# Correlation of the Photosynthetic Reduction of *p*-(Diazonium-)Benzenesulfonic Acid with the Increased Binding of the Probe to the Thylakoid Membrane

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The reactions of chloroplast thylakoid lamellae with the chemical probe *p*-(diazonium-)benzenesulfonic acid (DABS) in the light have been reinvestigated. In contrast to a previous report, electron transport from a photosystem I electron donor to methylviologen was found to be inhibited by this treatment. During the incubation of chloroplasts with DABS in the light, the probe is altered with high rates. Under aerobic conditions, a concomitant oxygen uptake is observed, which is stoichiometric to the amount of DABS altered. Under anaerobic conditions, the binding of the $^{35}$S-labeled probe to the membranes in the light is stimulated 2–3 fold as compared to the binding under aerobic conditions. The data are taken as evidence that the photoreduction of the probe rather than a conformational change of the membrane may be at least partially responsible for the increased reagent binding observed in the light.

## Introduction

Several methods have been used for the study of chloroplast membrane sidedness (for a recent review see [1]). One of the methods applied in such studies is the treatment of the membranes with chemical modifiers. Dilley and co-workers have introduced the water-soluble chemical probe *p*-(diazonium-)benzenesulfonic acid (DABS) into the study of the location of chloroplast membrane constituents [2]. This probe has been shown for other biological membrane systems to be non-penetrating [3, 4]. In subsequent studies, Dilley and co-workers [5, 6] found that when chloroplast membranes were treated with DABS in the light, the amount of modifier that could be bound significantly increased (3–4 fold). The increase in the amount of label bound was interpreted by these authors as indicating a membrane conformational change that took place in the light exposing otherwise buried groups to the diazonium reagent.

This conformational change was prevented by the electron transport inhibitior DCMU and could not be generated by known photosystem I cyclic reactions [5, 6]. Giaquinta *et al.* [7] have proposed that electron flow from Q, the primary electron acceptor of photosystem II, to plastoquinone is required to generate this large membrane perturbation.

In this communication we report, however, that the increased binding of the probe to the chloroplast membrane is associated with the photoreduction of the probe, prevented by DCMU. Evidence is presented to show that the reduced intermediate has an altered reactivity as compared to the parent compound, which may significantly contribute to the light-induced binding of the probe. It is also demonstrated that, under appropriate conditions, illuminated chloroplasts bind up to 10 fold more of the probe than dark-treated chloroplasts. In contrast to a previous report [8], the increased binding of the reagent can lead to a significant (70%) inhibition of electron transport through PS I.

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*Abbreviations:* DABS, *p*-(diazonium-)benzenesulfonic acid; DAD, diaminodurene; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3(3′,4′-dichlorophenyl)-1,1-dimethylurea; MV, methylviologen; PS I, PS II, photosystem I and II.

## Materials and Methods

Spinach chloroplasts were isolated according to the method of Nelson *et al.* [9], washed two times with 0.05 M $K_3PO_4$, 0.1 M NaCl (pH 7.2), and resuspended in the same buffer at a concentration of 1 mg chlorophyll/ml. Chlorophyll assays were according to Arnon [10].

Preparation of [$^{35}$S]DABS was carried out as described by Hoyer and Trebold [11]. [$^{32}$S]DABS was synthesized according to described procedures [12] as a solid crystalline compound. [$^{35}$S]sulfanilic acid was purchased from Amersham Buchler. Coupling

of DABS to the chloroplasts was carried out using the conditions described by Dilley *et al.*[2] in 0.05 M $K_3PO_4$, 0.1 M NaCl (pH 7.2). The temperature during the incubation was 0 – 4 °C unless otherwise stated. After the reaction of the chloroplasts with DABS, the reaction mixture was diluted 15 – 20 fold with 0.02 M Tris-HCl (pH 8.0) containing 5 mM $MgCl_2$, 10 mM KCl, 0.4 M sucrose and 2 mg/ml bovine serum albumine. The chloroplast lamellae were then centrifuged for 5 min at 10 000 rpm in a Sorvall SS 34 rotor at 0 – 4 °C, washed three times in the same buffer and finally resuspended in this buffer at a concentration of about 0.5 mg Chl/ml.

