The Possible Relationship between a Membrane Conformational Change and Photosystem II Dependent Hydrogen Ion Accumulation and Adenosine 5′-Triphosphate Synthesis†

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ABSTRACT: Data are presented which suggest that photosystem II dependent hydrogen ion accumulation and ATP synthesis can occur only after the lamellar membranes have undergone a conformational change. This membrane conformational change is detected by the electron transport dependent incorporation of diazonium benzene[^{35}S]sulfonate into membrane components. Previously it was established that electron flux from the photosystem II primary acceptor to plastoquinone is a necessary event for the occurrence of the diazonium-detected conformational change. These studies indicate that the release of hydrogen ions during photosystem II oxidation of the primary reductant is also a necessary event for the diazonium-detected conformational change. When iodide ions were substituted for water (or other proton-releasing donors) as the primary reductant of system II the conformational change did not occur even though a substantial rate of electron flow from the primary acceptor to plastoquinone occurred.

Recent research from this laboratory has established that photosystem II driven electron transport is accompanied by a conformational change of the lamellar membrane (Giaquinta et al., 1974a,c). Using the hydrophilic chemical modifier diazonium benzene[^{35}S]sulfonic acid (DABS),[1] we established that under electron transport saturating light intensities three- to fourfold more DABS is covalently bound (Giaquinta et al., 1974d) to lamellar membrane proteins when electron flux occurs through the Q[1] to plastoquinone redox segment than when it does not occur. Notably, electron transport through any segment of the photosynthetic redox chain not including the Q to plastoquinone region does not result in any incorporation of DABS over the level observed in the dark (Giaquinta et al., 1974a).

The work of Izawa et al. (1973a) and Trebst and Reimer (1973) has firmly established the existence of a second site of phosphorylation in isolated chloroplasts. The phosphorylation site, like the conformational change discussed above, is dependent on photosystem II driven electron transport. We have previously observed that any condition which does not result in the DABS-detected conformational change also does not support photosystem II dependent ATP synthesis. For instance, the DCMU-insensitive reduction of silicomolybdate by photosystem II does not result in an enhanced labeling of the chloroplast membrane with the diazonium reagent nor does this electron transport support H^+ accumulation within the thylakoid (Giaquinta and Dilley, 1975b) or ATP synthesis (Giaquinta et al., 1974b).

In this paper we have examined the relationship between the diazonium detected conformational change and photosystem II dependent hydrogen ion accumulation and ATP synthesis. In a previous publication (Giaquinta et al., 1974a) it was concluded that electron flow through the Q to plastoquinone redox segment is the only event in system II necessary for the diazonium-detected conformational change. However, those studies did not probe the possibility that protons, released upon oxidation of water or certain added electron donors, might be involved in some way in the conformational change. This report further examines that question employing iodide ions, which are oxidized without the release of protons (Izawa and Ort, 1974), as the primary reductant for photosystem II.

Experimental Procedures

Chloroplast Isolation. Chloroplasts (unfragmented naked lamellae) were isolated from commercial spinach (*Spinacia oleracea L.*). Leaves were washed with cold distilled water and ground in a Waring Blendor for 5 sec in a medium consisting of 0.3 *M* NaCl, 30 m*M* tricine–NaOH buffer (pH 7.8), 3 m*M* $MgCl_2$, and 0.5 m*M* EDTA. The homogenate was filtered through multiple layers of cheesecloth, and the chloroplasts were sedimented at 2500*g* for 2 min. The chloroplast pellet was then resuspended in a medium containing 0.2 *M* sucrose, 5 m*M* *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid (pH 7.5), 2 m*M* $MgCl_2$, and 0.05% bovine serum albumin. After a 45-sec centrifugation at 2000*g* to remove cell debris, the chloroplasts were spun down again (2000*g* 4 min) and finally suspended in a few milliliters of the above suspending medium. The chlorophyll concentration of this suspension was determined by the method of Arnon (1949).

