Functional Characterization of Four Thylakoid Membrane Polypeptides with Apparent Molecular Weights between 40 000 and 48 000

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Four polypeptides which exhibited apparent molecular weights from 40 000 to 48 000 were isolated from the thylakoid membrane. The isolation was achieved by gel filtration of polypeptides, solubilized by means of sodium dodecyl sulfate and, after removal of detergent by anion exchange chromatography in ethanolic solution. The polypeptide of the molecular weight 40 000 was identified as the γ-component of the coupling factor of photophosphorylation via the effect of its antiserum and by its serological cross reactions. The antiserum not only inhibits photophosphorylation reactions by 90% but also to the same extent the photoreduction of anthraquinone-2-sulfonate with tetramethyl benzidine as the electron donor, provided ADP, Pi and Mg^{2+} are present in the assay. The inhibition of electron transport is accompanied by a corresponding increase in the fluorescence yield. The other three antisera inhibit photosystem I reactions. In addition, the antiserum designated 45 000 PSI-1 inhibits phenazine methosulfate-mediated cyclic photophosphorylation. This antiserum as well as the two other sera do not contain any detectable anti-coupling factor activity. Antiserum 45 000 PSI-4, in contrast to 45 000 PSI-1, does not inhibit cyclic photophosphorylation. Moreover, it is the only one of the four antisera which does not affect the ratio of the fluorescence yields measured at 735 and 685 nm (F 735/F 685) at 77 °K. Hence, the antigen 45 000 PSI-4 probably plays a role on the acceptor side of photosystem I. On the other hand, the antiserum 48 000 PSI-3 seems to exert its effect on the donor side of photosystem I, because it inhibits the photoreduction of anthraquinone-2-sulfonate with low concentrations of the electron donor dichlorophenol indophenol. The prominent property of this antiserum is that the photophosphorylation with ferricyanide as the electron acceptor is stimulated more than two-fold. This, however, it not accompanied by an apparent change of the electron flux between water and ferricyanide. The antiserum causes a decrease of fluorescence yield which is probably due to an increased energy spill-over. All antisera exert their activity only after an illumination of the chloroplast preparations for several minutes. The dosis-effect curve is hyperbolic for the antiserum 48 000 PSI-3 and is sigmoidal with the other three antisera.

As the major part of the membrane proteins is insoluble, the methods which are usually applied to soluble proteins cannot be used for the functional and chemical characterization of proteins of the thylakoid membrane. In order to circumvent the difficulties arising from this fact, different possibilities exist (for ref. see [1]).

The attempt to classify which protein belonged to which photosystem, was carried out with mutants by subjecting the solubilized thylakoid membrane proteins of mutants and the wild type to gel electrophoresis [2–8]. A prerequisite of this method is that large numbers of mutants are available and that the defects are functionally sufficiently characterized.

An other possibility for the characterization of proteins is the investigation by means of gel electrophoresis of thylakoid fragments which still carry out photochemical reactions thus determining which polypeptides are contained and which ones are lacking [9–14]. However, it is difficult to decide whether the proteins of a given particle fraction belong to the reaction centers at all or in what type of reactions they are involved.

After having demonstrated that it is possible to inhibit electron transport in the thylakoid membrane by means of specific antisera [15, 16], we attempted to obtain more information on the molecular structure of the thylakoid membrane and its relationship to function by the use of serological methods. For this purpose it was necessary to isolate the individual polypeptides of the thylakoid membrane in substantial amounts. Since Smith and Pickels it is known that insoluble chloroplast pro-

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Abbreviations: DCPIP, 2,6-dichlorophenol indophenol; PMS, phenazine methosulfate; DCMU, N,N′-3,4-dichlorophenyl dimethylurea; Q, quencher of photosystem II; Q_{red}, reduced form; Q_{ox}, oxidized form; P_{680}, reaction center chlorophyll of photosystem II.

teins can be dissolved by means of detergents [17, 18]. As sodium dodecyl sulfate has the strongest dissociating properties, we used this detergent despite the fact that antigens after its removal are denatured [19]. It is somewhat surprising that the denatured polypeptides yielded antisera which reacted with the native thylakoid membrane.

We succeeded in functionally characterizing quite a number of polypeptides and localizing them in the thylakoid membrane [20 – 24]. Also lipids of the thylakoid membrane were serologically characterized [25 – 30, 32]. From determinations of the amount of antibodies adsorbed maximally out of monospecific antisera, information on the distribution of proteins and lipids in the thylakoid surface was obtained [31, 33]. In one case intermolecular interactions between lipids and proteins were observed and their consequences for the function were characterized [19].

In the following we report on the effects of four antisera which are directed towards polypeptides with the apparent molecular weights 48 000, 45 000 and 40 000. The polypeptides and their antisera are designated as 48 000 PSI-3, 45 000 PSI-1, 45 000 PSI-4 and 40 000 CF-96. As in the recent publication [24] the first number is the apparent molecular weight, PSI means that the antigen is to be localized in the region of photosystem I and the last number characterizes the antiserum. CF stands for coupling factor of photophosphorylation. In the region of apparent molecular weights 40 000 to 50 000 polyacrylamide gels show after staining at least five bands.

