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PHOTOSYSTEM I ELECTRON TRANSPORT AND PHOSPHORYLATION SUPPORTED BY ELECTRON DONATION TO THE PLASTOQUINONE REGION

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SUMMARY: In chloroplasts, tetramethyl-p-hydroquinone supports high rates of phosphorylation-coupled, noncyclic electron flow through Photosystem I to methylviologen. The reaction is totally sensitive to dibromothymoquinone, indicating an electron donation to the plastoquinone region of the photosynthetic chain. The uncoupled electron flow rate exceeds 1000 μequivalents per hour per mg chlorophyll. The phosphorylation efficiency (P/e_2) at the optimal pH of 8 is 0.6-0.65. Presumably this ratio represents the efficiency of energy coupling in the electron transfer step plastoquinone → cytochrome f.

INTRODUCTION

Phenylenediamines and reduced indophenol dyes represent artificial electron donors that support noncyclic electron flow through PS I*. Reactions mediated by these reductants are sensitive to the plastocyanin inhibitor KCN (1) but are insensitive to the plastoquinone analog DBMIB (2-4), indicating that electron donation by these substances occurs predominantly in the cytochrome f — plastocyanin region of the photosynthetic chain. Significantly, however, the photoreduction of NADP supported by thymohydroquinone (5) and anaerobic cyclic photophosphorylation catalyzed by low potential quinones (6) or by ferredoxin (3) have been shown to be sensitive to DBMIB, thus indicating an electron donation to the plastoquinone region. Unfortunately, the usefulness of these reaction systems for kinetic studies is limited due to the slow rate in the thymohydroquinone reaction (40 $\mu equiv \cdot hr^{-1} \cdot mg\ chlorophyll^{-1}$) and due to the unknown electron flux in the cyclic reaction.

In this communication we report that tetramethyl-p-hydroquinone ($TMQH_2$) supports a highly DBMIB-sensitive noncyclic electron flow through PS I to MV under aerobic condition. The electron flow is fast and is well coupled. We believe that this simple reaction system will provide new access to the mechanisms of electron transfer and energy coupling in the plastoquinone -- cytochrome f region of the photosynthetic chain.

*Abbreviations used: DAD, diaminodurene; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (dibromothymoquinone); DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MV, methylviologen; PS I (II), Photosystem I (II); SOD, superoxide dismutase; TMQ, tetramethyl-p-benzoquinone; $TMQH_2$, tetramethyl-p-hydroquinone.

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MATERIALS AND METHODS

Envelope-free (Class II) chloroplasts were prepared from market spinach (Spinacia oleracea) as described elsewhere (7) and finally suspended in a medium consisting of 0.2 M sucrose, 10 mM tricine-NaOH buffer (pH 7.5), 2 mM $MgCl_2$ and 10 mM NaCl. The chloroplast preparations were virtually free of catalase activity.

Tetramethyl-p-hydroquinone ($TMQH_2$) or durohydroquinone was prepared from duroquinone (Sigma) as follows: about 2 mg of $NaBH_4$ was added to 1 ml of ice-chilled stock solution of duroquinone (25 mM in ethanol-ethylene glycol 1:1 mixture) placed in a slender vial. After mixing, the solution was allowed to stand on ice for 5 min, during which time the reduction was complete. (There was enough moisture in the solvent to allow complete reduction of the quinone.) 5 µl of concentrated HCl was then added to the solution to stabilize the hydroquinone formed and to decompose the remaining $NaBH_4$. No detectable re-oxidation of $TMQH_2$ occurred in this stock solution during 5-6 hr experiments.

Bovine blood superoxide dismutase (SOD), purchased from Sigma, was dissolved in 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-NaOH buffer (pH 7.4). The solution (1 mg/ml or about 3000 units/ml) was dialyzed overnight against the same buffer and stored frozen. DAD (dihydrochloride) from Aldrich was recrystallized from aqueous ethanolic solution by adding excess HCl. DBMIB was a gift from Dr. N. E. Good.

Electron transport from $TMQH_2$ to MV was assayed as the O_2 uptake resulting from the reoxidation of reduced MV. A Clark-type O_2 electrode was used for assay. The actinic light used was a broad-band red light (620-700 nm: 500 $Kergs.sec^{-1}.cm^{-2}$). The reaction temperature was 21° C. Phosphorylation was measured as [^{32}P]ATP formation as detailed elsewhere (8).

RESULTS

TMQ (E_{m7} +0.07 V) is among those quinones which have been shown to support low rates of DBMIB-sensitive cyclic photophosphorylation under anaerobic conditions (6). We found that $TMQH_2$, prepared from TMQ as detailed in the Methods section, was sufficiently stable, even in a weakly basic reaction medium, to permit examination of the aerobic reaction system DCMU/$TMQH_2$ → MV (→O_2). Ascorbate, which is normally used as an electron reservoir for the direct donors, was useless in this system because ascorbate is too weak a reductant (E_{m7} +0.08 V) to keep $TMQH_2$ in its reduced form. As described below, not only was the $TMQH_2$ → MV reaction possible, the reaction turned out to be an unexpectedly fast one, even when allowance was made for the fact that above pH 7 the O_2 consumption rate was inflated (by ≤ 50%) due to the oxidation of $TMQH_2$ by O_2^-, which is the initial product of O_2 reduction by reduced MV.