Photochemical activities and radioactivity were then determined.

For $NADP^+$-reduction, the reaction mixture contained in 1 ml: 20 mM Tris-HCl (pH 8.0), 5 mM $NH_4Cl$, 10 $\mu$M DCMU, 5 mM Na-ascorbate, 0.1 mM DAD · 2 HCl, 1.5 mM $NADP^+$, 5 $\mu$M ferredoxin, excess ferredoxin-$NADP^+$-reductase and chloroplasts equivalent to 10 – 20 $\mu$g Chl. The cuvettes were illuminated in air at room temperature for 4 min with heat-filtered white light of an intensity of about $1 \times 10^5$ ergs $cm^{-2}$ $sec^{-1}$ at the position of the cuvette. The reduction of $NADP^+$ was determined by measuring the increase of absorbance at 365 nm. Spinach ferredoxin and spinach ferredoxin-$NADP^+$-reductase were isolated by described procedures [13, 14].

MV reduction was followed in a Clark-type oxygen electrode similar to that described by Delieu and Walker [15]. The reaction mixture contained in 2 ml: 30 mM Tris-HCl (pH 8.0), 5 mM $NH_4Cl$, 10 $\mu$M DCMU, 5 mM Na-ascorbate, 0.1 mM DAD · 2 HCl, 0.2 mM MV, 0.5 mM $NaN_3$ and chloroplasts equivalent to 10 – 20 $\mu$g Chl. The reaction vessel was thermostated at 20 °C and illuminated with heat-filtered white light of approximately $2 \times 10^5$ ergs $cm^{-2}$ $sec^{-1}$ at the position of the reaction vessel.

Light intensities were measured with an YSI Kettering Model 65 Radiometer.

For the determination of radioactivity, the procedure of Giaquinta *et al.*[7] was used. Radioactivity was counted by liquid scintillation using the Aquasol Universal L.S.C. Cocktail (New England Nuclear) in a Packard Tri-Carb liquid scintillation spectrometer Model 3385.

For the quantitative determination of DABS in solution, a method similar to that for the determination of nitrite [16] was used: An aliquot of the diazonium salt solution was allowed to react with an excess amount of α-naphthylamine (0.1 M in 6 M acetic acid) and after 30 min the extinction of the red coloured azo compound was measured at 530 nm. The concentration of DABS was determined using a calibration curve. When DABS was determined in a chloroplast suspension, an aliquot of the suspension prior to the addition of DABS was mixed with the α-naphthylamine solution in the same way and used as a blank.

## Results

### 1. *Light-dependent inhibition of PS I electron transport*

Selman *et al.*[8] have shown that illumination of chloroplast lamellae during incubation with DABS increases the inhibition of $NADP^+$-reduction at the expense of an artificial PS I electron donor, reduced DCIP, over the dark level. Similar results are shown in Table I for the DAD-dependent $NADP^+$-reduction. The rather high inhibition of $NADP^+$-reduction seen for chloroplasts incubated with DABS in

Table I. Inhibitions of PS I partial reactions caused by incubation of chloroplast lamellae with DABS in the dark and in the light.

a. $NADP^+$ reduction at the expense of a PS I electron donor, DAD.

b. MV-catalyzed oxygen uptake with DAD or DCIP/ascorbate as electron donors. The assay conditions were as described in Materials and Methods. "Dark DABS" stands for incubation of chloroplasts with DABS in the dark, "Light DABS" for incubation in the light. Incubation conditions: Chloroplasts at a concentration of 1 mg Chl/ml were allowed to react with 2 mM DABS for 1 min at 0–4 °C. The "Light DABS" sample was preilluminated for 15 sec before the addition of the reagent. Illumination was carried out with white light of approximately $1 \times 10^6$ ergs $cm^{-2}$ $sec^{-1}$. The control chloroplasts were light-treated similarly without the addition of DABS. Electron transport rates are given in $\mu$equivalents/mg Chl/h.