Cyanide Treatment. Cyanide-treated chloroplasts were prepared by incubating chloroplasts at 0° for 90 min in a 30 m*M* KCN solution buffered at pH 7.8 as described by Ouitrakul and Izawa (1973). The treatment was terminated by

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[1] Abbreviations used are: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; DABS, diazonium benzenesulfonate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Q, primary acceptor of electrons from Photosystem II; P/e_2, the ratio of the number of molecules of ATP formed to the number of pairs of electrons transported; tricine, *N*-tris(hydroxymethyl)glycine.

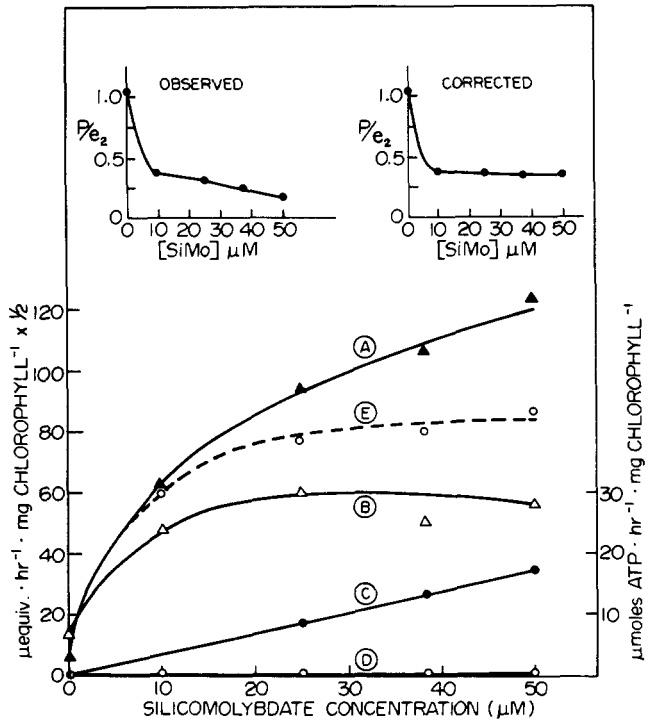

FIGURE 1: Efficiency of phosphorylation during silicomolybdate-mediated electron transport in the absence and presence of DCMU using KCN-treated chloroplasts. Inhibition of photosystem I by KCN treatment was according to previously described procedures (Ouitrakul and Izawa, 1973). The reaction mixture for the electron transport and phosphorylation assays contained in 2 ml: 0.1 *M* sucrose, 50 m*M* tricine–NaOH (pH 8.0), 3 m*M* $Na_2H^{32}PO_4$, 0.8 m*M* ADP, 0.25 m*M* ferricyanide (FeCy), and chloroplasts equivalent to 40 μg of chlorophyll. Silicomolybdate concentrations were as indicated and the DCMU concentration (when present) was 2.5 μM. Illumination was with heat-filtered white light of approximately 1000 kergs cm^{-2} sec^{-1}. Electron transport and ATP formation supported by silicomolybdate are shown by curves A and B, respectively. Curves C and D are for electron transport and ATP formation in the presence of DCMU. Curve E is drawn from points obtained by subtracting curve C from A. Note that electron flow from water to SiMo in the absence of DCMU has a K_m of 10 μM for SiMo whereas the K_m for SiMo in the presence of DCMU in the 35–40 μM. These data are suggestive of two sites of SiMo reduction with markedly different affinities for the electron acceptor.

