Effect of an Antiserum to a Thylakoid Membrane Polypeptide on the Primary Photoreaction of Photosystem II

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As was described previously, an antiserum to polypeptide 11000 inhibited photosynthetic electron transport on the oxygen evolving side of photosystem II. The effect of the antiserum on chloroplasts from two tobacco mutants also clearly showed that the inhibition site is on the photosystem II-side of the electron transport chain. One of the two tobacco mutants lacks the oxygen evolving capacity but exhibits some electron transport with tetramethyl benzidine, an artificial donor to PS II. In this mutant electron transport was barely inhibited. The effect of the antiserum on the primary photoevents showed that the initial amplitude of the absorption change of chlorophyll a_{II} at 690 nm and that of the primary electron acceptor X_{320} at 334 nm both diminished in the presence of the antiserum. Both signals were restored upon addition of diphenylcarbazide another artificial donor to photosystem II. Comparison of the degree of inhibition on the amplitudes of the fast and slow components of the 690 nm absorption change with the manometrically measured inhibition of electron transport shows that besides a full inactivation of a part of the reaction centers of photosystem II another part apparently mediates a fast cyclic electron flow around photosystem II as reported by Renger and Wolff earlier for tris-treated chloroplasts. Moreover, the antiserum affects the low temperature fluorescence in a way which is opposite to Murata's effect of the Mg^{2+}-ion induced inhibition of energy spill-over from photosystem II to photosystem I. The antiserum under the condition in which the Hill reaction is inhibited lowered the 686 nm emission and enhanced the 732 nm emission which indicates an enhanced energy spill-over to photosystem I.

In previous publications we have reported on the effect of an antiserum to a polypeptide fraction on the oxygen evolving side of photosystem II [1, 2]. It appeared that the antiserum inhibited electron transport near the Tris-block between the sites of electron donation of tetramethyl benzidine and diphenyl carbazide. The inhibition by the antiserum was produced in the course of the light reaction. As this data implies that a component from the vicinity of photosystem II becomes exposed to the outer surface of the thylakoid membrane and as this view is seemingly opposed to some of the current concepts on the topology of the photosystems in the thylakoid membrane, we felt that further substantion of our results would be necessary. This was attempted by the use of tobacco mutants which differed with respect to electron transport properties and photosynthetic unit sizes and by use of the repetitive flash spectroscopy in the electron transport systems described previously [1, 2]. This method allows the direct measurement of the photoreaction of the reaction center chlorophyll Chl a_{II} [3] and that of the primary electron acceptor X_{320} [2, 8] of photosystem II. Moreover, the effect of the antiserum on the low temperature fluorescence emission at 77 °K was investigated.

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Abbreviations: Polypeptide 11000 stands for a polypeptide fraction with the apparent molecular weight 11000; DCMU, dichlorophenyl dimethyl urea.

Materials and Methods

The isolation and characterization of the polypeptide fraction 11000 from stroma-freed chloroplasts of *Antirrhinum majus* was described previously [1].

The isolation of photochemically active stroma-free swellable chloroplasts from Nicotiana tabacum var. John William's Broadleaf was also described earlier [4, 5]. In addition to this tobacco we used chloroplasts from the tobacco aurea mutant Su/su[2] which is a chlorophyll deficient mutant and yields chloroplast preparations with 5 – 10 times higher

electron transport rates on a chlorophyll basis than the green control. The mutant is a more pronounced type of the earlier described tobacco mutant Su/su [6]. Properties of the chloroplasts from yellow leaf sections of the variegated tobacco mutant NC95 have been described earlier [4, 7]. This type of chloroplasts exhibits only photosystem I reactions.

Light reactions are described and referred to in the previous publication [2]. Diphenyl carbazide as a donor to photosystem II was used in this publication in the concentration range from 3.3×10^{-5} to 3.3×10^{-4} molar in the assay. Flash spectroscopy of X_{320} and P_{690} was carried out according to the techniques described by Rüppel and Witt and by Witt [8, 9].

