PHOTOPHOSPHORYLATION NOT COUPLED TO DCMU-INSENSITIVE PHOTOSYSTEM II OXYGEN EVOLUTION

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Received June 17,1974

SUMMARY

An abbreviated Photosystem II electron transport sequence from water to silicomolybdate plus ferricyanide functions in the presence of DCMU. This electron transfer was not coupled to photophosphorylation, and therefore places phosphorylation associated with Photosystem II (Site II) after the site of DCMU inhibition. Silicomolybdate *per se* was not inhibitory to phosphorylation in either the cyclic or noncyclic (water to methylviologen) electron transport mode.

INTRODUCTION

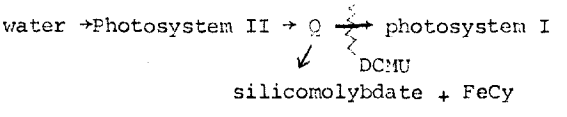
The coupling of photosynthetic electron transport to photophosphorylation (and the oxidative phosphorylation analoque) is an energy transduction process not yet understood (1). Izawa and co-workers (2-4) have extensively studied energy conservation associated with non-cyclic electron transport and postulate the existence of two separate ATP coupling sites in the photosynthetic electron transport chain. They conclude that one coupling site (site II) is associated with photosystem II somewhere between water oxidation and plastoquinone. Site I phosphorylation is believed to be associated with the electron transport between plastoquinone and cytochrome f (5). Site II phosphorylation was shown to display different characteristics than Site I, such as pH optima, lack of photosynthetic control and different uncoupler sensitivity.

Izawa *et al.* (2-4) interpret the Site II phosphorylation in terms of the

Abbreviations: DCMU, 3-(3-4-Dichlorophenyl)-1,1-dimethylurea; PMS, Phenazene methosulfate; DBMIB, 2-5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; SiMo, silicomolybdate; FeCy, ferricyanide.

chemiosmotic hypothesis, which postulates that the water protons are deposited within the grana membrane thereby establishing a proton gradient that can directly drive ATP synthesis. More recent evidence from these workers (6) suggests that the protons do not necessarily have to be generated from water oxidation, since artificial electron donors to photosystem II will catalyze site II ATP formation as long as the oxidation of such compounds releases protons. The implication being that the protons released are deposited within the membrane. No direct evidence is available that establishes that the protons from water oxidation are released within the membrane, but the Gould and Izawa (7) results are consistent with that postulate as are data of Fowler (8) and Junge and Auslander (9). Recent reviews by Trebst (1) and Dilley and Giaquinta (10) discuss this aspect further.

It has been reported that in the presence of silicotungstate, ferricyanide can accept electrons at photosystem II prior to the site of DCMU inhibition with concomitant water oxidation (11). A similar DCMU insensitive oxygen evolution was found by Barr and Crane (to be published separately) using silicomolybdate and ferricyanide, giving faster rates of electron transfer compared to silicotungstate. This is an ideal system with which to test whether the site II ATP formation is coupled to electron transport and/or photochemistry in the abbreviated electron transfer sequence shown below:



The data indicate that this DCMU insensitive photosystem II electron flow is not coupled to ATP formation, and that silicomolybdate is not an inhibitor *per se* of phosphorylation associated either with cyclic or non-cyclic electron flow when the latter utilizes electron transfer from water to methyl viologen.

MATERIALS AND METHODS

Chloroplast isolation from *Spinacia oleracea* was as described previously (12). The isolation medium contained, 0.4 M Sucrose, 20 mM Tricine-KOH (pH 7.8),

3mM $MgCl_2$, 10mM KCl, 3 mM sodium ascorbate and bovine serum albumin (2 mg/ml final concentration). Oxygen evolution or consumption was determined polarographically (12). Photophosphorylation as measured by light dependent incorporation of ^{32}P into ATP was according to the procedure of Neuman et al. (13).

RESULTS

The rates of oxygen evolution mediated by ferricyanide at various silicomolybdate concentrations in the presence of DCMU are shown in Table 1.

Table I. DCMU insensitive oxygen evolution in the presence of silicomolybdate.

Assay:	Silicomolybdate Concentration μM	Oxygen Evolution $\mu eqe^- (hr \cdot mgChl)^{-1}$
$H_2O \rightarrow$ FeCy + SiMo + DCMU	0	0
	25	28
	37	65
	50	100
	75	128
	100	148
	125	143

The reaction mixture contained in 2 ml: 100mM KCl, 5mM $MgCl_2$, 20 mM Tricine-KOH (pH 8.1), 0.5 mM ferricyanide (FeCy), 5 μM DCMU and chloroplasts equivalent to 35 ug Chl/ml. Actinic illumination was with heat filtered white light of intensity 2×10^5 ergs/cm^2/sec. The control rate of oxygen evolution in the water to ferricyanide reaction (minus DCMU and silicomolybdate) was 201/μeqe^- $(hr \cdot mgChl)^{-1}$.

In the presence of silicomolybdate and DCMU the rate of oxygen evolution approaches 75% of the control rate (water to ferricyanide), indicating that ferricyanide and silicomolybdate are being reduced prior to the site of DCMU inhibition. As with silicotungstate (11) this oxygen evolution does result from chloroplast water oxidation as evidenced by the lack of oxygen

Table 2. Electron transport and photophosphorylation in the presence of DCMU and silicomolybdate

Assay	Oxygen Evolution $\mu eq(hr \cdot mgChl)^{-1}$	ATP formation $\mu moles\ (hr \cdot mgChl)^{-1}$
1. $H_2O \rightarrow FeCy$	368	138
2. $H_2O \rightarrow FeCy$ + DCMU	29	4
3. $H_2O \rightarrow FeCy$ + DCMU + SiMo	162	6
4. $H_2O \rightarrow FeCy$ + SiMo	400	25
5. $H_2O \rightarrow FeCy$ (low light)	200	90

Reactions conditions were as described in Table 1 and Methods and Materials. Silicomolybdate and FeCy concentrations were 33 μM and 0.5 mM, respectively.

evolution under these conditions when tris-washed chloroplasts (which lack oxygen evolution capability) were employed.