O_2 traces shown in Fig. 1 are from experiments run at pH 8 in which a sufficient amount of SOD (see legend) was included in the reaction mixture to maximally suppress the O_2 uptake (approx. 40% suppression in this case). As has been discussed for ascorbate-involving donor reactions (9), the light-induced consumption of 1 molecule of O_2 under these conditions should correspond to the transfer of a pair of electrons ($2e^- \equiv O_2$) according to the formula:

$$TMQH_2 + O_2 \xrightarrow[MV,\ SOD]{2e^-\ \text{transport}} TMQ + H_2O_2 \qquad \text{(Eqn. 1)}$$

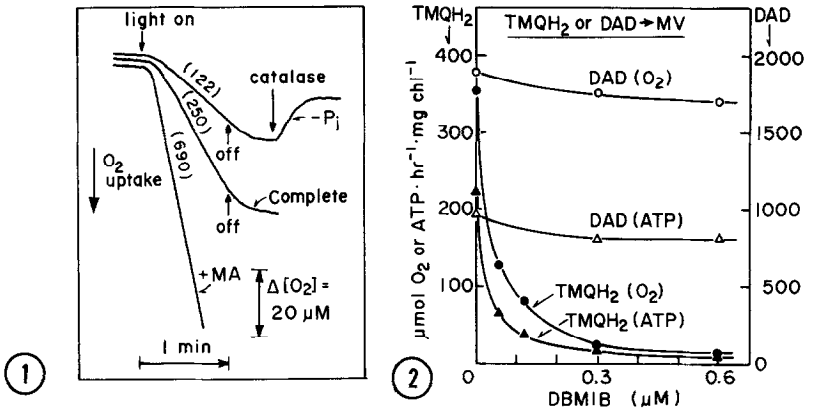

Fig. 1. O_2 traces for the $TMQH_2$ → MV reaction. The complete reaction mixture (2 ml) contained 0.1 M sucrose, 40 mM tricine-NaOH buffer (pH 8.0), 5 mM $MgCl_2$, 5 mM [^{32}P]Na_2HPO_4 (P_i), 0.8 mM ADP, 5 μM DCMU, 0.1 mM MV, 0 5 mM $TMQH_2$, chloroplasts equivalent to 12 μg chlorophyll/ml, and 75 μg (220 units) SOD/ml. The amount of SOD used was about 50% more than the minimum amount required to maximally suppress O_2 uptake. When added, methylamine-HCl (MA) was 10 mM. In the top trace, a small amount of catalase (approx. 1 μg) was added at the time indicated by the arrow. The numbers given along the traces are O_2 uptake rates in μmol.hr^{-1}.mg chlorophyll^{-1}.

Fig. 2. Comparison of the effects of DBMIB on the $TMQH_2$ → MV reaction and the DAD → MV reaction. The reaction mixture used for the $TMQH_2$ reaction was as in Fig. 1. For the DAD reaction, $TMQH_2$ was replaced by 0.5 mM DAD plus 1 mM D-ascorbate and less chloroplasts were used (6 μg chlorophyll/ml). In both reactions SOD (220 units/ml) was present in the reaction mixture. The reaction time was 30 to 120 sec depending on the rate.

The stoichiometric reduction of O_2 to H_2O_2 was demonstrated by the experiment of Fig. 1 (top trace) which shows that the addition of catalase after turning off the light released an amount of O_2 which was equivalent to one half of the amount of O_2 taken up during the preceding illumination. The traces of Fig. 1 also depict that the reaction rate is markedly increased by the addition of ADP and phosphate (trace labelled "complete") or of the uncoupler methylamine (+ MA). The rate of phosphorylating electron transport ranged from 500 to 700 μequiv (250 to 350 μmol O_2)·hr^{-1}.mg chlorophyll^{-1} depending on the chloroplast preparation. Uncoupled rates were 2 to 3 times as high.