Low temperature fluorescence was measured with a modified Perkin-Elmer Fluorescence Spectrophotometer MPF-3. For the recording of the spectra in Fig. 1 tobacco leaves were mashed and the brei was immediately frozen to liquid nitrogen temperatures in glass tubes (inner diameter 3.7 mm). The spectra of Fig. 1 are not corrected. For these fluorescence spectra of chloroplast preparations we used the assay system described earlier [2]. After measurement of the degree of inhibition of the light reaction by the antiserum, the samples were diluted 1:5 v/v with 0.2 M tricine buffer pH 7.6 and frozen to the liquid nitrogen temperature. For the determination of the base line we used the complete assay mixture but without chloroplasts.

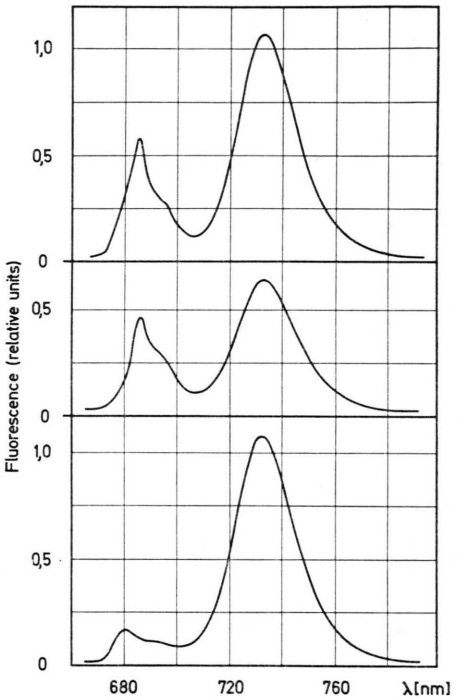

Fig. 1. Low temperature fluorescence emission spectra at 77 °K of tobacco chloroplasts. Excitation wavelength 440 nm; excitation and emission slit 10 nm; upper spectrum: Wild type tobacco *Nic. tabacum* var. John William's Broadleaf; middle spectrum: Tobacco aurea mutant Su/su²; lower spectrum: Yellow leaf patch from variegated tobacco mutant NC95.

Results

1. *Effect of the antiserum to polypetide 11000 on electron transport in mutant chloroplasts from tobacco*

The general electron transport properties of the wild type tobacco and the two tobacco mutants used are characterized by the low temperature fluorescence emission spectra shown in Fig. 1. The green type shows the usual peak ratios at 685 nm *versus* 732 nm, known from the literature [10]. The aurea mutant Su/su² which has a much smaller photosynthetic unit than the green type, namely 300 Chl/O_2 evolved/flash in comparison to 2400 Chl/O_2 evolved/flash with the wild type has a much higher ratio of the short wavelength emission bands *versus* the long wavelength emission, indicating an increased photosystem II/photosystem I ratio. The yellow leaf patch from the variegated tobacco mutant NC95 shows a clear preponderance of the long wavelength emission band which is attributed in the literature to photosystem I [10]. The effect of the antiserum on these three types of chloroplasts in the electron transport system $H_2O \rightarrow$ anthraquinone-2-sulfonate or tetramethyl benzidine $\rightarrow$ anthraquinone-2-sulfonate is depicted in Fig. 2. The antiserum inhibits the green type chloroplast to the extent described earlier [2] whereas the aurea mutant despite its much higher rates of electron transport through photosystem II is inhibited to a lesser extent by the antiserum than the green control. Electron transport in chloroplasts from the yellow leaf patch of the tobacco mutant NC95 is not inhibited by the antiserum. This chloroplast type gives no O_2-evolution but has a low activity for the photosystem II-mediated anthraquinone-2-sulfonate reduction with tetramethyl benzidine as the electron donor. Again, these results with the NC95 mutant clearly demonstrate that the inhibitory action of the antiserum lies on the oxygen evolving side of photosystem II whereas photosystem I reactions are not affected by the antiserum [2].

