organic solvents. In order to obtain information on the chemical nature of the antigenic determinants, photosystem II preparations were treated with 5% sodium periodate. The subsequent SDS gel electrophoresis, the transfer of the peptides to cellulose nitrate membranes and incubation with antisera led to the result, that neither the sulfolipid not the galactolipid label had been removed by this procedure, although the sugar molecules had been oxidatively removed from the lipid molecule. This means that the antigenic region is not exclusively made up by sugar molecules, but that the antibody specificity is directed beyond the sugar molecule towards the glycerol-fatty acid region. In a further experiment the effect of lipase from *Rhizobis arrhizus* (Boehringer) on the antigenic determinant was studied. In this case not the photosystem II prepa-

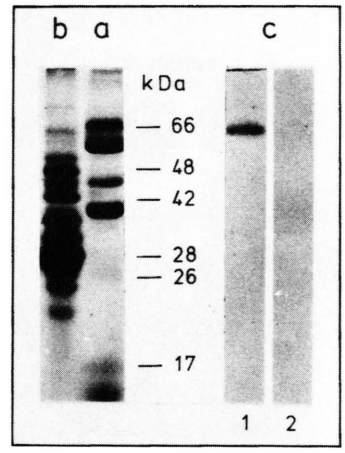

Fig. 8. Reaction of an antiserum to a 66 kDa polypeptide with the 66 kDa polypeptide (*i.e.* the D_1/D_2 dimer) of a photosystem II preparation from the mutant *Nicotiana tabacum* Su/su in the Western blot procedure. a. Marker proteins in a 7–12% polyacrylamide gradient gel: bovine serum albumin 67 kDa, glutamate dehydrogenase 56 kDa, ovalbumin 45 kDa, D-aminoacid oxidase 37 kDa, hexokinase 25 kDa and myoglobin 17 kDa. b. Photosystem II polypeptides in the SDS polyacrylamide gel. c. Nitrocellulose membrane with the photosystem II peptides after the reaction with 1) the antiserum to a 66 kDa peptide (serum dilution 1 : 1000) 2) the control serum (serum dilution 1 : 100).

rations but the cellulose nitrate membranes containing the transferred peptides of the photosystem II complexes of the yellow-green Su/su mutant were treated with the enzyme. It is seen that the reaction of the 66 kDa peptide with the sulfolipid antiserum was lost by this treatment, which means that separation of the sulfoquinovosyldiglyceride portion from the fatty acids removes the antigenic property of the sulfolipid molecule.

From the results obtained by SDS gel electrophoresis and the evaluation of the densitograms it is concluded that the occurrence of the 66 kDa peptide in photosystem II preparations is the result of an aggregation of the D_1 and D_2 peptide, and hence represents a heterodimer. This result fits an earlier observation of Koenig *et al.* who showed that an antiserum to a 66 kDa peptide of the thylakoid membrane inhibited photosystem II reactions [28]. And indeed, we were able to show as seen in Fig. 8, that the described 66 kDa peptide reacts with this antiserum. However, this antiserum does not react with the isolated D_1 or the D_2 peptide.

Discussion

By means of the Western blot technique and by using monospecific polyclonal antisera to glycolipids we have shown for the first time that glycolipids are bound onto polypeptides of photosystem II. Whereas the digalactolipid antiserum and that to sulfolipid reacts in all the *Nicotiana tabacum* varieties which are *N. t.* var. John William's Broadleaf (wild type), the yellow-green mutant *N. tabacum* Su/su and the yellow mutant *N. t.* Su/su var. Aurea, exclusively with the 66 kDa peptide, the antiserum to monogalactolipid does not react with it, but reacts with all other peptides of the photosystem II complex which are the D_1, D_2 peptides the chlorophyll-binding peptides of the proximal antenna, the 3 extrinsic peptides of the oxygen-evolving complex and the peptides of the light-harvesting complex. As the experiments show that lipase treatment, that is treatment with a lipid-splitting enzyme, removes the antigenic determinants, it must be concluded that the lipids are bound and are not just unspecifically adsorbed onto the peptides. It therefore appears, that the lipids are specifically bound to defined peptides.