a. DAD/ascorbate → $NADP^+$

| Treatment | Addition | Electron transport rate | Inhibition [%] |
|---|---|---|---|
| Control | – | 133 | – |
| | +reductase | 185 | – |
| Dark DABS | – | 36 | 72 |
| | +reductase | 73 | 60 |
| Light DABS | – | 3 | 98 |
| | +reductase | 5 | 97 |

b. DAD or DCIP/ascorbate → MV

| Treatment | Donor | Electron transport rate | Inhibition [%] |
|---|---|---|---|
| Control | DAD | 658 | – |
| Dark DABS | (100 $\mu$M) | 600 | 9 |
| Light DABS | | 187 | 71 |
| Control | DCIP | 517 | – |
| Dark DABS | (50 $\mu$M) | 517 | 0 |
| Light DABS | | 262 | 49 |
| Control | DCIP | 635 | – |
| Light DABS | (150 $\mu$M) | 337 | 47 |

the dark (72% without and 60% with added ferredoxin-$NADP^+$-reductase, respectively) is increased to almost complete inhibition when incubation is carried out in the light.

Table I also shows the results for MV-mediated $O_2$ consumption using either DAD or reduced DCIP as electron donors for PS I. Unlike $NADP^+$-reduction, incubation of chloroplast lamellae with DABS in the dark results in only a marginal inhibition of electron transport in this PS I partial reaction. In contrast, MV reduction in chloroplast membranes treated with DABS in the light is markedly inhibited to about 30 – 50% of the control electron transport rate. The level of inhibition apparently depends on the electron donor used, the reaction with DAD being more sensitive than the reaction with reduced DCIP.

### 2. *Reduction of DABS by illuminated chloroplasts*

When a solution of DABS is illuminated in the presence of chloroplasts, this solution rapidly loses its ability to couple to α-naphthylamine to give an azo compound (Fig. 1). The initial rate of alteration of the diazonium salt during the first 10 sec of illumination equals 240 μmol DABS altered/mg Chl/h. The time courses for this light-dependent alteration of DABS by chloroplasts are similar under aerobic and anaerobic conditions (Fig. 1).

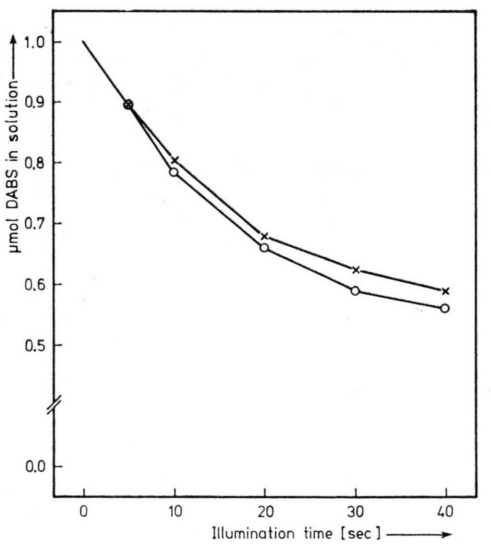


Fig. 1. DABS destruction by illuminated chloroplasts measured as the loss in ability to couple to α-naphthylamine. ○—○, under aerobic, ×—×, under anaerobic conditions. Reaction conditions: The reaction mixture contained in 2 ml: 0.05 M $K_3PO_4$ (pH 7.2), 0.1 M NaCl, chloroplasts equivalent to 0.26 mg Chl, and 1 μmol DABS. 5 sec after the addition of DABS to the stirred reaction mixture the samples were illuminated for the indicated times. 1 min after the addition of DABS, aliquots were withdrawn and mixed with the α-naphthylamine solution. Anaerobic conditions were generated by $N_2$ before the addition of the chloroplasts. After the addition of the chloroplasts, the air-water interface was flushed with $N_2$. Anaerobic conditions were checked with an oxygen electrode. Illumination was with white light of approximately $2 \times 10^5$ ergs $cm^{-2}$ $sec^{-1}$, $T = 20$ °C.