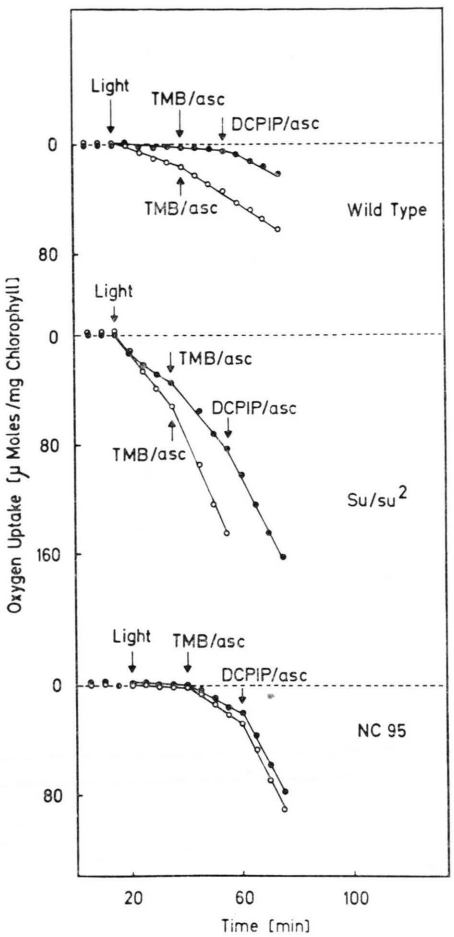

Fig. 2. Electron transport measured as oxygen uptake in an anthraquinone-2-sulfonate mediated Mehler reaction with various electron donors in tobacco chloroplasts. TMB/asc, tetramethyl benzidine/ascorbate; DCPiP/ascorbate, 2,6-dichlorophenol indophenol. (○) Assay in the presence of control serum; (●) assay in the presence of antiserum. Upper curve: Wild type tobacco; middle curve: tobacco aurea mutant Su/su^2; lower curve: chloroplasts from yellow leaf patch of the variegated tobacco mutant NC95. Red light 580 nm$<\lambda<$700 nm 25 °C.

2. *Influence of the antiserum on the primary photoreactions in photosystem II*

In chloroplasts from wild type tobacco, electron transport from water to the artificial electron acceptor anthraquinone-2-sulfonate is inhibited by the antiserum (Fig. 3). This inhibition is not relieved by tetramethyl benzidine an artificial donor to photosystem II[11]. However, addition of diphenyl carbazide another artificial donor to photosystem II restores electron transport through photosystem II. Reaction sequences of this type have led us to the

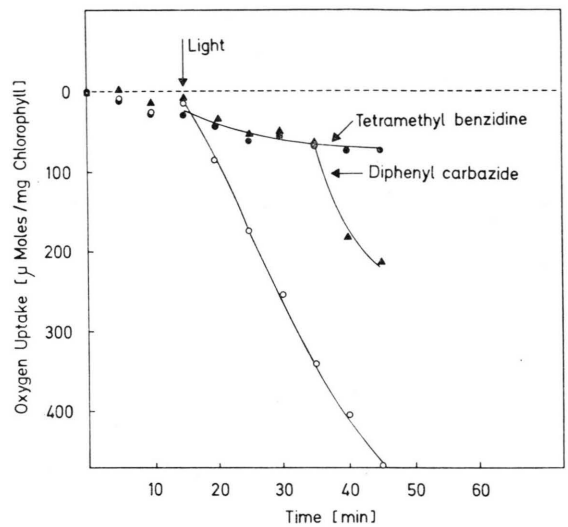

Fig. 3. Electron transport reaction measured as oxygen uptake in an anthraquinone-2-sulfonate mediated Mehler reaction in wild type tobacco chloroplasts. (○) Hill reaction in the presence of control serum; (●) Hill reaction in the presence of antiserum, addition of tetramethyl benzidine does not relieve inhibition. (▲) Hill reaction in the presence of antiserum, diphenyl carbazide relieves inhibition.

interpretation that the antiserum to this polypeptide inhibits electron flow through photosystem II between the sites of electron donation of tetramethyl benzidine and diphenyl carbazide[1, 2]. In the same assay shown in Fig. 3 we have measured the absorbance change at 334 nm reflecting the turnover of X_{320}, which is the primary electron acceptor to photosystem II[28]. With addition of tetramethyl benzidine as the electron donor, the amplitude of the X_{320} signal was diminished by 60 per cent in the presence of the antiserum. The signal was partly restored upon addition of diphenyl carbazide (Fig. 4). This data means that reaction centers of photosystem II are blocked by the antiserum and that the site of inhibition must be before X_{320} as deduced from the effect of diphenyl carbazide on the signal amplitude.