Material and Methods

Stroma-freed chloroplasts of *Antirrhinum majus* were isolated as described earlier [34]. The polypeptide fraction 48 000 PSI-3 originates from chloroplasts which were extracted with a 1 mM EDTA solution, containing 10 mM sodium chloride (pH 7.7). The dissolution was carried out in the earlier described dodecyl sulfate containing phosphate buffer [20]. The fraction 40 000 CF-96 was isolated from the portion of the chloroplasts which was dissolved in 0.01 M sodium phosphate buffer (pH 8.4) containing 0.08% dodecyl sulfate.

The polypeptide fractions 48 000 PSI-3 and 40 000 CF-96 were isolated by means of gel filtration on Sepharose 6B (Pharmacia). Rechromatography was done either on Sepharose 6B or on Sephadex G-150 (Pharmacia).

The starting material for the isolation of the antigens 45 000 PSI-4 and 45 000 PSI-1 was a polypeptide fraction of the apparent molecular weight 45 000. This fraction was obtained by gel filtration on Sepharose 6B. After removal of dodecyl sulfate by chromatography with the anion exchanger AG1-X2 (Bio-Rad) the preparation was subjected to diafiltration against water. The turbid suspension was cleared by the addition of an equal volume of ethanol. To 192 ml of this solution, containing approximately 150 mg of polypeptide, 2 ml pyridine, 4 ml picoline, 3 ml N-ethylmorpholine and 2 ml triethanol amine were added, which resulted in a complete clearing of the solution. The separation was achieved on a column (90 × 2.5 cm), containing the anion exchanger AGl-X8 (Bio-Rad). The column was equilibrated with the above described ethanol containing buffer, which, however, did not contain triethanol amine [35]. The elution was carried out with a pH-gradient of increasing acidity, which was achieved by increasing amounts of acetic acid. The elution of the antigen 45 000 PSI-4 occurs at pH 10.5 to 9.0, that of 45 000 PSI-1 at pH 8.0 to 6.0. The eluates were concentrated in the rotation evaporator at 30 °C after several additions of water and subsequently dialyzed against water, which leads to the precipitation of the protein.

The preparation of the antisera was carried out as described previously [31]. 3 to 7 mg of antigen were necessary for the immunization of one rabbit.

Partial reactions of photosynthesis and photophosphorylation reactions were carried out in the assay conditions described earlier [22, 36, 37]. Chloroplasts of wild type tobacco and of yellow leaf sections of the variegated tobacco mutant NC95 were used [38].

Fluorescence rise curves were measured with a modified Perkin Elmer Fluorescence Spectrophotometer MPF-3 equipped with a Siemens Oscillograph model Oscillar MO 7114. Light was switched on by means of a photographical shutter. Excitation wavelength was 435 nm, with an excitation slit of 40 nm; emission wavelength was 685 nm with the emission slit of 40 nm. Scattered light was eliminated with a filter absorbing radiation below 630 nm. Incident radiant energy was 42 W/m², which is termed "high intensity" in the following.

For low excitation energy the above indicated energy was cut down by use of several scattering filters to approximately 1/500. By this device the exciting beam is broadened to such an extent that it illuminated most of the cuvet. This way it is achieved that the emitted energy is still well measurable. The chloroplast concentration corresponded to 12 μg chlorophyll/ml; the suspension buffer contained 0.05 M Tricine/0.1 M KCl pH 7.2; the assay was 10^{-4} M of anthraquinone-2-sulfonate and 2.7×10^{-3} M of ascorbate. Time resolution for the fluorescence rise kinetics was 5 sec per scale. Initial fluorescence F_0 was measured with a time resolution of 20 msec per scale.

Low temperature fluorescence emission spectra were measured with a Farrand Spectrofluorometer MK 1 equipped with a device for corrected spectra. Because of the strong drift the spectra were recorded in the uncorrected mode and the registration was carried out with a Siemens Oscillograph model Oscillar MO 7114. The registration speed was 150 nm/min. Excitation wavelength was 435 nm, the excitation slit 10 nm. Registration was carried out from 650 nm to 770 nm, with an emission slit of 1 nm. Every registration was repeated 5 times. The chloroplast concentration corresponded to 3 μg chlorophyll/ml assay. The suspension buffer was 0.05 M Tricine/0.1 M KCl pH 7.2, and contained 3.75×10^{-5} M anthraquinone-2-sulfonate and 10^{-3} M ascorbate. Prior to freezing all samples were kept for 15 min in absolute darkness. This was also valid if samples were preilluminated. Preillumination was done by irradiating samples for 3 min with 190 W/m^2, transmitted through a red plexiglas filter with transmission from 580 nm upwards. The light has the same wavelength distribution as that used in the photochemical assay. For the low temperature fluorescence measurements the samples were frozen in quartz tubes (inner diameter 3 mm) in liquid nitrogen.