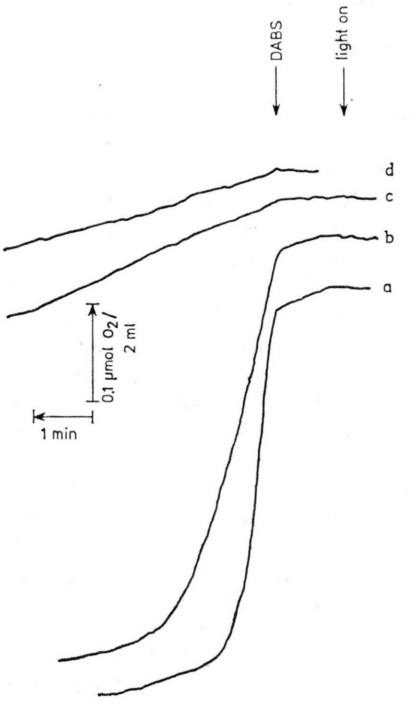


Fig. 2. Changes in oxygen concentration in the reaction mixture during the incubation of DABS with illuminated chloroplasts. Traces from an oxygen electrode are shown. The reaction mixture contained in 2 ml: 0.05 M $K_3PO_4$ (pH 7.2), 0.1 M NaCl and chloroplasts equivalent to 0.4 mg Chl. At the indicated times, the light was turned on (white light with about $2 \times 10^5$ ergs $cm^{-1}$ $sec^{-1}$ intensity) and then 0.5 μmol DABS were added with a syringe. $T = 20$ °C. Trace a: without additions; trace b: plus 1 μM DBMIB; trace c: plus 10 μM DCMU; trace d: without additions, but in the dark. The initial rate in trace a after the addition of DABS equals 110 μmol $O_2$/mg Chl/h.

Fig. 2 shows the change of oxygen concentration during incubation of DABS with chloroplasts measured with an oxygen electrode. In the light and in the absence of any added electron acceptor, oxygen is very slowly taken up, the rate being about 5 μmol $O_2$/mg Chl/h (trace a). The addition of DABS to the illuminated reaction mixture causes a rapid oxygen consumption with an initial rate of about 110 μmol $O_2$/mg Chl/h (trace a), although the reaction mixture contains only buffer, chloroplasts and

DABS. The response time of the electrode used is probably too slow to measure the fast transient rate during the first 10 sec seen in Fig. 1.

That this oxygen consumption is not just a chlorophyll photosensitized reaction is shown by its sensitivity to the electron transport inhibitors DBMIB (trace b) and DCMU (trace c). DCMU (10 $\mu$M) almost completely prevents the DABS stimulation of oxygen consumption (trace c, for comparison the reaction in the dark is shown in trace d), while DBMIB (1 $\mu$M) is less effective and only inhibits the rate of oxygen consumption to apparently 70% of the uninhibited rate (trace b).

Fig. 3 shows that the total amount of $O_2$ consumed during the illumination of chloroplasts in the presence of DABS is linearly correlated to the amount of DABS added to the reaction mixture. From this figure, a stoichiometry of about 0.8 $O_2$ consumed per DABS added can be calculated.

It is possible to reduce DABS chemically with K-ferrocyanide. This leads to an oxygen uptake similar to that seen when the reduction is carried out by illuminated chloroplasts. The stoichiometry of this oxygen uptake is about 1 $O_2$ taken up per DABS added (see Fig. 3).

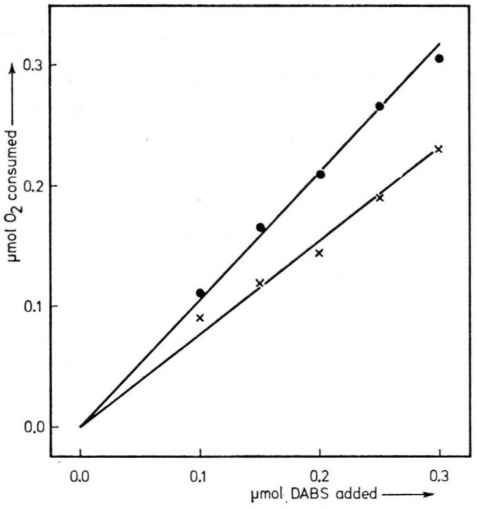


Fig. 3. Oxygen consumption upon reduction as a function of the amount of DABS added. ○—○, reduction by K-ferrocyanide. The reaction mixture contained in 2 ml: 0.05 M $K_3PO_4$ (pH 7.2), 0.1 M NaCl, 2 $\mu$mol K-ferrocyanide, and the indicated amounts of DABS. ×—×, reduction by illuminated chloroplasts. The reaction mixture contained in 2 ml: 0.05 M $K_3PO_4$ (pH 7.2), 0.1 M NaCl, chloroplasts equivalent to 0.2 mg Chl, and DABS as indicated. Illumination was with white light of an intensity of approximately $2 \times 10^5$ ergs $cm^{-2}$ $sec^{-1}$. The oxygen uptake was measured with an oxygen electrode, $T = 20$ °C.