The absorbance change of $Chla_{II}$ at 690 nm was measured in the same assay system. Fig. 5 shows the effect of the antiserum and control serum on the absorbance change at 690 nm. The two fast decay phases of the signal are attributed to $Chla_{II}$[29, 30] and the slow phase to $Chla_I$[9]. It is clearly seen that the antiserum inhibits the amplitude of the fast signals to approximately the same extent as the X_{320} signal (Fig. 4). The inhibition shown in Fig. 4 represents 51 per cent. However, the slow phase signal is suppressed

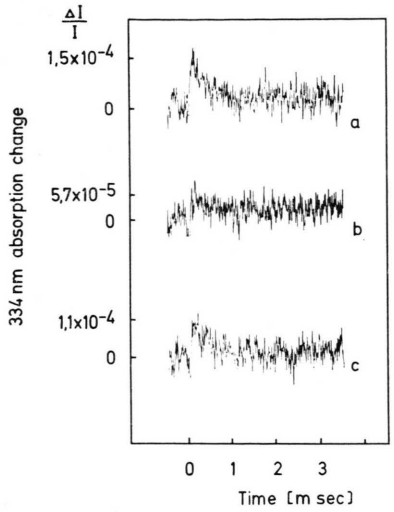

Fig. 4. Absorption changes at 334 nm in wild type tobacco chloroplasts. a) In the presence of control serum, b) in the presence of antiserum, c) in the presence of antiserum + 3.3×10^{-3} M diphenyl carbazide. Chloroplasts corresponding to 6.4×10^{-5} M chlorophyll in the assay. Excitation with 1024 flashes of 20 μsec duration; dark time between flashes 250 msec; optical path length 1 mm.

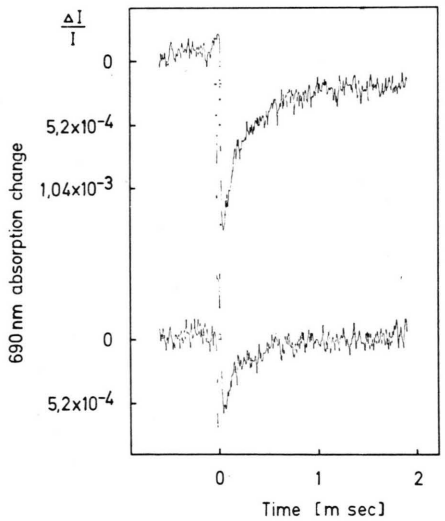

Fig. 5. Absorption changes at 690 nm in wild type tobacco chloroplasts. Upper curve in the presence of control serum; lower curve in the presence of antiserum. Chloroplasts corresponding to 3.2×10^{-6} M chlorophyll in the assay. Excitation with 1024 flashes of 500 nsec duration; dark time between flashes 250 msec; optical path length 2 cm.

by more than 90 per cent. Measurements of absorption changes at 703 nm (data not shown) confirm the results obtained for the slow phases of the 690 nm absorption changes. This high inhibition of $Chla_I$ which reflects the overall decrease of electron transport corresponds precisely to the degree of inhibition measured manometrically in the same assay system (Fig. 3). It can obviously mean that reaction centers of photosystem II are affected in two ways by the antiserum: 50 – 60 per cent are fully put out of function and the remaining 40 – 50 per cent change their way of functioning mediating either a fast cyclic electron flow as proposed by Renger and Wolf for Tris-washed chloroplasts [12] or a direct charge recombination [12]. However, it follows from the facts that the initial amplitudes of the 320 nm and 690 nm absorption changes are decreased by the antiserum by more than 50 per cent, which is a clear difference to Tris-washed chloroplasts [12, 13], that the inhibition site of the antiserum must be closer towards the reaction center than the Tris-block.