Results

Effect of the antisera on photochemical reactions with chloroplasts

The four antisera, described in the following, inhibit the photoreduction of anthraquinone-2-sulfonate with the electron donor couple dichlorophenol indophenol (DCPIP)/ascorbate. However, no influence of the antisera on the photoreduction of ferricyanide with water as the electron donor or another suitable artificial donor is observed. Hence, all antisera exert their effect in the region of photosystem I. In detail, however, the antisera differ in very characteristic ways.

The antisera 45 000 PSI-1 and 40 000 CF-96 inhibit phenazine methosulfate (PMS)-mediated cyclic photophosphorylation whereas the antisera 45 000 PSI-4 and 48 000 PSI-3 do not (Table I). The antiserum 48 000 PSI-3 stimulates the photophosphorylation with ferricyanide as the electron acceptor by more than two-fold [2]. However, electron transport between water and ferricyanide in the absence or in the presence of uncouplers is not affected by this antiserum (Table II). The antiserum 48 000 PSI-3 inhibits the photoreduction of anthraquinone-2-sulfonate with DCPIP/ascorbate

Table I. Effect of the four antisera on photophosphorylation reactions in tobacco chloroplasts.

Serum	[μmol [^{32}P] ATP-formed · (mg chlorophyll)$^{-1} \cdot h^{-1}$]		
	PMS cyclic	$H_2O \rightarrow K_3Fe(CN)_6$ non-cyclic	$H_2O \rightarrow$ A-2-sulf non-cyclic
48 000 PSI-3	484 ± 29	180 ± 11	4 ± 2
Control serum	480 ± 28	75 ± 5	55 ± 3
45 000 PSI-4	491 ± 30	76 ± 5	17 ± 1
Control serum	493 ± 30	76 ± 4	57 ± 4
45 000 PSI-1	340 ± 20	75 ± 5	30 ± 2
Control serum	482 ± 29	78 ± 6	55 ± 4
40 000 CF-96	40 ± 2	23 ± 1	17 ± 1
Control serum	486 ± 29	76 ± 3	55 ± 6

The reaction was carried out at 20 °C. Illumination with 550 000 ergs $sec^{-1} \cdot cm^{-2}$ white light. Values are averages of at least 7 individual determinations. The mean error of the average value is indicated.

Table II. Effect of the antiserum 48 000 PSI-3 on the ferricyanide Hill reaction.

	[μmol Ferricyanide reduced · (mg chlorophyll)$^{-1} \cdot h^{-1}$]		
	without uncoupler	+$(NH_4)_2SO_4$	+Gramicidin
48 000 PSI-3	200	1060	830
Control serum	204	1073	821

$(NH_4)_2SO_4$, 10^{-3} M in the assay; gramicidin 0.3 μg/ml assay pH 7.0. Each value is the average of the least 7 individual determinations.

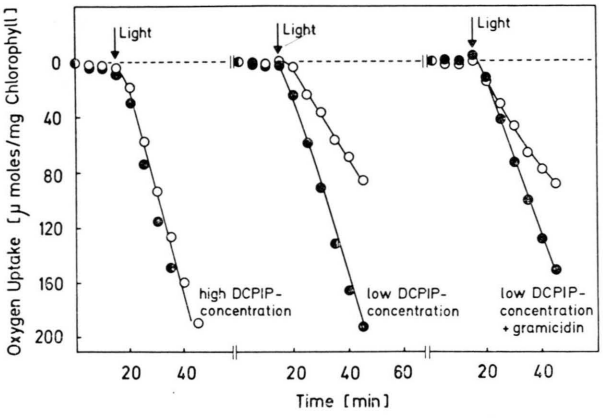

Fig. 1. Effect of the antiserum 48 000 PSI-3 on the anthraquinone-2-sulfonate photoreduction in wild type tobacco chloroplasts with the electron donor couple DCPIP/ascorbate; high concentration of DCPIP is 0.9 mM in the assay, low concentration 0.15 mM. Gramicidin concentration if indicated is 1 μg/ml. The assays contained 10^{-6} M DCMU. The experiment shown in the middle of the graph had been preilluminated, therefore, the inhibition is seen immediately.

as the electron donor if the concentration of DCPIP is low (Fig. 1). This is an indication that the antigen is to be localized between the two photosystems before plastocyanin on the donor side of photosystem I if the interpretation of Fujita and Murano is valid [39]. As the antiserum 45 000 PSI-4 inhibits electron transport only at high DCPIP-concentrations and as no influence of this antiserum on PMS-mediated cyclic photophosphorylation is observed, we conclude that the antigen plays its role on the acceptor side of photosystem I. The localization of the antigens 48 000 PSI-3 and 45 000 PSI-4 outside the reaction center of photosystem I is further supported by the fact, that these antisera do not affect electron transport in chloroplasts of the tobacco mutant NC95 whereas the antisera 45 000 PSI-1 and 40 000 CF-96 inhibit electron transport in this mutant (Fig. 2). In NC95 chloroplasts photosystem II is defective and their photochemical activity in the leaf is probably restricted to cyclic photophosphorylation. The antigen 40 000 CF-96 is identified as the γ-component of the coupling factor [40, 41]. It inhibits electron transport by 90% if the assay contains ADP, Pi and Mg^{2+}. Addition of uncouplers, such as gramicidin, valinomycin and ammonium salts, relieved the inhibition of electron transport by the antiserum. If ADP, Pi and Mg^{2+} are omitted from the assay, electron transport is inhibited by approximately 10% only. The antiserum affects the ATP-ase activity [21] whereas no effect on proton translocation was detected. From these results it might follow that under phosphorylating conditions not more than 10% of the total electron transport are basal electron flow, not coupled to photophosphorylation.