In Fig. 4 the quantitative correlation between the amount of DABS altered by illuminated chloroplasts (measured as the loss of DABS able to couple to α-naphthylamine to give the corresponding azo compound, see Materials and Methods and Fig. 1) and the amount of oxygen taken up during the reaction

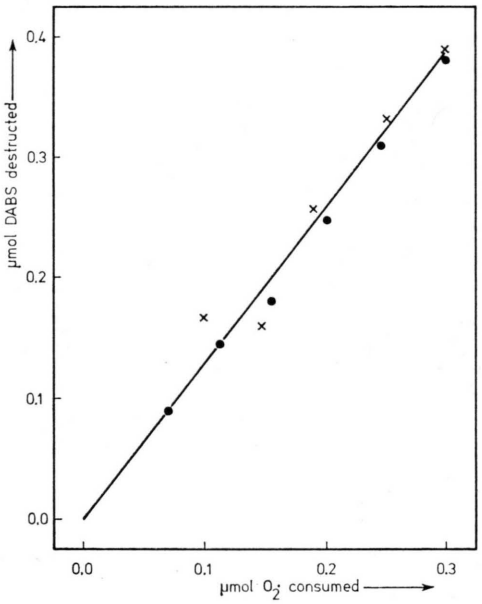


Fig. 4. Correlation between DABS destruction and oxygen uptake upon reduction with illuminated chloroplasts. The reaction mixture contained in 2 ml: 0.05 M $K_3PO_4$ (pH 7.2), 0.1 M NaCl, chloroplasts equivalent to 0.2 mg Chl and 0.5 mM DABS. 10 sec after the addition of DABS to the stirred reaction mixture, the samples were illuminated for various times. After a total incubation time of 2 min the oxygen uptake was determined and aliquots mixed with the α-naphthylamine solution. DABS destruction was measured by its loss in ability to couple to α-naphthylamine as described in Materials and Methods. Oxygen uptake was measured with an oxygen electrode, $T = 20$ °C. The samples were illuminated with white light of about $2 \times 10^5$ ergs $cm^{-2}$ $sec^{-1}$.

is shown. DABS was added to stirred chloroplast suspensions in the oxygen electrode, and 10 sec thereafter the suspensions were illuminated for different times. The total exposure time of the chloroplasts to DABS was kept constant at 2 min. The amount of $O_2$ consumed was recorded, samples were withdrawn and mixed with the α-naphthylamine solution. The results displayed in Fig. 4 clearly show that the amount of oxygen taken up during the incubation of chloroplasts with DABS in the light is proportional to the amount of DABS altered during this procedure. One can calculate from this figure a stoichio-

metry of about 0.8 $O_2$ taken up per DABS altered, confirming the results shown in Fig. 3.

These results indicate that the oxygen uptake is associated with a chemical alteration of the diazonium compound. Although the reaction products have not been identified, we interpret these data as a reduction of the diazonium compound by illuminated chloroplasts (or by K-ferrocyanide) followed by a stoichiometric oxygen uptake.

Assuming from the chemical reduction of DABS that 1 $O_2$ is taken up per DABS reduced and, in addition, that DABS reduction requires 1 electron per molecule, an overall stoichiometry of 0.75 $O_2$ taken up per DABS reduced would be predicted when the reduction is carried out by illuminated chloroplasts (because of the oxygen evolved in the water splitting reaction). The measured overall stoichiometry of 0.8 $O_2$ taken up per DABS reduced is in close agreement to the calculated value.