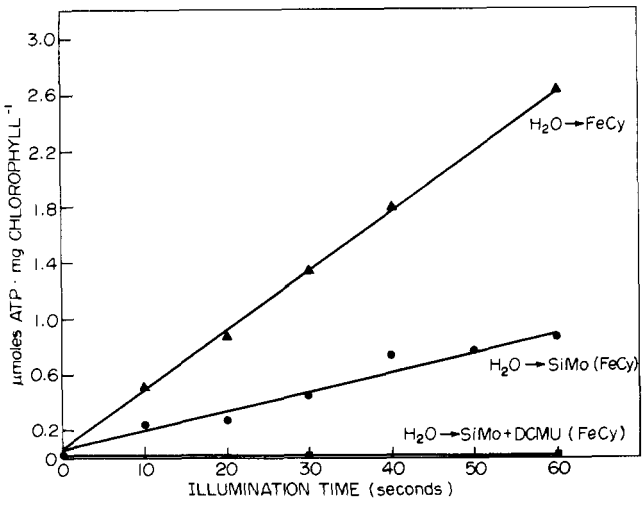

FIGURE 2: Time course of ATP synthesis during electron transport from water to silicomolybdate in the absence and presence of DCMU. The reaction mixture contained in 2 ml: 100 m*M* KCl, 5 m*M* $MgCl_2$, 20 m*M* tricine–NaOH (pH 8.0), 3 m*M* $Na_2H^{32}PO_4$, 0.8 m*M* ADP, 0.5 m*M* ferricyanide (FeCy), 0.5 μM 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone and chloroplasts equivalent to 40 μg of chlorophyll. ATP formation supported by H_2O → ferricyanide (Δ), H_2O → 20 μM silicomolybdate → FeCy (○), and H_2O → silicomolybdate → FeCy with 2.5 μM DCMU (■) are shown. Illumination with heat-filtered white light of approximately 1000 kergs cm^{-2} sec^{-1} was for the time indicated. Incorporation of ^{32}P into ATP was determined as described in Experimental Procedures.

diluting and lowering the pH of the mixture with two volumes of a medium containing 0.1 *M* sucrose, 30 m*M* *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (pH 7.2), and 2 m*M* $MgCl_2$. Izawa et al. (1973b) have shown this procedure is effective in inactivating plastocyanin.

Hydroxylamine Treatment. The ability of chloroplasts to oxidize water was abolished according to the technique of Ort and Izawa (1973). Chloroplasts, at a final chlorophyll concentration of approximately 100 μg/ml, were suspended in the following medium: 0.2 *M* sucrose, 2 m*M* $MgCl_2$, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (pH 7.55), 1 m*M* ethylenediaminetetraacetate, and 10 m*M* NH_2OH. After a 20-min incubation in the dark at 21° the chloroplasts were washed with cold suspending media (described above) to remove the amine.

Binding of Diazonium Benzene[^{35}S]sulfonate. Determination of the electron transport dependent covalent binding of diazonium benzene[^{35}S]sulfonate to chloroplast membranes was accomplished according to the procedures outlined in an earlier paper (Giaquinta et al., 1974c). Evidence that the electron transport dependent DABS binding is primarily to membrane protein via covalent bonds was presented in previous publications (Bering et al., 1973; Giaquinta et al., 1974d, Figure 2).

Measurements. Electron transport was measured with a membrane covered Clark-type electrode as either O_2 evolution or as O_2 consumption when autoxidizable electron acceptors were employed. The samples were illuminated with either orange light (600–700 nm) at an intensity of approximately 600 kerg sec^{-1} cm^{-2} or heat-filtered white light of an intensity of approximately 1000 kergs sec^{-1} cm^{-2}. The reaction temperature was 19°. ATP formation was measured as the residual radioactivity after the extraction of the ^{32}P-labeled orthophosphate as phosphomolybdic acid in butanol–toluene. Radioactivity was determined from the Čerenkov radiation (Gould et al., 1972).