Characteristic differences between the antisera are seen if one plots the effect of the respective antiserum against the amount of antiserum added. The dosis-effect curve for the antiserum 48 000 PSI-3 is hyperbolic (Fig. 3) whereas the curve shape with the other antisera is sigmoidal (Figs 4, 5). From this we infer that with 48 000 PSI-3 the modification of one antigen molecul in a func-

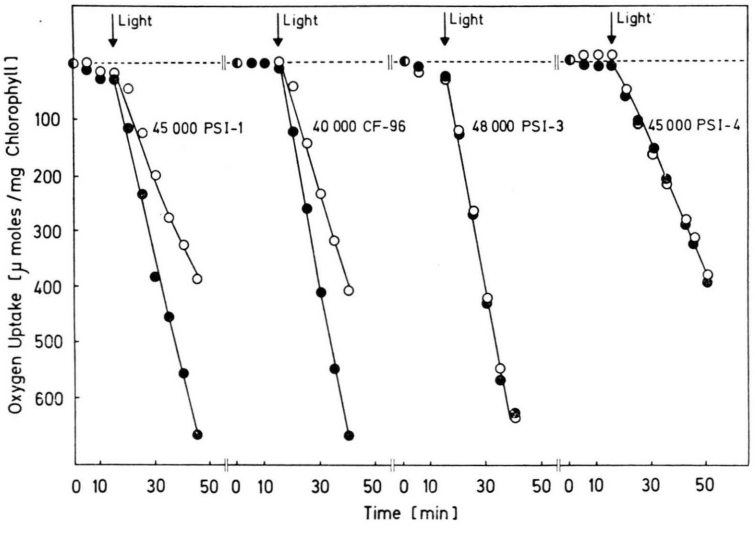

Fig. 2. Effect of the four antisera on the anthraquinone-2-sulfonate photoreduction in chloroplasts of the tobacco mutant NC95 with DCPIP/ascorbate as the electron donor couple. The DCPIP-concentration is 0.9 mM in the assay.

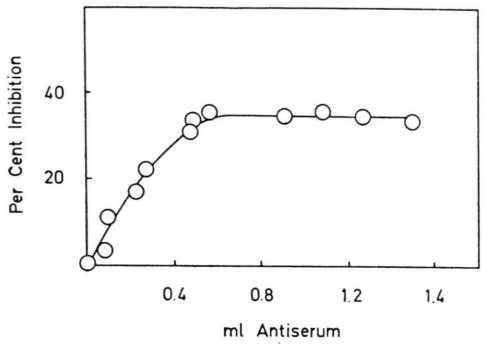

Fig. 3. Dependence of the degree of inhibition of electron transport caused by the antiserum 48 000 PSI-3 on the amount of antiserum added, in wild type chloroplasts. Electron transport reaction DCPIP/ascorbate → anthraquinone-2-sulfonate. DCPIP-concentration 0.15 mM.

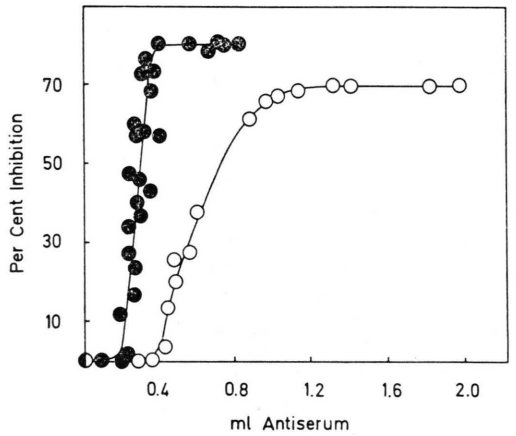

Fig. 4. Dependence of the degree of inhibition of electron transport in wild type tobacco chloroplasts caused by the antiserum 45 000 PSI-1 (○) and 45 000 PSI-4 (●) on the amount of antiserum added. Electron transport reaction DCPIP-ascorbate → anthraquinone-2-sulfonate. DCPIP-concentration 0.9 mM.