### 3. *Kinetics of DABS binding to chloroplast lamellar systems and of inhibition of electron transport through PS I*

DABS binding to chloroplast membranes under various conditions has been studied intensively by Dilley and co-workers [5–8]. The purpose of the experiments reported here is to determine whether there is a correlation between the reduction of the diazonium compound during the incubation with illuminated chloroplasts to the binding of the compound to the membrane.

It was shown in section 2 that aerobic reduction of DABS is associated with a stoichiometric oxygen consumption. DABS is reduced, however, with similar kinetics under both aerobic and anaerobic conditions. Thus, oxygen uptake is probably due to the reaction of the reduced DABS species with molecular oxygen rather than a reduced oxygen species reacting with DABS. If the increased DABS binding to the thylakoid membranes in the light is at least partially due to the presence of this reduced DABS species, molecular oxygen and membranes should compete for this species and, therefore, the binding of the probe to the membranes in the light should be increased under anaerobic conditions.

Fig. 5 shows the time course of the light-dependent binding of the probe to chloroplast membranes and of the inhibition of electron transport through PS I under aerobic and anaerobic conditions. In the dark, the lamellar systems bind about the same

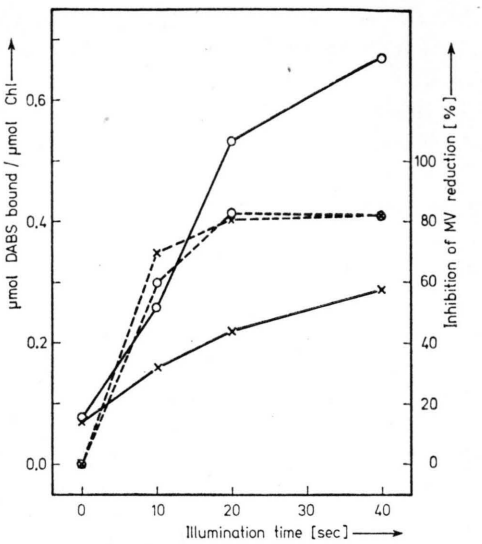


Fig. 5. Time courses of light-dependent DABS binding and PS I inhibition under aerobic and anaerobic conditions. Solid lines: probe binding to the membranes: ×—×, binding under aerobic conditions; ○—○, binding under anaerobic conditions. Dashed lines: inhibition of MV reduction with the DAD/asc. donor couple: ×- - -×, inhibition of chloroplasts treated with DABS under aerobic conditions. ○- - -○, inhibition of chloroplasts treated with DABS under anaerobic conditions. Incubation conditions: The incubation mixture contained in 3 ml: 0.05 M $K_3PO_4$ (pH 7.2), 0.1 M NaCl, 2 mM DABS and chloroplasts equivalent to 0.8 mg Chl. Anaerobic conditions were generated and checked as described in the legend to Fig. 1. The incubation mixtures were illuminated for the indicated times with white light of approximately $2 \times 10^5$ ergs $cm^{-2}$ $sec^{-1}$, $T = 20$ °C. The total incubation time of chloroplasts with the probe was 2 min. The assay conditions for the MV-catalyzed $O_2$ uptake are described in Materials and Methods.

amount of DABS. With illumination, there is significantly (2 – 3 fold) more reagent binding under anaerobic than under aerobic conditions. The amount of probe bound after 40 sec of illumination under anaerobic conditions is about 0.7 bound/1 Chl. This is a 10 fold increase in binding as compared to the dark level.

Inhibition of MV reduction seems not to be affected by the difference in treatment, *i. e.* the extent of inhibition is not correlated to the amount of probe per chlorophyll which is bound to the membranes.

## Discussion

1. The reduction of azo compounds by isolated chloroplasts has been described previously [17]. In this paper we describe the photosynthetic reduction of DABS, a diazonium salt. Upon reduction by either illuminated chloroplasts or chemically by K-ferro-

cyanide, the diazonium salt loses its ability to couple to α-naphthylamine to give the corresponding azo compound and takes up a stoichiometric amount of oxygen. It is well known from organic chemistry that diazonium salts may lose their diazonium group upon reduction and hence the ability to carry out azo coupling [18]. The reduction of the diazonium salt by illuminated chloroplasts is completely inhibited by DCMU and only partly by DBMIB indicating that the diazonium salt is probably reduced by both PS I and PS II.