Results

Relationship between Silicomolybdate-Mediated Electron Transport and Energy-Linked Reactions in the Presence and Absence of DCMU. It has been established that photosystem II can reduce silicomolybdate in the presence of DCMU and that this reaction is not coupled to ATP synthesis (Giaquinta et al., 1974b; Giaquinta and Dilley, 1975a). This observation is confirmed in Figures 1 and 2. All experiments depicted in Figure 1 were carried out using chloroplasts in which plastocyanin had been inactivated by KCN treatment (Ouitrakul and Izawa, 1973). This procedure ensured that no photosystem I activity occurred. When DCMU was omitted from the reaction the rate of silicomolybdate reduction was substantially increased, more than fivefold at 25 μM silicomolybdate (cf. traces A and C). Figure 1 shows that the electron transport mediated by silicomolybdate in the absence of DCMU supports a substantial rate of phosphorylation which is sustained at a constant rate for at least 60 sec (Figure 2). This electron transport is coupled to NH_4^+ uptake whereas the electron flux in the presence of DCMU is not (Table I). The addition of 2,5-di-

Table I: NH_4^+ Uptake during Electron Transfer from Water to Silicomolybdate in the Presence and Absence of DCMU.[a]

System	μmoles of NH_4^+ mg of Chl^{-1}
1. $H_2O \rightarrow$ no acceptor	0.49
2. $H_2O \rightarrow$ SiMo	3.19
3. $H_2O \rightarrow$ no acceptor + DCMU	0.0–0.1
4. $H_2O \rightarrow$ SiMo + DCMU	0.0–0.1

[a] The reaction mixture contained in 3 ml: 0.1 *M* choline chloride, 10 m*M* Tris-tricine (pH 7.0), 0.33 m*M* NH_4Cl, and chloroplasts equivalent to 66 μg of chlorophyll. Concentrations of silicomolybdate (SiMo) and DCMU were 33 and 5 μ*M*, respectively. Illumination was with heat-filtered red light (Corning filter 2103) of 200 kergs cm^{-2} sec^{-1} intensity. NH_4^+ uptake was determined with a Beckman Cationic electrode as the decrease in NH_4^+ concentration in the suspending medium.

bromo-3-methyl-6-isopropyl-*p*-benzoquinone, which probably prevents the oxidation of plastohydroquinone (Böhme et al., 1971; Böhme and Cramer, 1972), did not inhibit electron transport to silicomolybdate or its attendant ATP synthesis (data not shown).

It appears that the DCMU-sensitive silicomolybdate reduction is superimposed upon an inhibitor-insensitive reduction. When silicomolybdate reduction (trace A of Figure 1) is corrected for the DCMU insensitive electron flux (trace C), the true concentration dependence of the phosphorylating electron transport is obtained (trace E). When the uncorrected rate (trace A) of silicomolybdate reduction is used to calculate a phosphorylation efficiency (P/e_2) the ratio is diminished as the concentration of silicomolybdate is increased (upper left inset, Figure 1). However, a P/e_2 ratio of 0.35–0.38 is obtained when the "corrected" rate of silicomolybdate reduction (trace E) is used in the calculation and this ratio does not change significantly as the concentration of silicomolybdate is varied (upper right inset, Figure 1). It is important to note that these corrected efficiencies are about the same as the efficiencies of photosystem II phosphorylation accompanying electron transport from water to more conventional lipophilic oxidants such as oxidized *p*-phenylenediamine, oxidized diamonodurene, dimethylbenzoquinone, or 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (Ouitrakul and Izawa, 1973; Izawa et al., 1973a).

Effect of DCMU on Silicomolybdate-Mediated Binding of Diazonium Benzene[^{35}S]sulfonate to Chloroplast Membranes. [^{35}S]DABS treatment of illuminated chloroplasts during electron flux from water to ferricyanide (Table II, line 1) resulted in more than a threefold increase in the amount of DABS bound to the chloroplast membranes compared to that bound by chloroplasts reacted in the light but with DCMU present (line 2) or in the dark (line 5). The amount of diazonium benzenesulfonate bound to the lamellar membranes during electron flow from water to silicomolybdate (line 3) is approximately 2.5-fold greater than that bound in the dark (line 6) or in the light with DCMU added (line 4). These results are consistent with the DABS binding observed when dimethylbenzoquinone or 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone are employed as the acceptors of electrons from photosystem II (Giaquinta et al., 1974a) and indicate that electron flow through a part of the chain beyond the DCMU block is required for the extra binding.