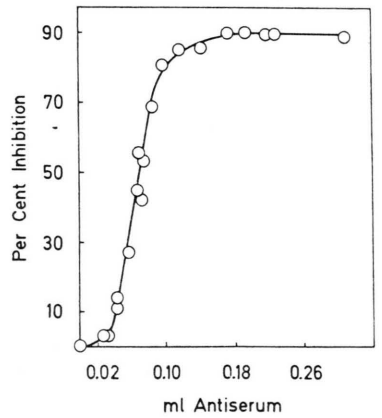

Fig. 5. Same plot as Fig. 4 but for the antiserum 40 000 CF-96.

tional unit is sufficient for the inhibition of electron transport. If, however, the curve shape is sigmoidal, an effect is only observed if in a functional unit, several antigens have reacted with antibodies. With all investigated antisera inhibition is only observed after a few minutes of illumination provided that during the preparation procedure the chloroplasts were sufficiently protected from light. If the illuminating intensity is sufficiently low no effect at all is seen.

Finally, it should be noted that a number of antisera to polypeptide fractions with the apparent molecular weight 45 000 have no effect on electron transport, despite the fact that the antisera agglutinate stroma-freed chloroplasts, that is, the antisera contain antibodies to native proteins. Therefore, it appears probable that the thylakoid membrane either contains polypeptides which play no role in electron transport or that there are antigenic determinants whose reaction with antibodies has no consequence on electron transport.

Effect of the antisera on chlorophyll a fluorescence of chloroplasts

As variable fluorescence depends on the ratio Q_{red}/Q_{ox} and as Q_{red} is oxidized by photosystem I a decrease of electron flux behind Q should result in an increase of the fluorescence yield. This is what is observed with the antisera 45 000 PSI-4, 45 000 PSI-1 and 40 000 CF-96. The increase in fluorescence yield caused by these antisera is considerable (Fig. 6 b and e). At low intensities of the exciting light fluorescence yield is increased approximately two-fold, with an even three-fold increase of the variable portion (Fig. 6 b). At high intensity of the exciting light smaller effects are observed (Fig. 6 e). If DCMU blocks electron transport completely between the photosystems, the fluorescence of the control should come up to the level of the antiserum assay, increasing in turn even the fluorescence of the antiserum assay if the antiserum did not fully inhibit electron transport. After addition of DCMU the predicted increase of the fluorescence yields was observed (Fig. 6 c and f). From this it follows, that these increases of the fluorescence yield depend on Q and that the three antisera inhibit electron transport behind Q. In addition, it is sometimes observed that in the presence of DCMU the assay with control serum fluoresces somewhat more than the assay with anti-