The data in Table II show that this DCMU insensitive water oxidation is not coupled to phosphorylation. As expected for the control reaction water to ferricyanide, the presence of 5 μM DCMU inhibits both oxygen evolution and ATP synthesis. Silicomolybdate plus DCMU substantially restored the rate of oxygen evolution (approximately 50% of the control rate) but no ATP was formed. In order to determine that the low level of phosphorylation was not simply reflecting the 50% reduction of net electron flow, the rate of electron flow in the control reaction ($H_2O \rightarrow FeCy$) was lowered (by reducing incident light intensity) so as to approximate the rate of electron transfer in the $H_2O \rightarrow SiMo$ + FeCy + DCMU case. As can be seen, substantial phosphorylation occurs during this slower electron transfer rate from water to ferricyanide.

In the absence of DCMU, electron transfer from water to ferricyanide in the

presence of SiMo was increased but again there was no significant phosphorylation (Table II). Apparently ferricyanide in the presence of silicomolybdate can accept electrons at sites before and after the DCMU block, but before the ATP coupling site (Site II). This electron flow from water to silicomolybdate plus ferricyanide does not involve electron transfer past the region of plastoquinone into photosystem I since the inhibitor dibromothymoquinone (DBMIB), a plastoquinone antagonist (14) has no effect on this electron transfer reaction (Table 3).

Table 3. Effect of DBMIB on electron transport from water to ferricyanide in the presence of silicomolybdate.

Assay	Oxygen evolution $\mu eq\,(hr \cdot mgChl)^{-1}$
$H_2O \rightarrow FeCy$	207
$H_2O \rightarrow FeCy$ + DBMIB	71
$H_2O \rightarrow FeCy$ + SiMo	300
$H_2O \rightarrow FeCy$ + DBMIB + SiMo	290

Reaction mixture as in Table 2 and 3. DBMIB concentration was 0.5 μM.

Silicomolybdate is not an inhibitor of photophosphorylation in either the cyclic PMS mediated system or the water to methylviologen non-cyclic system (Table 4). The P/e_2 ratio of near 1.2 for the control water to methylviologen reaction and near 1.0 for the water to methylviologen plus silicomolybdate system shows that silicomolybdate does not inhibit either the Site I or Site II phosphorylation. Methylviologen, unlike ferricyanide, because of its low redox potential cannot accept electrons at the level of photosystem II and the DCMU inhibited methylviologen reduction cannot be restored by silicomolybdate, consistent with the data reported for silicotungstate (11).

Table 4. Electron transport and photophosphorylation with ferricyanide and methylviologen in the presence of silicomolybdate.

Assay	Oxygen Evolution $\mu eq\,(hr \cdot mgChl)^{-1}$	ATP formation $\mu moles\,(hr \cdot mgChl)^{-1}$	P/2e
$H_2O \rightarrow FeCy$	301	108	0.72
$H_2O \rightarrow FeCy$ + SiMo	518	24	0.09
$H_2O \rightarrow MV$	192	111	1.15
$H_2O \rightarrow MV$ + SiMo	220	109	0.99
PMS	---	170	----
PMS + SiMo	---	172	----

Reaction conditions described in Tables 1 and 2. Methylviologen (MV) concentration was 0.5mM. The methylviologen assays contained 0.5 mM sodium azide. PMS concentration was 30 μM.

DISCUSSION

These results show that electron transport from water to a site prior to the DCMU block is not coupled to ATP formation. However, a dilemma is presented when comparing these results with other recent developements in research on photophosphorylation. Izawa and colleagues (2-4,7) interpret their data on site II phosphorylation as consistent with water oxidation depositing protons inside the membrane and that proton deposition is the primary driving force or condition needed for Site II ATP formation. Here we have shown (a) water oxidation occurs in th presence of SiMo + FeCy + DCMU, (b) that electron transfer from water to FeCy + SiMo does not support ATP formation, (c) that SiMo itself is not inhibitory to ATP formation, and (d) nor does it interfere with the "site II" ATP formation believed to occur in the water to methylviologen case (the P/e_2 stays near 1).

Following the logic pattern we suggest: either (1) that water oxidation does not result in internal deposition of protons, or (2) that silicomolybdate

+ FeCy, but not silicomolybdate + methylviologen gives an anomolous situation wherein water protons are not deposited inside or (3) that water protons are deposited inside in the water to FeCy + silicomolybdate + DCMU case, but that internal water proton deposition alone is not enough to drive ATP formation, another energetic or catalysis event being necessary that is linked to a redox step(s) not activated by the abbreviated water to silicomolybdate electron transfer chain. Further work is in progress to test these alternatives.

The authors gratefully acknowledge the expert technical assistance of Barbara J. Anderson. This work supported by NSF Grant GB30998 and NIH Grant 5R01GM19595 and Career Development Award to R.A.D. and NSF Grant GB-27501 to F.L.C. Drs. Barr and Crane have recently shown that silicomolybdate can accept electrons in the absence of ferricyanide as evidenced by formation of reduced silicomolybdate.

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