Lack of Light-Dependent Binding of Diazonium Benzene[^{35}S]sulfonate to Chloroplast Membranes during Oxidation of I^- by PS II. When water oxidation has been selectively abolished by hydroxylamine treatment the chloroplasts are able to use alternative sources of electrons. Unique among exogenous photosystem II reductants are ferrocyanide and iodide ions (Izawa and Ort, 1974) and *N,N,N',N'*-tetramethylbenzidine (Harth et al., 1974) which do not release protons upon oxidation.

The level of DABS binding during electron donation by either water (using control chloroplasts) or iodide (using NH_2OH-treated chloroplasts) is presented in Table III. Attempts to use ferrocyanide or *N,N,N',N'*-tetramethylbenzidine were foiled by undefined chemical incompatabilities of the reductants and the diazonium reagent. DABS treatment of illuminated chloroplasts in which water served as the primary electron donor ($H_2O \rightarrow$ MV) resulted in nearly a tripling of the amount of [^{35}S]DABS incorporated over chloroplasts treated in the dark (compared lines 1 and 2, Table III). The selective inhibition of water oxidation by hydroxylamine diminished the amount of DABS bound in the light (line 7) to nearly the dark level (lines 2 and 11). Although the addition of I^- restored significant electron transport to the NH_2OH-treated chloroplasts (105 μequiv hr^{-1} mg of $chlorophyll^{-1}$) there was no increase in the amount of DABS incorporated into the chloroplast membranes (line 8). The inclusion of DCMU completely abolished electron donation by iodide but scarcely affected the level of DABS incorporation (line 9). The small amount of light-dependent DABS binding we did observe in NH_2OH-treated chloroplasts was probably due to residual water oxidation (10 μequiv hr^{-1} mg of $chlorophyll^{-1}$). We cannot absolutely dismiss the possibility that iodide in some fashion prevents light-dependent DABS incorporation. However, we feel that it is unlikely since the addition of I^- to control chloroplasts did not abolish the electron transport stimulated DABS labeling of chloroplast membranes (lines 3 and 4). Moreover, the addition of I_2 did not diminish the amount of [^{35}S]DABS incorporated into control chloroplast membranes (lines 5 and 6). I_2 is a very reactive oxidant and has been shown by Izawa and Ort (1974) to be rapidly reduced by unidentified protonated reductants present in chloroplast suspensions reforming I^-.

Discussion

Figure 1 shows that silicomolybdate can accept electrons at two different sites along the redox chain, only one of which is coupled to ATP formation. One site of silicomolybdate reduction is apparently quite close to the system II phototrap and Q since it is not inhibited by DCMU (cf. Giaquinta and Dilley, 1975a). The second site occurs closer to photosystem I, since it is sensitive to DCMU inhibition, but not beyond plastoquinone because 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone does not inhibit the reaction (Giaquinta et al., 1974b). Only when electrons are permitted to flow to the second site of silicomolybdate reduction is there an increase in the diazonium binding indicative of the membrane conformational change (Table II). The failure of electron transfer from H_2O to silicomolybdate (+DCMU) to result in light-dependent DABS binding (or internal H^+ accumulation) apparently is not due to a membrane disruption caused by silicomolybdate, since silicomolybdate reduction at its second site of acceptance does result in NH_4^+ uptake, phosphorylation and light-dependent DABS binding (Figure 1 and Table I). Thus, these data establish a correlation of photosystem II dependent

Table II: Incorporation of Diazonium Benzene[^{35}S] sulfonate into Chloroplast Membranes during Activation of Electron Transport from Water to Silicomolybdate in the Presence and Absence of DCMU.[a]

System	Reaction Conditions	Electron Transport Rate Prior to DABS Treatment (μequiv hr^{-1} mg Chl^{-1})	[^{35}S]DABS Binding (nmol mg of Chl^{-1}
1. $H_2O \rightarrow$ ferricyanide	Light	151	174
2. $H_2O \rightarrow$ ferricyanide + DCMU	Light	<2	47
3. $H_2O \rightarrow$ ferricyanide	Light	166	90
4. $H_2O \rightarrow$ SiMo + DCMU + ferricyanide	Light	61	39
5. $H_2O \rightarrow$ ferricyanide	Dark		36
6. $H_2O \rightarrow$ SiMo + ferricyanide + or − DCMU	Dark		37

