Hg^{++} - A DCMU* INDEPENDENT ELECTRON ACCEPTOR OF PHOTOSYSTEM II**

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SUMMARY

Mercuric chloride functions as a direct electron acceptor from the quencher of fluorescence in Photosystem II. The photoreduction of ferricyanide, dichlorophenol-indophenol or methyl viologen is inhibited by mercuric ion while oxygen evolution is uneffected. Mercuric chloride supported oxygen evolution (mercury Hill reaction) is not prevented by DCMU or other similar electron transport inhibitors.

INTRODUCTION

Mercury containing compounds are known to inhibit photosynthesis in algae (1-3) as well as various photosynthetic reactions of isolated chloroplasts (4-6). Most of the experimental work reported with mercurials employed organic compounds and a minimum of information exists on the effects of mercuric salts toward photosynthesis. Using spinach chloroplasts MacDowall (6) noted that $HgCl_2$ stimulated phenol-indophenol reduction at concentrations less than 1μM but inhibited dye reduction at higher concentrations with a point for 50% inhibition at about 50μM. Izawa and Good (4), studying the effect of $HgCl_2$ at 1μM, concluded that mercuric ion acts as an energy transfer inhibitor which prevents ATP synthesis while it stimulates ferricyanide reduction in the absence of ADP. They did not use high enough concentrations of $HgCl_2$ to observe the inhibition of uncoupled electron transport previously reported (6) but did note that an "unspecific" inhibition appears at higher concentrations. We have studied the inhibition of photosynthetic electron transport in isolated spinach chloroplasts at concentrations high enough to limit photoreduction of Hill oxidants. This paper presents evidence that $HgCl_2$, at 100μM

*DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

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inhibits electron transport by acting as a preferred electron acceptor at the site of the quencher (Q) of chlorophyll fluorescence in Photosystem-II.

METHODS

Once washed spinach *(Spinacia oleracea L.)* chloroplasts were prepared from market leaves, as described previously (7), in a grinding medium containing 20mM hydroxyethylpiperacine ethanesulfonic acid (HEPES) buffer (pH 7.8), 0.8 M sucrose and 10mM NaCl. Chloroplasts were resuspended in fresh grinding medium and the amount of chlorophyll determined by a modification of a standard method (8).

Electron transport was measured using previously described techniques (9). The reaction mixture for oxygen evolution contained, in millimolar concentrations, HEPES buffer, 50 (pH 7.8); NaCl, 30; $MgCl_2$, 2.5; and 100µg chlorophyll. Either $K_3Fe(CN)_6$, 1mM; 2,6-dichlorophenol-indophenol (DCIP), 33µM; or $HgCl_2$, 100µM were added as the electron acceptor. For methyl viologen reduction the above mixture was used with 100µM viologen and 0.5mM NaN_3 replacing the dye. DCIP photoreduction was recorded at 590nm in a Beckman DK2-A spectrophotometer equipped for side illumination of the sample curvette. Blue actinic light was provided through a Corning 4303 filter at $1x10^5$ ergs/cm^{2}sec. The photomultiplier was blocked from the actinic light by a Turner 590nm interference filter. The reaction mixture used was that for oxygen evolution with DCIP except that 25µg of chlorophyll was used. The sensitive ferrocyanide assay used here was as described by Avron and Shavit (10).

Fluorescence induction kinetics of isolated chloroplasts were recorded as described before (11) except that, as above, HEPES was the buffer included.

RESULTS

Figure 1 illustrates the effect of $HgCl_2$ on various electron transport reactions in chloroplasts. When the photoreduction of common Photosystem I (methyl viologen) or Photosystem II (ferricyanide or DCIP) electron acceptors was followed, there was a clear inhibition with a 50% point about 200µM (lower curves). However, when oxygen evolution was measured with a Clark-electrode using ferricyanide or DCIP as the electron acceptor, there was no inhibition by $HgCl_2$ (upper curves). This inhibition of

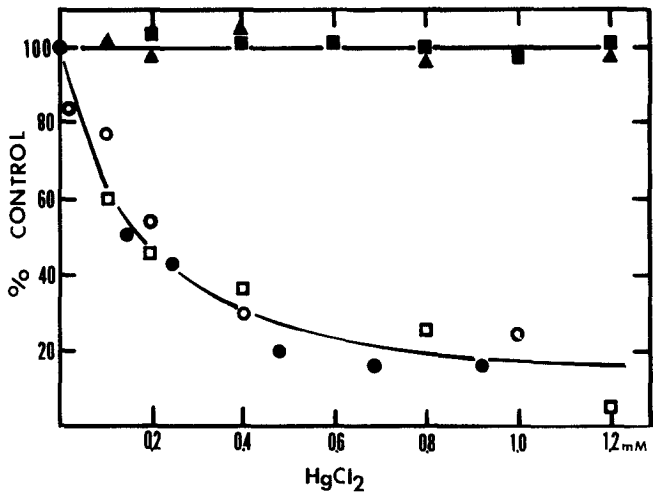

Fig. 1. The effect of $HgCl_2$ on electron acceptor reduction of oxygen evolution with spinach chloroplasts. Symbols represent (with 100% rate in μmoles/mg Chl/hr in brackets): ○, ferricyanide reduction [71]; ●, DCIP reduction [123]; □, methyl viologen reduction [201]; ■, DCIP oxygen evolution [104]; and ▲, ferricyanide oxygen evolution [77]. Other conditions are described in METHODS.