The tobacco aurea mutant Su/su^2 contains a smaller photosynthetic unit than the wild type tobacco and hence much more reaction center chlorophyll per total chlorophyll. Moreover, the lamellar system of the mutant is characterized by very extended intergrana regions with only occasional protrusions and invaginations of thylakoids forming partitions which were formerly called doublings [4]. In this condition where the lamellar system is swollen partition regions should be much better accessible to antibodies than in the wild type. However, in the aurea mutant Su/su^2 electron transport in the electron transport system $H_2O \rightarrow$ anthraquinone-2-sulfonate or tetramethyl benzidine $\rightarrow$ anthraquinone-2-sulfonate is affected to a lesser extent by the antiserum than in the green type chloroplasts (Fig. 2). Instead of 90 per cent inhibition with the wild type only 50 to 60 per cent inhibition are observed with the mutant. The X_{320}-signal and the fast 690 nm absorbance change are inhibited under the same conditions by only approximately 20 per cent (Tables I and II). However, if P_{700} is the indicator of electron transport the inhibition is as in the experiments of Fig. 2 about 60 to 70 per cent (Tables I and II). The data of Tables I and II compared to that of Figs 4 and 5 with the wild type could mean that fewer reaction centers in this mutant are totally inhibited by the antiserum. After antiserum addition most of the photosystem II reaction centers are apparently in the condition in which the fast cyclic electron flow through photosystem II is operative [12]. The situation in the mutant after inhibition of electron transport by the antiserum is

Table I. Effect of the antiserum to polypeptide 11000 on the amplitude of the absorption change of the primary electron acceptor X_{320} of photosystem II in tobacco chloroplasts. Electron transport in the system TMB/asc → anthraquinone-2-sulfonate. Assay mixture as described earlier [2]. The listed data is a representative experiment out of 4 similar ones. The amplitude units were calculated for a chlorophyll concentration of 10^{-4} M. Measurement at 334 nm. Excitation with 1024 flashes, of 20 μsec duration and saturating intensity. Dark time between the flashes 250 msec, optical path length 1 mm.

		Amplitude ΔA_0 [rel. units]	% Inhibition
Wild type	antiserum	3.6	62
	control serum	9.4	
Aurea mutant Su/su²	antiserum	36.5	25
	control serum	48.7	

Table II. Effect of the antiserum to polypeptide 11000 on the amplitude of the absorption change of the reaction center chlorophyll $Chla_{II}$ and $Chla_{I}$ at 690 nm in tobacco chloroplasts. Electron transport system and assay mixture as in Table I. The given data is a representative experiment out of a set of 4. The amplitude units were calculated for a chlorophyll concentration of 5×10^{-6} M in the assay. 1024 flashes of 300 erg·sec^{-1}·cm^{-2} intensity; optical path length 2 cm. Fluorescence subtracted.

		Amplitude A_0 [rel. units] fast phases	% Inhibition	Amplitude A_0 [rel. units] slow phase	% Inhibition
Wild type	antiserum	2.1	50	0.17	80
	control serum	4.25		0.83	
Aurea mutant Su/su²	antiserum	16.9	13	1.56	75
	control serum	19.4		6.25	

more comparable to the effect of Tris-washing than in the wild type.

3. *Effect of the artificial donor diphenyl carbazide on electron transport reactions in tobacco chloroplasts*