[a] The reaction mixture contained in 2 ml: 100 m*M* KCl, $MgCl_2$, 20 m*M* *N*-2-hydroxyethylpiperazine-*N*'-ethanesulfonic acid (pH 7.2), 0.5 m*M* ferricyanide, and chloroplasts equivalent to 250 μg of chlorophyll. The concentrations of silicomolybdate (SiMo) and DCMU were 50 and 20 μM, respectively. The reaction mixtures were illuminated for 5 sec with heat-filtered red light approximately 500 kergs cm^{-2} sec^{-1} prior to addition of 1 m*M* [^{35}S]DABS and illumination was continued for an additional 15 sec. The rates of electron transport just prior to DABS addition were determined in identical reaction mixtures. After the DABS reaction, the unreacted [^{35}S]DABS was removed by washing and the amount of ^{35}S bound to the membranes was determined (Giaquinta et al., 1974c).

Table III: Incorporation of Diazonium Benzene[^{35}S] sulfonate during Electron Transport to Methylviologen in Hydroxylamine-Treated Chloroplasts.

System	Reaction Conditions	Electron Transfer Rate Prior to DABS Treatment (μequiv hr^{-1} mg of Chl^{-1})	[^{35}S]DABS Binding (nmol mg Chl^{-1})
1. $H_2O \rightarrow$ methylviologen	Light	84	38
2. $H_2O \rightarrow$ methylviologen	Dark		14
3. $H_2O + I^- \rightarrow$ methylviologen	Light	84	28
4. $H_2O + I^-$ methylviologen	Dark		11
5. $H_2O + I_2 \rightarrow$ methylviologen	Light	91	41
6. $H_2O + I_2 \rightarrow$ methylviologen	Dark		14
Hydroxylamine-Treated Chloroplasts			
7. $H_2O \rightarrow$ methylviologen	Light	10	18
8. $I^- \rightarrow$ methylviologen	Light	105	18
9. I^- + DCMU $\rightarrow$ methylviologen	Light	2	12
10. $I^- \rightarrow$ methylviologen	Dark		11
11. $H_2O \rightarrow$ methylviologen	Dark		12

[a] Hydroxylamine treatment of chloroplasts and electron donation from iodide were as described in Experimental Procedures. The reaction mixture used for electron transport and DABS reactions contained in 2 ml: 0.1 *M* sucrose, 50 m*M* tricine–NaOH (pH 8.0), 3 m*M* $MgCl_2$, 0.5 m*M* methylviologen, and chloroplasts equivalent to 300 μg of chlorophyll. Iodide and DCMU at 25 m*M* and 15 μM, respectively, were added as indicated. The amount of I_2 added was 0.20 μmol in 2 ml (lines 5 and 6). This is the amount of I_2 which would be generated from 20 sec of I^- oxidation at a rate of 105 μequiv mg of Chl^{-1} hr^{-1}. Illumination conditions for the DABS reaction and determination of electron transport rates at the time of DABS treatment were as described in Table II. The light intensity was adjusted to lower the rate of the $H_2O \rightarrow$ methylviologen reaction to approximate the rate of the $I^- \rightarrow$ methylviologen reaction.

H^+ accumulation and ATP synthesis with the membrane conformational change.