dye reduction agrees with earlier work (4,6) on $HgCl_2$ but the lack of an effect on concommitant oxygen evolution was perplexing. A possible explanation of the differential effect of $HgCl_2$ was that chloroplasts photoreduced mercuric ions at a site between Photosystem II and the point at which various dyes accept electrons. This theory explains why $HgCl_2$ prevents dye reduction while it supports oxygen evolution. To test this idea we measured the extent of oxygen evolution with increasing concentrations of $HgCl_2$ in a reaction mixture containing no other electron acceptor. With no, or very low concentrations of $HgCl_2$, there was little detectable oxygen evolution (Fig. 2). However, in the concentration range from 10 to 100μM, there was a linear increase in electron transport with either $HgCl_2$ or $Hg(CH_3COO)_2$ as the electron acceptor.

If Hg^{++} accepts electrons from Photosystem II at a site before the usual dyes, it would be of immediate interest to determine whether DCMU or other known inhibitors of electron transport would limit the Hg^{++} stimulated oxygen evolution. Table I shows the amount of Hg^{++} supported oxygen evolution with various inhib-

TABLE I - Effect of Electron Transport Inhibitors on $HgCl_2$ Supported Oxygen Evolution

Inhibitor	Conc. (μM)	μmoles oxygen/mg Chl/hr
None	-	45
DCMU	1	56
CMU	10	64
o-phenanthroline	100	45
HOQNO	10	41
$PbCl_2$	200	9
Tris-Wash	0.8M	22

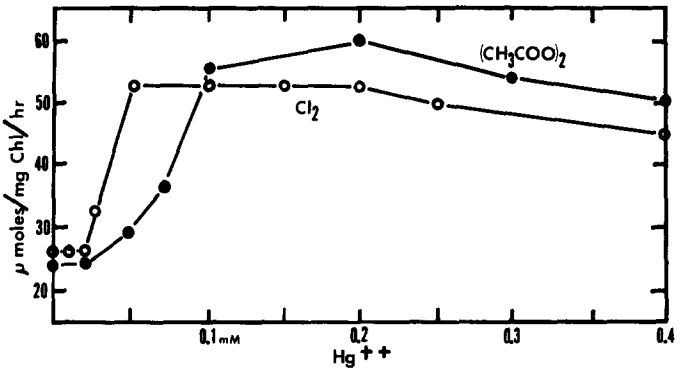

Fig. 2. The effect of $HgCl_2$ or $Hg(CH_3COO)_2$ as an electron acceptor for oxygen evolution in spinach chloroplasts. No other electron acceptor was added. Condition as described in METHODS.

itors. Surprisingly, these compounds (DCMU;3-(p-chlorophenyl)-1, 1-dimethylurea, (CMU); 2-heptyl-4-hydroxyquinoline-N-oxide, (HOQNO); o-phenanthroline) have no inhibitory effect. This was even observed for those inhibitors such as DCMU which block very near Photosystem II. However, treatments which limit electron transport on the oxidizing side of Photosystem II, such as 200μM $PbCl_2$ (11) or a 0.8M Tris-wash of the chloroplasts (12), reduce electron transport. These observations could best be explained if Hg^{++} accepts electrons from the chain on the reducing side of Photosystem II before the DCMU-block, i.e., at the point of Q.