The artificial donor diphenyl carbazide was introduced by Vernon and Shaw [14]. The donor properties have, in comparison to other donors, been characterized by Shneyour and Avron [15]. From all the donors to photosystem II its properties are peculiar. For instance, it does not support photophosphorylation in any electron transport system and on the other hand the donor seems to require vey little structural integrity of the thylakoid membrane. Chlorophyll-protein preparations of low molecular weight such as "Fraction 3" described by Koenig *et al.* support diphenyl carbazide mediated photoreduction of $K_3Fe(CN)_6$ (below pH 7), DCPiP, anthraquinone-2-sulfonate and other acceptors [16]. The reactions are DCMU-sensitive, although relatively high concentrations of DCMU also with Tris-washed chloroplasts have to be used in order to suppress the photooxidations of diphenyl carbazide. From this it might appear that this donor feeds in its electrons very close to the reaction center of photosystem II if it does not something else. Harnischfeger has deduced from his experiments that it changes the functionability of photosystem II by inducing probably some structural change [17]. Since the localization of the inhibition site of our antiserum depends to a large extent on the donor functions of diphenyl carbazide we have tested the donor properties on a complete Hill reaction in the assay system $H_2O \rightarrow$ DCPiP. In such a reaction diphenyl carbazide stimulates O_2-evolution by 20 per cent (Table III). This simple experiment already shows the difference to the donor tetramethyl benzidine which under the same conditions apparently suppresses O_2-evolution (Table III). If this stimulating property of diphenyl carbazide is to be interpreted in Harnischfeger's way it would be understandable that after the inhibition by the antiserum addition of

Table III. Influence of diphenylcarbazide on the oxygen evolution in the DCPiP-Hill reaction in wild type tobacco chloroplasts. DCPiP, 2,6 dichlorophenolindophenol, 0.86×10^{-3} M in the assay. The Hill reaction was measured as O_2-evolution with a Clark type oxygen electrode. Temp. 20 °C. The photoreduction of anthraquinone-2-sulfonate was measured in a Mehler type reaction as oxygen uptake. Assay system in ref. 2. Temp. 25 °C. Red light 25000 ergs sec^{-1} cm^{-2} 580 nm$<\lambda<$700 nm. The DCPiP-Hill reaction rate has under our conditions an optimum at the external pH 7.8 and falls steeply down towards higher pH-values.

Electron transport system	pH	Oxygen evolution or consumption[a] [μmol mg Chl^{-1}·h^{-1}]
$H_2O \rightarrow$ DCPiP	6.2	72.5
$+2.9\times10^{-4}$ M diphenylcarbazide		87
$H_2O \rightarrow$ DCPiP		82
$+2.9\times10^{-4}$ M diphenylcarbazide	7.0	95
$+2.9\times10^{-5}$ M diphenylcarbazide		87
$H_2O \rightarrow$ DCPiP	8	48.3
$+2.9\times10^{-5}$ M diphenylcarbazide		62.8
$H_2O \rightarrow$ anthraquinone-2-sulfonate	7.6	
$+1.6\times10^{-4}$ M tetramethyl benzidine		46.7 [a]
$+3\times10^{-3}$ M ascorbate		95.4 [a]

diphenyl carbazide always shows some reappearing electron transport. In this case the real inhibitory effect of the antiserum would be directed rather towards the reaction center itself (Figs 4 and 5).

4. *Effect of the antiserum on the low temperature fluorescence emission at 77 °K*

Fluorescence emission spectra of chloroplasts at low temperatures are interpreted in the literature [18, 19]. The emission bands at 685 and 695 nm are mainly attributed to photosystem II. In particular the 695 nm emission is supposed to originate from the reaction center of photosystem II [20]. Correspondingly, the 732 nm emission is associated with photosystem I. According to the literature, changes in the low temperature emission spectra reflect different redox states in which the photosystems were in at the time of freezing of the sample but reflect also changes of energy spill-over between the two photosystems [10, 21]. Thus, Murata has shown that cations like Mg^{2+} inhibit energy spill-over from photosystem II to photosystem I which leads in the low temperature fluorescence emission to an enhancement of the 685 and 695 nm bands and to a lowering of the 732 nm emission [22]. In this condition Mg^{2+} enhances Hill reactions but inhibits typical photosystem I reactions [22].

With our antiserum to polypeptide 11000 the effect on low temperature fluorescence emission is opposite to Murata's observation (Table IV).