Electron flux through the Q to plastoquinone segment is not in itself sufficient to cause the membrane conformational change. The oxidation of I^- by photosystem II in NH_2OH-treated chloroplasts was not effective in inducing the additional DABS binding even though there was a substantial rate of electron flow through the Q to plastoquinone region (Table III). Iodide or I_2 apparently did not inactivate DABS chemically, since additional DABS binding was induced by water to methylviologen electron flow in the presence of I^- as well as I_2 (lines 3 and 5 of Table III). The photosystem II oxidation of proton-liberating substances, H_2O in control chloroplasts, or Mn^{2+} in Tris-treated chloroplasts [$H_2O + Mn^{2+} \rightarrow Mn(OH)^{2+} + H^+ + e^-$ (Davies, 1969)] (Giaquinta et al., 1974c) does result in the conformational change.

Although both the photosystem II dependent conformational change and photosystem II dependent ATP formation appear to require the hydrogen ions which are released upon oxidation of the photosystem II donor, the data indicate that it is not simply the internal accumulation of protons within the thylakoid space which is critical for either of these processes. Activation of photosystem I electron transport by the cyclic cofactors *N*-methylphenazonium methosulfate or menadione in the presence of DCMU did not result in any light-dependent DABS binding, even though these systems support a substantial internal accumulation of protons and yield high phosphorylation rates (Giaquinta et al., 1974a). Moreover, electron flow from I^- to methylviologen supports system I phosphorylation and results in proton accumulation (Izawa and Ort, 1974), presumably via plastoquinone involvement, but does not support the additional DABS binding. Perhaps the protons released upon photosystem II oxidation of hydrogen donors are deposited in a region within the membrane that is not in reversible equilibrium with the inner osomotic space. Izawa et al. (1975) presented data which suggest a compartmentation

of the protons released upon water oxidation from those protons translocated by photosystem I dependent reactions.

The proton requirement of the conformational change differs from that of photosystem II ATP synthesis. Gramicidin collapses transmembrane proton gradients and abolishes ATP synthesis at both sites I and II in chloroplasts, but gramicidin (or other uncouplers tested) does not prevent the extra DABS binding which accompanies electron flow from water to methylviologen (Giaquinta et al., 1973).

The obligatory requirement of a membrane conformational change in photosystem II dependent hydrogen ion accumulation and ATP synthesis is an intriguing premise. Possible mechanisms to explain the function of the DABS-detected conformational change in these processes are conjectural. It may be that electron flux in the Q to plastoquinone region produces a condition or state that is required to position the water oxidation system so that protons are released to the inside of (or within) the membrane. One plausible general mechanism for such events can be suggested; electron transport from Q to plastoquinone may cause exposure of amino acid functional groups which are otherwise masked. Once exposed, the release of H^+ from water or exogenous proton liberating electron donors may result in protonation of the amino acid functional groups. It is easy to imagine how the protonation of otherwise ionized groups (e.g., glutamic or aspartic carboxyl groups) could lead to changes in electrostatic interactions among membrane polyelectrolytes, with subsequent rearrangements. Since the rearrangement of membrane proteins could result in alterations of membrane conformation it would not be surprising that new sites for diazonium coupling would be exposed[2] (Dilley and Giaquinta, 1975).

Another possibility which must be considered is that the conformational change is involved as an intermediate in the storage of redox energy, along the lines suggested by Boyer et al. (1973) and Green and Ji (1972). Our data provide no direct evidence which supports this notion. Moreover, we have made two observations which are difficult to reconcile with a model proposing that the DABS-detected conformational change is involved in energy storage: (1) the light-dependent binding of DABS is not prevented by uncouplers of ATP synthesis (Giaquinta et al., 1973); (2) the ability of chloroplast membranes to bind DABS in a "postillumination" fashion is completely gone in less than 1 sec (unpublished data) whereas postillumination ATP synthesis (X_E) can occur for more than 30 sec after the light has been turned off (Hind and Jagendorf, 1963).

[2] It was previously demonstrated that the amount of DABS bound to chloroplast membranes in the light is directly proportional to the rate of electron transport occurring immediately prior to the reaction of the membrane with the chemical modifier (Giaquinta et al., 1974c). Thus it would seem unlikely that the binding can be explained in terms of one DABS molecule binding and in so doing partially "unzipping" the membrane, initiating a type of chain reaction of DABS binding.

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