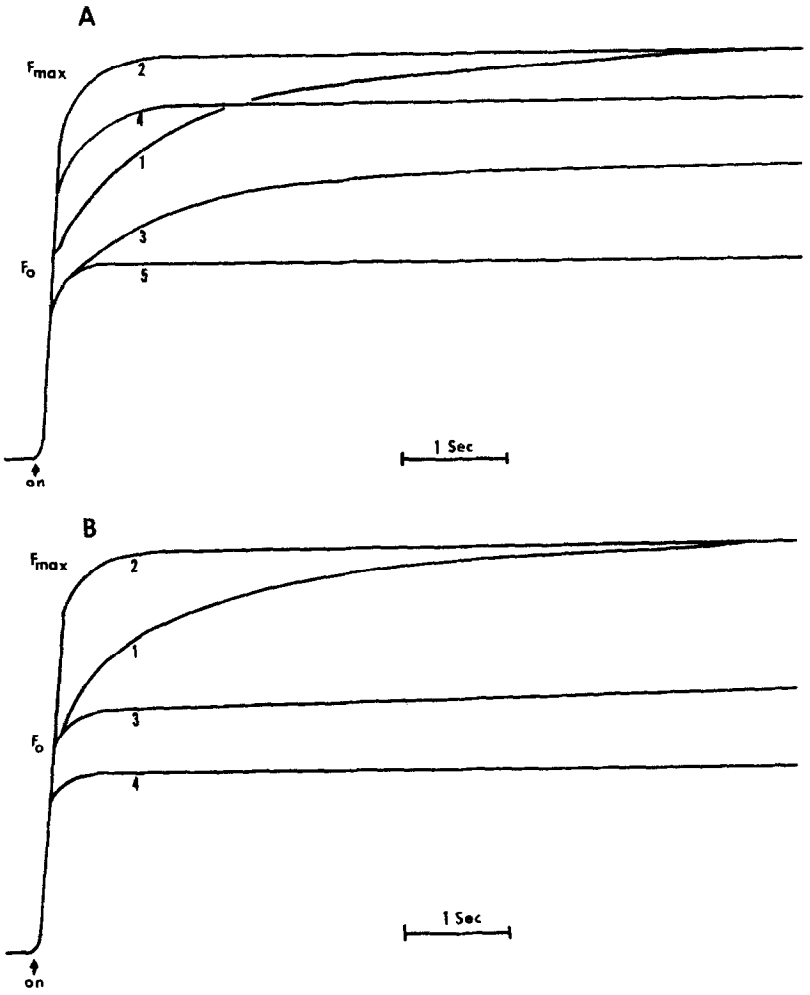

Fig. 3. Fluorescence induction kinetics of isolated chloroplasts. Trace 1, control responses; trace 2, 3μM DCMU. Fig. 3A. Trace 3, 430μM ferricyanide; trace 4, 430μM ferricyanide and 3μM DCMU; trace 5, 430μM ferricyanide and 280μM $HgCl_2$. Fig. 3B. Trace 3, 280μM $HgCl_2$; trace 4, 280μM $HgCl_2$ and 3μM DCMU. Technique as in ref. 11.

If Hg^{++} is directly accepting electrons from Q, or in lieu of Q, this could be confirmed by observing the oxidation-reduction state of Q during fluorescence induction. Figure 3 illustrates the comparative effects of $HgCl_2$ and $K_3Fe(CN)_6$ (known to oxidize Q) (13) on fluorescence changes of dark adapted spinach chloroplasts. Both $K_3Fe(CN)_6$ (Fig. 3A) and $HgCl_2$ (Fig. 3B) oxi-

dize Q and suppress fluorescence to near the initial level (F_o). DCMU blocks the oxidation of Q by $K_3Fe(CN)_6$ and a near maximum fluorescence emission (F_{max}), much like the effect of DCMU alone is seen. Hg^{++} also oxidizes Q and keeps fluorescence low but DCMU does not prevent this oxidation. When both DCMU and Hg^{++} are added together (Fig. 3B, trace 4) fluorescence remains at F_o or lower. Therefore, Hg^{++} must accept electrons directly from Q or from the Photosystem II reaction center chlorophyll.

CONCLUSIONS

Synthetic electron acceptors have been very important to the study of photosynthesis and other oxidation-reduction reactions. A standard assay for Photosystem II activity was to measure ferricyanide reduction or oxygen evolution supported by ferricyanide. Ferricyanide Hill reaction involves a phosphorylation site (14) and is inhibited by DCMU. Hg^{++} supported oxygen evolu-

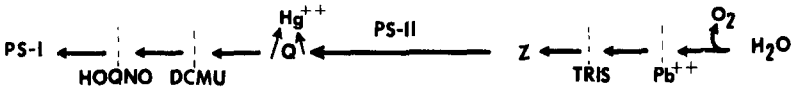

Fig. 4. Scheme for site of Hg^{++} function.

tion or the mercury Hill reaction involves a much shorter segment of the electron transport chain; is not inhibited by DCMU, but is limited by blocking electron transport between the oxidizing side of Photosystem II and water (Fig. 4). Standard uncouplers of ATP synthesis do not stimulate mercury Hill reaction (our data not shown), therefore a coupling site is not involved.

This new electron acceptor, functioning at or before Q, provides us with a new and very important assay for Photosystem II activity. Already we can conclude that models of Photosystem II which places the DCMU sensitive site on the oxidizing side of the photoact (15) are not supported by our observations. It should be quite interesting to examine the effect of Hg^{++} on Q, on the plastoquinones, on C-550, on cytochrome b_{559}, on P-680 and other components of Photosystem II.

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