The affiliation of the peptide with the molecular mass 66 kDa to photosystem II has been demonstrated by the present studies. Thus, densitometric analysis of the peptide distribution pattern have clearly established the interrelationship between the 66 kDa peptide and the hydrophobic, diffuse D_1 and D_2 bands, identifying the 66 kDa peptide as the heterodimer of D_1 and D_2. An explanation, however, has to be found for the fact the antiserum to the sulfolipid and that to digalactolipid do not label the monomeric hydrophobic peptides and that the antiserum to the monogalactolipid does not label the 66 kDa peptide. From investigations of several laboratories it is well known that during SDS gel electrophoresis of hydrophobic peptides of the photosynthesis membrane of bacteria and of higher plants polypeptide aggregations occur formed by a mixed aggregation of peptides with lower molecular masses. In addition to this, decomposition of peptides into species with lower molecular masses might occur. Therefore, the provenance of such polypeptide aggregates is relatively easily traced back by immunological cross reactions if the basic structure and thus the antigenic determinants are conserved during aggregation. Thus, Menke and Koenig report already in 1980 [35] that during gel chromatographic separation and characterization of the total peptides of the thylakoid membrane of *Antirrhinum majus* on Cl-6B Sepharose and hydroxylapatite, 66 kDa fractions, when subjected to pH changes, led to peptide fractions with lower molecular masses. Satoh describes the formation of higher polypeptide aggregates from peptides with the molecular masses 43 and 27 kDa [36]. Greenberg *et al.* [37] as well as Wälzlein and Pistorius [38] show that during the analysis of peptides from *Spirodella oligorrhiza* and *Anacystis nidulans* a D_1 core peptide cleavage product of the masses 23.5 and 25 kDa respectively is observed. However, in both cases these degradation products with the lower molecular mass reacted with the antiserum to the core peptide D_1. Nanba and Satoh [39] and Marder *et al.* [40] as well as Barber *et al.* [41] have isolated the core complex "D_1 D_2 cytochrome b_{559}" from higher plants. It should be noted that during these isolation procedures dimers with the molecular mass of 60 kDa occurred. This core complex of higher plants (*Spinacia* and *Pisum*) contains approx. 4 chlorophyll molecules, 2 pheophytin molecules, 1 β-carotin as well as 2 non-heme iron atoms and cytochrome b_{559}.

The amino acid sequences of D_1 and D_2 have impressing homologies with the L and M subunit of photosynthetic bacteria [42–45]. These peptides form in the reaction center a dimer and the same is assumed for D_1 D_2 in the photosystem II complex of higher plants. Under certain conditions of the SDS polyacrylamide gel electrophoresis this dimer can apparently be isolated as such, exhibiting a molecular mass of 66 kDa. Our investigations clearly show that the conformation of this dimer must be drastically different from that of the monomeric peptides D_1 and D_2. In the native condition bound sulfolipid and digalactolipid are localized as prosthetic groups in a way in the surface of the heterodimer, as to be accessible to antibodies. This also explains that an earlier described antiserum to a 66 kDa peptide which inhibited photosystem II reactions [28, 34] also reacts in the Western blot analysis with our 66 kDa peptide. Decomposition of the dimer either leads to the cleavage of the lipids or these hydrophobic groups are in the course of a conformational change turned to the inside of the monomeric peptides. This might lead to what is experimentally observed namely that certain antigenic determinants are altered, rearranged or covered in such a way that neither the glycolipid antisera nor the antiserum to the 66 kDa peptide react with D_1 and D_2. In this sense it might seem thinkable that the two bound lipid types namely glycolipid and sulfoquinovosyldiglyceride finally serve to hold the dimer together.

The monogalactolipid seems functionally related to the light-harvesting complex. According to investigations of Siefermann-Harms [46] this lipid might play a role in energy transfer in the light-harvesting complex. Our studies do not permit yet to estimate the number of lipid molecules bound to the respective peptides.

With the observation that the glycolipids mono- and digalactosyldiglyceride as well as the sulfolipid are bound as prosthetic groups onto the core peptides of photosystem II, our earlier experiment in which we were able to show that antisera to these lipids affect electron transport reactions on the donor side of photosystem II, find an explanation.

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