We determined a stoichiometry of 1 $O_2$ taken up per DABS reduced using K-ferrocyanide as a reductant and a stoichiometry of about 0.8 $O_2$ taken up per DABS reduced for the reduction by illuminated chloroplasts (see section 2). The determination of the latter stoichiometry is somewhat complicated by the fact that DABS is concurrently bound to the chloroplast membranes. However, only up to 4% of the total amount of DABS added to the incubation mixture was found to be bound under aerobic conditions and, thus, should not interfere severly with the determination of the stoichiometry.

2. Giaquinta *et al.* [7] have proposed that electron flow from Q to plastoquinone causes a membrane conformational change which is indicated by extra DABS binding to the chloroplast membrane. Our observation is that under conditions where electron transport through this region is possible, the chloroplasts are able to reduce the probe. Conditions which prevent the light induced extra DABS binding (*i. e.* in the presence of DCMU but not DBMIB) almost completely inhibit the reduction of the probe (Fig. 2).

Attempts to increase the binding of the reagent to the membranes by chemically reducing DABS in the presence of chloroplasts in the dark were unsuccessful (data not shown). It seems possible to us that DABS has to be reduced at or very close to the membrane to allow the reduced intermediate to react efficiently with the membrane because of side reactions which may occur.

One of these side reactions is the oxidation of the reduced intermediate by molecular oxygen. When we exclude this reaction by anaerobic conditions, the velocity of DABS (or "intermediate") binding to the membranes increases 2 – 3 fold (Fig. 5), although the time course for the reduction by illuminated chloroplasts remains similar (Fig. 1). This result suggests that oxygen and chloroplasts compete for the reduced intermediate generated by chloroplasts in the light. The extra probe binding to the membranes caused by chloroplast electron transport in the light may be due, therefore, to an altered reactivity of the reduced probe, *i. e.* an increased unspecificity. the proposed reactions are summarized in Table II.

Table II. Altered reactivity of DABS after illumination with chloroplasts.

| Compound | Reacts with | Consequence |
|---|---|---|
| DABS | chloroplast membrane | binding<br>partial inhibition of $NADP^+$ reduction |
| "Reduced intermediate" (generated from DABS by chloroplasts in the light) | chloroplast membrane | increased binding<br>complete inhibition of $NADP^+$ reduction<br>inhibition of MV reduction |
| | molecular oxygen | quench of the increase of probe binding to the chloroplast membrane |

Our results do not, of course, exclude the possibility of the concomitant occurence of a conformational change, as hypothesized by Giaquinta *et al.* [7]. It seems unlikely, however, that an up to 10 fold increase in probe binding can be explained by a conformational change alone.

3. Selman *et al.* [8] have investigated the effect of chemical modification with DABS on electron transport on the reducing side of PS I. This site of PS I is known to be localized on the outer surface of the chloroplast thylakoid lamellae [1]. Inhibition of ferredoxin reduction by isolated chloroplasts is caused by different treatments including antibodies [19, 20], lactoperoxidase-catalyzed iodination [21], and treatment with diazonium salts (DABS [8] and diazonium-1,2,4-triazole [22]), reagents which are supposed to be unable to penetrate the thylakoid membrane (with the exception of the triazole derivative). Some of these treatments also inhibit the reduction of MV or anthraquinone-sulfonic acid, *e. g.* antibodies [23–25], and to a small extent iodination [21].

An outstanding feature of DABS inhibition of ferredoxin reduction is its potentiation by electron transport during the incubation of the chloroplasts with the reagent [8]. The authors interpreted the increase in inhibition of ferredoxin reduction in terms of an electron transport induced conformational

change of the membrane which increases the inhibition of electron transport at the same site(s).

In contrast to these previously reported results [8], we find an inhibition of electron transport from a PS I electron donor to MV when the incubation of the chloroplasts with DABS is carried out in the light. Our results, therefore, allow another interpretation. The electron transport induced increase in the inhibition of ferredoxin reduction by DABS may be due to the inhibition at an additional site also affecting electron transport to MV. This site can be identical to the inhibition site of the antibodies [23–25].