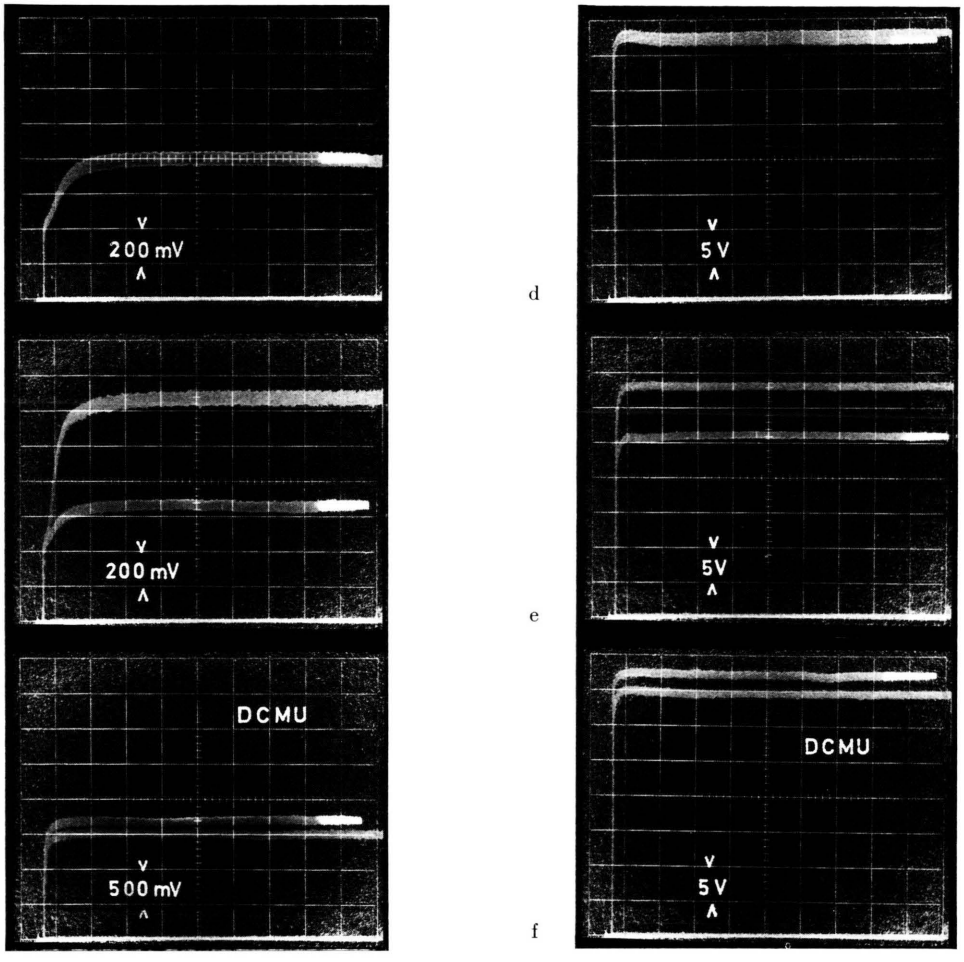

Fig. 6. Effect of antiserum 45 000 PSI-4 on the fluorescence rise. a) and d) not illuminated prior to fluorescence excitation; b) and e) recording after 3 min of preillumination; c) and f) as b) and e) but in the presence of 10^{-6} M DCMU. a), b), and c) low exciting light intensity; d), e) and f) high exciting light intensity. Low exciting light intensity approximately 0.08 W/m²; high light intensity 40 W/m². 1 scale on the abscissa is equivalent to 5 sec. Controls are marked at the end of the curve. Note the different scales with the ordinate. All samples contained the same amount of wild type tobacco chloroplasts.

serum. Changes in the fluorescence yield in the presence of DCMU are often due to changes in the spill-over of excitation energy from photosystem II to photosystem I. If this is valid in the present case, the antisera cause in addition to an increase of the fluorescence yield which is due to the inhibition of electron transport, a slight increase in the efficiency of energy migration. This in turn might be due to an unspecific alteration of the molecular structure of the thylakoid membrane by the antibodies. Whether this alteration occurs only in the presence of DCMU is not entirely clear. With specific effects of the individual antisera on spill-over which are described below, no relationship seems to exist. The described fluorescence effects are not seen in chloroplasts which are well adapted to the dark (Fig. 6 a and d). Just as the inhibition of photochemical reactions these effects are only observed after the illumination during several minutes and persist also after a prolonged dark period.

In contrast to expectations 48 000 PSI-3 lowers the fluorescence yield despite the fact that chloroplasts were well adapted to the dark (Fig. 7 a and e). Initial fluorescence F_0 might be lowered somewhat. DCMU increases the fluorescence yield in both samples to a considerable extent (Fig. 7 b