From comparison with the control serum it appeared that in the presence of antiserum the 686 nm and the 695 nm bands were lowered whereas the 732 nm band was enhanced (Table IV). The effect was observed in the absence and presence of Mg^{2+}-ions but was more pronounced in the absence of Mg^{2+}-ions which appears self-evident as Mg^{2+} counteracts our effect. All our interpretations are based on the emission ratio F 732/F 686. In Tris-washed chloroplasts the effect was less apparent probably because the emission ratio F 732/F 686 was already considerably increased in comparison to untreated chloroplasts. This effect was measured in the electron transport system $H_2O \rightarrow$ anthraquinone-2-sulfonate or tetramethyl benzidine/ascorbate $\rightarrow$ anthraquinone-2-sulfonate in which electron transport was inhibited to more than 60 per cent by the antiserum at the moment of freezing of the sample. It appeared in the course of these experiments that absence of Mg^{2+} in the sample retards the timely onset of inhibition induced by the antiserum in the course of the light reaction.

Table IV. Influence of the antiserum to polypeptide 11000 on the fluorescence emission at 77 °K in wild type tobacco chloroplasts. Reaction in the electron transport system TMB/asc $\rightarrow$ anthraquinone-2-sulfonate. At the time of freezing the sample to liquid nitrogen temperatures the sample with magnesium and antiserum exhibited 66 per cent inhibition. Excitation slit 10 nm, Emission slit 5 nm. The relative emission was measured against the base line of the complete reaction mixture but without chloroplasts.

Assay	Excitation wavelength [nm]	F 732 [rel. units]	F 686 [rel. units]	F 732 / F 686
Antiserum + Mg^{2+}	445	119	93	1.28
Control serum + Mg^{2+}		125	117	1.06
Antiserum − Mg^{2+}	445	122	93	1.42
Control serum − Mg^{2+}		102	94	1.08
Tris-buffer washed chloroplasts:				
Antiserum + Mg^{2+}	445	122.5	92.5	1.32
Control serum + Mg^{2+}		99	75	1.28
Antiserum + Mg^{2+}	470	155	155	1
Control serum + Mg^{2+}		127	153.5	0.83
Antiserum − Mg^{2+}	470	98.5	86.5	1.14
Control serum − Mg^{2+}		111	123	0.9
Tris-buffer washed chloroplasts:				
Antiserum + Mg^{2+}	470	114	91.5	1.25
Control serum + Mg^{2+}		96	92	1.04

The results of Table IV are interpreted to mean that the antiserum inhibits on the photosystem II side of the electron transport chain. From the fluorescence data alone it would appear that the antiserum acts on or near the reaction center of photosystem II inducing an enhanced energy spill-over to photosystem I. From comparison with Tris-washed chloroplasts and the Murata effect it appears that our antiserum effect is similar to Tris-treatment where the ratio F 732/F 686 is also increased.

Discussion

In this publication we attempted to further substantiate our previous finding that an antiserum to polypeptide 11000 inhibits photosynthetic electron transport on the oxygen evolving side of photosystem II [1, 2]. By use of different chloroplast mutants, with the repetitive flash spectroscopy techniques and low temperature fluorescence emission the earlier data was essentially confirmed. This does not

imply that the water splitting reaction occurs on the outer surface of the thylakoid membrane [2]. However, it appears that the formal localization of the inhibition site in the electron transport scheme depends to a large extent on the functioning mode of diphenyl carbazide (Figs 3 and 4, Table III). On the other hand one cannot easily question the donor functions of this component as the chloroplast-mediated photooxidations of diphenyl carbazide are duely DCMU-sensitive. At this point we should like to note that if the interpretation of the diphenyl carbazide data was not correct, a direct effect of the antiserum on the reaction center of photosystem II would be the only possible interpretation of our results. In this context it should be mentioned that Radunz *et al.* by means of antibodies to chlorophyll had observed that part of the reaction center chlorophyll of photosystem II was surface located [23]. The effect of the antiserum on the photosystem II-reaction center could be a distance change between the primary photosystem II donor and acceptor due to a change of the molecular structure in the thylakoid membrane induced by the antibody binding. In this case diphenyl carbazide would not loose its capabilities of being a donor in the antiserum treated chloroplasts in contrast to tetramethyl benzidine. This latter interpretation would partly fit into the observation of Diner and Joliot on the transformation of reaction centers of photosystem II from an active into an inactive state by an electric field [24]. However, at this state of the investigation polypeptide 11000 could well also be part of an electron transport component on the oxygen evolving side of photosystem II such as for example the manganese protein [25, 26]. Moreover, very recently on the basis of trypsin treated chloroplasts it was inferred that the reaction centers of photosystem II are covered by a protein component which can act as a regulatory component for electron efflux [27].