This qualitative difference of DABS inhibition of electron transport through PS I can be explained by assuming a conformational change. Alternatively, it is possible to attribute this effect to the altered reactivity of the reduced form of the probe generated by the chloroplasts in the light. The time courses of the inhibition of MV reduction caused by the treatment under aerobic and anaerobic conditions are very similar although the time courses of the binding of the probe to the membranes are different (Fig. 5). This may be explained by the fact that maximal inhibition of MV reduction requires only a limited increase in binding of the probe in the light. Therefore, the obvious difference in the time courses of the binding of the probe to the whole membrane under aerobic and anaerobic conditions cannot be seen in the time course of the inhibition, which may only require binding of the probe at a specific site. Thus, we think that, as far as PS I is concerned, it is possible to explain the inhibition caused by treatment of chloroplasts with DABS in the light without assuming the occurence of a conformational change, but such a conformational change is an alternative explanation.

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[1] A. Trebst, Ann. Rev. Plant Physiol. **25**, 423 [1974].
[2] R. A. Dilley, G. A. Peters, and E. R. Shaw, J. Membrane Biol. **8**, 163 [1972].
[3] H. C. Berg, Biochim. Biophys. Acta **183**, 65 [1969].
[4] D. L. Schneider, Y. Kagawa, and E. Racker, J. Biol. Chem. **247**, 4074 [1972].
[5] R. T. Giaquinta, R. A. Dilley, and B. J. Anderson, Biochem. Biophys. Res. Commun. **52**, 1410 [1973].
[6] R. T. Giaquinta, R. A. Dilley, B. R. Selman, and B. J. Anderson, Arch. Biochem. Biophys. **162**, 200 [1974].
[7] R. T. Giaquinta, R. A. Dilley, B. J. Anderson, and P. Horton, Bioenergetics **6**, 167 [1974].
[8] B. R. Selman, R. T. Giaquinta, and R. A. Dilley, Arch. Biochem. Biophys. **162**, 210 [1974].
[9] N. Nelson, Z. Drechsler, and J. Neumann, J. Biol. Chem. **245**, 143 [1970].
[10] D. I. Arnon, Plant Physiol. **24**, 1 [1949].
[11] L. W. Hoyer and N. C. Trebold, J. Clin. Invest. **49**, 87 [1970].
[12] Chromatographie, p. 146, E. Merck AG, Darmstadt.
[13] K. Tagawa and D. I. Arnon, Nature **195**, 537 [1962].
[14] M. K. Shin, K. Tagawa, and D. I. Arnon, Biochem. Z. **38**, 84 [1963].
[15] T. Delieu and D. A. Walker, New Phytol. **71**, 201 [1972].
[16] L. Kolditz, Anorganikum, 6. Auflage, p. 808, VEB Deutscher Verlag der Wissenschaften, Berlin 1974.
[17] O. K. Ash, W. S. Zaugg, and L. P. Vernon, Acta Chem. Scand. **15**, 1629 [1961].
[18] R. T. Morrison and R. N. Boyd, Organic Chemistry, 2nd Edition, p. 778, Allyn and Bacon, Inc., Boston 1971.
[19] R. J. Berzborn, W. Menke, A. Trebst, and E. Pistorius, Z. Naturforsch. **21 b**, 1057 [1966].
[20] C. Bengis and N. Nelson, J. Biol. Chem. **250**, 2783 [1975].
[21] C. J. Arntzen, P. A. Armond, C. S. Zettinger, C. Vernotte, and J. M. Briantais, Biochim. Biophys. Acta **347**, 329 [1974].
[22] I. Maruyama, K. Nakaya, K. Ariga, F. Obata, and Y. Nakamura, FEBS Letters **47**, 26 [1974].
[23] G. Regitz, R. Berzborn, and A. Trebst, Planta **91**, 8 [1970].
[24] E. Racker, G. Hauska, S. Lien, R. J. Berzborn, and N. Nelson, Proc. 2nd Int. Congr. Photosyn. Res., Stresa (G. Forti, M. Avron, A. Melandri, eds.), **Vol. 2**, p. 84, Dr. W. Junk, N. V., The Hague 1971.
[25] W. Menke, F. Koenig, A. Radunz, and G. H. Schmid, FEBS Letters **49**, 372 [1975].