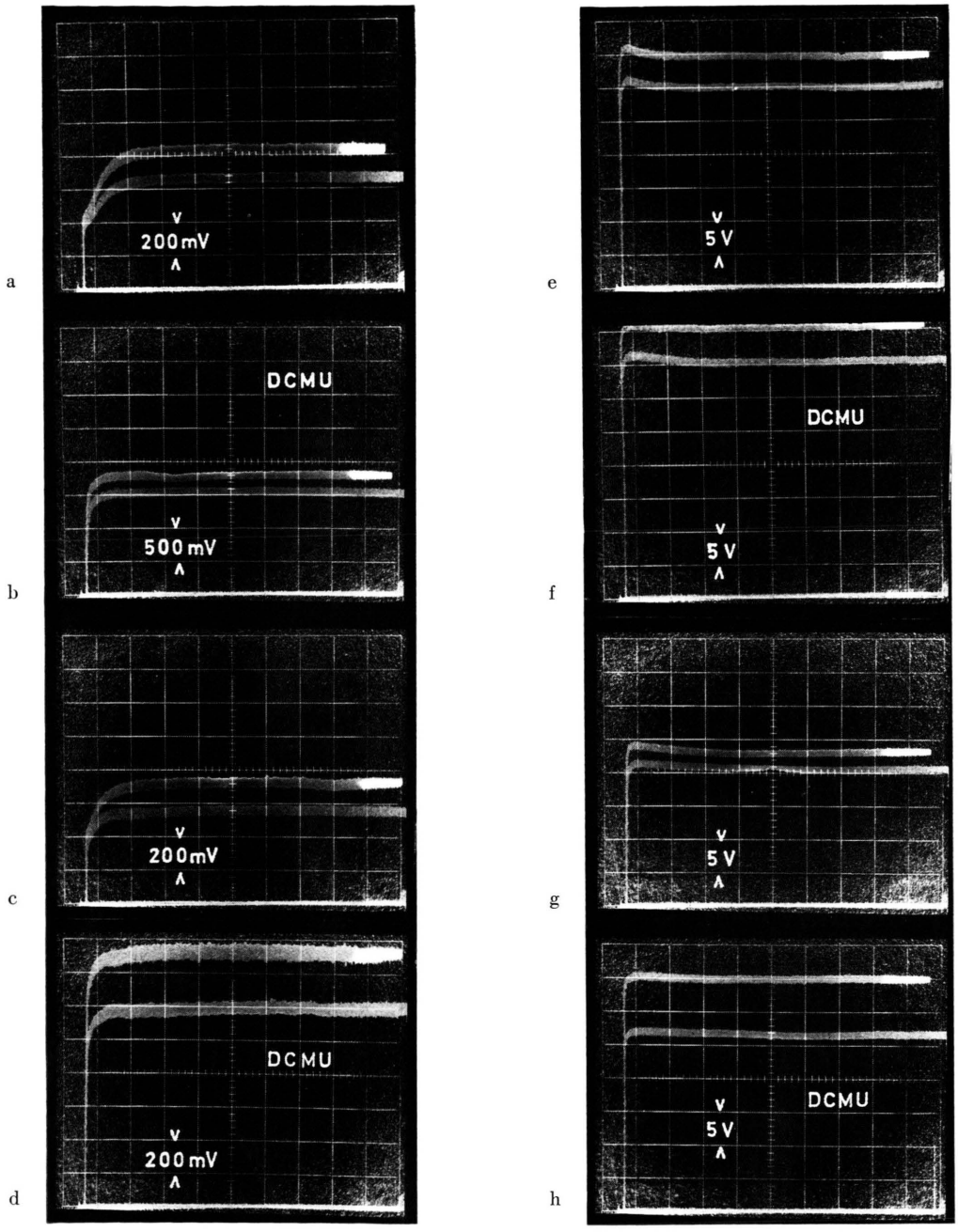

Fig. 7. Effect of the antiserum 48 000 PSI-3 on the fluorescence rise. a), e), b), and f) not illuminated prior to fluorescence excitation. c), d), g), and h) recording after 3 min of preillumination; b), f), d), and h) in the presence of 10^{-6} M DCMU. a), b), c), and d) (left row) low light intensity; e), f), g), and h) (right row) high light intensity; 1 scale on the abscissa corresponds to 5 sec.

and f). The difference, however, between the assay with control serum and antiserum remains. After preillumination the difference between the two samples is even increased by DCMU (Fig. 7 c and d, g and h). In the described experiments ascorbate was the electron donor and anthraquinone-2-sulfonate the electron acceptor. The lowering of the fluorescence yield by the antiserum

is observed also in the presence of ADP, Pi and Mg^{2+}. This is equally valid if water is the electron donor and if ferricyanide or anthraquinone-2-sulfonate are the electron acceptors. The lowering of the fluorescence yield is also seen with dithionite (Fig. 8). Consequently, it must be assumed that the fluorescence yield lowering is caused by a change in the efficiency of energy migration.

As at the temperature of liquid nitrogen, electron transport between the photosystems is interrupted and as not only the antenna chlorophylls of photosystem II but also those of photosystem I exhibit fluorescence emission, this method provides in-

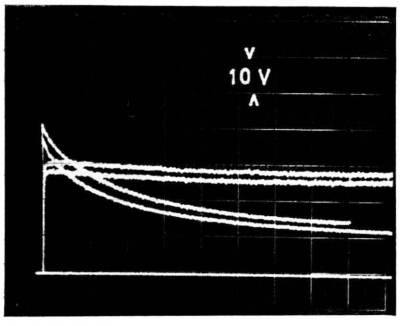

Fig. 8. Effect of the antiserum 48 000 PSI-3 on the fluorescence rise in the presence of 10^{-2} M and in the absence of dithionite. High intensity of the exciting light; 1 scale on the abscissa corresponds to 5 sec. Fluorescence in the presence of dithionite rises immediately upon excitation above that of the assay in the absence of dithionite and subsequently falls below this assay. The control curves lies above those with antiserum.

formation on the energy spill-over between the photosystems [42, 43]. Antiserum 48 000 PSI-3 causes an increase of the ratio F 735/F 685 up to approximately 20% irrespective whether the samples had been illuminated prior to freezing or not (Table III). Consequently, the lowering of the room temperature fluorescence by the antiserum 48 000 PSI-3 can be due to an increased migration of excitation energy from photosystem II to photosystem I. On the other hand, the antiserum 45 000 PSI-4 does not affect the ratio F 735/F 685 significantly. The serum 45 000 PSI-1 influences the spill-over of dark adapted and preilluminated chloroplasts differently. With dark adapted chloroplasts the ratio F 735/F 685 is up to approximately 10% lower than in the controls, whereas with preilluminated chloroplasts the ratio F 735/F 685 is up to 10% higher than in the controls. The serum 40 000

Table III. Effect of the four antisera on low temperature fluorescence emission in tobacco chloroplasts at 77 °K.

Serum	F 735 / F 685	
	Dark [%]	Preilluminated [%]
45 000 PSI-1	−9±2	12±3
	−9±2	13±2
	−11±1	6±1
45 000 PSI-4	2±3	−4±2
	4±2	4±4
	−4±2	−4±2
48 000 PSI-3	22±1	18±1
	15±1	18±2
40 000 CF-96	−3±1	23±2
	−4±1	18±3

Every value is the average of five recordings. The mean error of the average value is indicated.

CF-96 changes the spill-over only after preillumination. In this case the ratio F 735/F 685 is increased by approximately 20%. Thus, it is demonstrated that the antisera influence in a specific way the spill-over of excitation energy although we feel that the alteration of the membrane structure, by which these spill-over changes are induced, are of secondary nature. After the demonstration that the lowering of the fluorescence yield by the serum 48 000 PSI-3 is due to an increase of the spill-over, it is to be investigated whether the observed difference between the fluorescence level of sample and control contains beside the negative component caused by spill-over, a positive component due to an inhibition of electron transport. If this was the case the positive contribution to the difference should become zero after DCMU-addition. This in turn would increase the difference. In dark adapted chloroplasts the difference between the fluorescence level of the antiserum sample and the control stays unchanged, an increase, however, of the difference after preillumination is observed.