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Note added in proof: While this paper was in press K. Satoh, R. Strasser, and W. L. Butler, Biochim. Biophys. Acta **440**, 337 [1976] also reported that the energy spill-over from photosystem II to photosystem I was enhanced when the photosystem II reaction centers were closed.

[1] F. Koenig, G. H. Schmid, A. Radunz, B. Pineau, and W. Menke, FEBS Lett. **62**, 342 [1976].
[2] G. H. Schmid, W. Menke, F. Koenig, and A. Radunz, Z. Naturforsch. **31 c**, 304 [1976].
[3] G. Döring, G. Renger, J. Vater, and H. T. Witt, Z. Naturforsch. **24 b**, 1139 [1969].
[4] P. H. Homann and G. H. Schmid, Plant Physiol. **42**, 1619 [1967].
[5] G. H. Schmid, A. Radunz, and W. Menke, Z. Naturforsch. **30 c**, 201 [1975].
[6] G. H. Schmid, Planta **77**, 77 [1967].
[7] G. H. Schmid, Methods in Enzymology (ed. A. San Pietro), **Vol. 23**, p. 171, Acad. Press, New York 1971.
[8] H. Rüppel and H. T. Witt, Meth. Enzym. **16**, 316 [1970].
[9] H. T. Witt, Quart. Rev. Biophysics **4**, 365 [1971].
[10] J. C. Goedhcer, Annu. Rev. Plant Physiol. **23**, 87 [1972].
[11] E. Harth, W. Oettmeier, and A. Trebst, FEBS Lett. **43**, 165 [1974].
[12] G. Renger and Ch. Wolff, Biochim. Biophys. Acta **423**, 610 [1976].
[13] G. Döring, Biochim. Biophys. Acta **376**, 274 [1975].
[14] L. P. Vernon and E. R. Shaw, Plant Physiol. **44**, 1645 [1969].
[15] A. Shneyour and M. Avron, Biochim. Biophys. Acta **253**, 412 [1971].
[16] F. Koenig, W. Menke, H. Craubner, G. H. Schmid, and A. Radunz, Z. Naturforsch. **27 b**, 1225 [1972].
[17] G. Harnischfeger, Z. Naturforsch. **29 c**, 705 [1974].
[18] Govindjee, G. Papageorgiou, and E. Rabinowitch, in Practical Fluorescence: Theory, Methods and Techniques ed. George G. Guilbault), Marcel Dekker, 1973.
[19] Govindjee and R. Govindjee, Scientific Amer. **231**, 68 [1974].
[20] B. Kok, Photosynthetic Mechanism of Green Plants 45 — 55. Nat. Acad. Sci. — Nat. Res. Counc. Publ. 1145, Washington D.C. 1963.
[21] W. L. Butler, Accounts Chem. Res. **6**, 177 [1973].
[22] N. Murata, Biochim. Biophys. Acta **189**, 171 [1969].
[23] A. Radunz, G. H. Schmid, and W. Menke, Z. Naturforsch. **26 b**, 435 [1971].
[24] B. Diner and P. Joliot, Biochim. Biophys. Acta **423**, 479 [1976].
[25] P. Joliot and B. Kok, in Bioenergetics of Photosynthesis (ed. Govindjee), 387, Acad. Press, New York, San Francisco, London 1975.
[26] G. Renger, Z. Naturforsch. **25 b**, 966 [1970].
[27] G. Renger, Biochim. Biophys. Acta **440**, 287 [1976].
[28] H. H. Stiehl and H. T. Witt, Z. Naturforsch. **23 b**, 220 [1968].
[29] M. Gläser, C. Wolff, H. E. Buchwald, and H. T. Witt, FEBS-Letters **42**, 81 [1974].
[30] M. Gläser, C. Wolff, and G. Renger, Z. Naturforsch. **31 c**, in press.