It is just this very dependance on the preillumination which speaks in favor of the above interpretation, as an inhibition of electron transport is also only observed after preillumination. The resulting positive contribution, however, appears too small if one considers that with an inhibition degree of approximately 35% it should amount to 1/3 to 1/2 of the fluorescence increases, which are observed with the antisera 45 000 PSI-1 and 45 000 PSI-4. A quantitative evaluation of the situation is difficult

as the influence of DCMU on the spill-over at room temperature with the antiserum sample and the control is unknown.

In Tris-washed chloroplasts absorption changes of P_{680} were observed which were not accompanied by fluorescence changes which indicates that part of the electron transport under certain conditions does not pass through Q (44, further ref. [45 – 47]). Therefore, the question must be asked whether the antiserum 48 000 PSI-3 deviates part of the electron transport in such a way that Q is by-passed. This part of the electron transport which is activated by the antiserum might be the one which causes the strong stimulation of photophosphorylation when ferricyanide is the electron acceptor. If this was indeed the case, it would be understandable why no influence on fluorescence is observed. Hence, the antiserum 48 000 PSI-3 would exert two effects, the second of them would affect reaction center II. The action on photosystem II would not result in a changed electron transport speed if ferricyanide was the electron acceptor. One could assume that for both electron transport pathways ferricyanide could serve as the electron acceptor.

For the evaluation of experiments in which the efficiency of excitation energy migration is changed it must be borne in mind that such changes are caused if distances between chlorophyll molecules or their orientations are altered. As to the antiserum 45 000 PSI-4 it should be noted that this antiserum does not cause an appreciable change of the energy spill-over from photosystem II to photosystem I which is understandable from what has been said before if the antiserum exerts its effect far enough beyond photosystem I on the acceptor side. On the other hand, it is surprising that binding of antibodies to the γ-component of the coupling factor strongly affects energy spill-over.

Concluding Remarks

The results, which were reported above, show again that antisera which are directed to membrane proteins are useful tools for the localization of the proteins in the thylakoid membrane and for their functional characterization despite the fact that the antigens used for immunization are denatured. The effect of the individual antisera on electron transport proofed to be very specific. The exact localization of components in the electron transport scheme is not so much limited by the specificity of the antisera but rather by the lack of suitable test methods. With all this it is presumed that the antisera are monospecific with respect to their action.

However, some uncertainties inherent to serological methods should be mentioned. Thus, antisera with differing actions are sometimes obtained if different rabbits are immunized with the same antigen. In the extreme case one animal produces no antibodies reacting with native antigenic determinants whereas the other makes antibodies which agglutinate stroma-freed chloroplasts but exert no influence on electron transport. A third animal, however, might produce an active antiserum which agglutinates stroma-freed chloroplasts and inhibits photosynthetic electron transport. In addition, antisera obtained from blood withdrawals after different times of the immunization might differ considerably with respect to their effects. This is the reason why we cannot decide whether in the 40 000 – 50 000 molecular weight region polypeptides exist which do not play a role in electron transport. From 30 individual antigen fractions which were used for immunization 12 antisera only agglutinated chloroplasts whereas 18 antisera also inhibited electron transport.

It should be emphasized, however, that the uncertainty affects only conclusions if the outcome of reactions is negative. With a positive result the advantage of the high specificity of the antisera remains. Moreover, a purity test of the antigen preparations is not possible by serological methods alone, above all since the usual double diffusion and immune electrophoretic tests do not yield reasonable results. An unequivocal characterization of the antigens is only possible by investigating their primary structure.

Good evidence for the usefulness of our method is that we isolated from the mixture containing many solubilized polypeptides, components which later upon testing revealed themselves as being components of the coupling factor of photophosphorylation. The components exhibited the same properties as the coupling factor preparations obtained by other methods [40, 41]. Up to now we have characterized the following polypeptides: 11 000 PSII-15 (real molecular weight 6300) plays a role on the oxygen-evolving side of photosystem II [21 – 23, 48]. The polypeptide 66 000 PSII-42 belongs probably to the reaction center II [24]. The polypeptides 33 000 PSI-60 [20] and 48 000 PSI-3

were found to act on the donor side of photosystem I. The polypeptides 66000 PSI-88 and 45000 PSI-1 are apparently components of the reaction center I. The polypeptide antiserum 66000 PSI-88 is until now the first serum which inhibits electron transport only after ultrasonication [24] whereas all other polypeptides are accessible from the outside. In turn the polypeptides 66000 PSI-96 [24] and 45000 PSI-4 seem to be involved on the acceptor side of photosystem I. Among proteins with known function, plastocyanin [37], cytochrome f [49], ferredoxin-$NADP^+$-reductase [50, 51] and carboxydismutase [52] were localized in the outer surface of the thylakoid membrane.

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