The experiment shown in Fig. 2 demonstrates that the $TMQH_2$ → MV reaction and associated phosphorylation are highly sensitive to DBMIB, in sharp contrast to the typical PS I reaction DAD → MV which is almost completely insensitive. The sensitivity of the $TMQH_2$ reaction to DBMIB shown in Fig. 2 may even seem unusually high (90% inhibition by 0.2 μM DBMIB), but presumably this is due

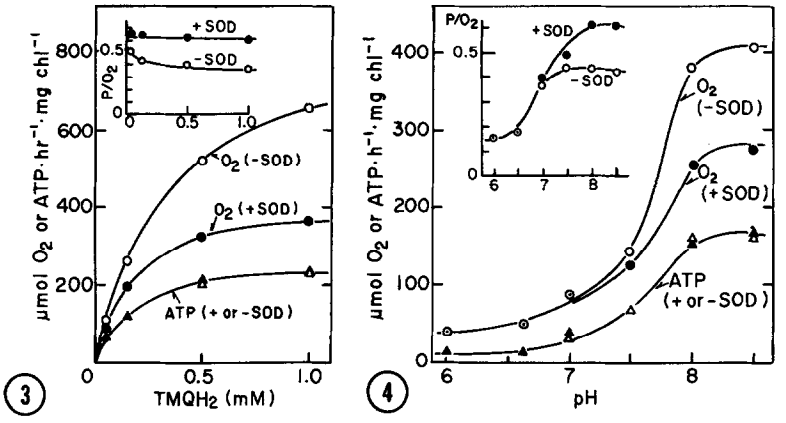

Fig. 3. The $TMQH_2 \rightarrow MV$ reaction and associated phosphorylation as a function of the $TMQH_2$ concentration. The reaction mixture used was as in Fig. 1 except for the varied concentrations of $TMQH_2$ and the omission of SOD in one set of experiments (-SOD curves).

Fig. 4. The pH dependence of the $TMQH_2 \rightarrow MV$ reaction and associated phosphorylation. The basic composition of the reaction mixture was as in Fig. 1 except for the varied pH and the omission of SOD in one set of experiments (-SOD curves). The buffers used were: 2-(N-morpholino)ethanesulfonic acid (MES)-NaOH (pH 6-6.6), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-NaOH (pH 7-7.5) and tricine-NaOH (pH 8-8.5).

to the dilute chloroplast suspension used for the reaction (12 μg chlorophyll per ml).

Fig. 3 shows that both the O_2 uptake rate and the phosphorylation rate increase sharply with the increase in $TMQH_2$ concentration and saturate approximately at 1 mM $TMQH_2$. The apparent K_m value found is about 0.15 mM (from +SOD data). The degree of suppression of O_2 uptake by SOD also increases with the $TMQH_2$ concentration and approaches 50% at 1 mM $TMQH_2$. This suggests that at 1 mM $TMQH_2$ the O_2^- radical formed is almost all reduced to H_2O_2 by the $TMQH_2$ unless forced to dismutate to O_2 and H_2O_2 by added SOD (cf. ref. 9). The P/e_2 ratio, that is the P/O_2 ratio computed on the basis of O_2 data obtained with excess SOD, was practically constant (0.6 to 0.65) over the entire range of $TMQH_2$ concentrations tested (+SOD data in Fig. 3, inset).

pH profiles for the $TMQH_2 \rightarrow MV$ reaction and associated phosphorylation are shown in Fig. 4. Below pH 7 the electron transport rate, phosphorylation rate and phosphorylation efficiency are all very low but they increase rapidly, as the pH is raised, to reach maximum values at pH 8-8.5. Measurements above pH 8.5 were unreliable because of the increased rate of autoxidation of $TMQH_2$ (data not shown). The cause for the apparent lack of SOD effect on O_2 uptake

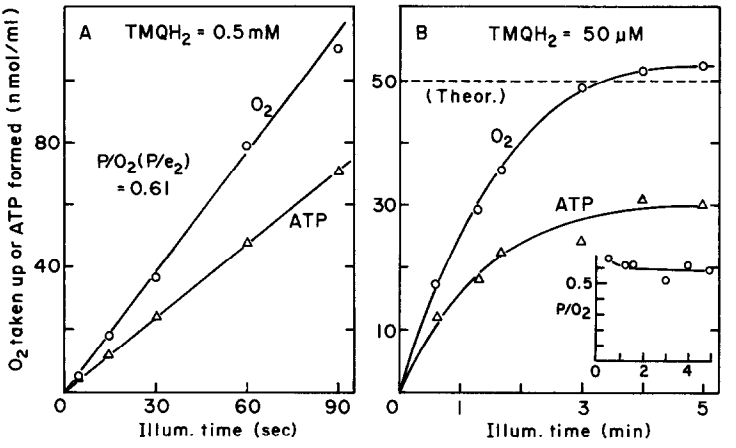

Fig. 5 (A,B). The kinetics of the $TMQH_2 \rightarrow MV$ reaction and associated phosphorylation at two different $TMQH_2$ concentrations. A: $TMQH_2$ = 0.5 mM where the reaction is close to zero order with respect to the $TMQH_2$ concentration; B: $TMQH_2$ = 50 μM where the reaction is close to first order. The basic composition of the reaction mixture was as in Fig. 1 (with 220 units SOD) except that the mixture for B contained twice as much chloroplasts (24 μg chlorophyll/ml). The amounts of O_2 taken up and ATP formed were determined using a series of identical reaction mixtures illuminated for different periods of time.

below pH 7 has not been pursued. It may be due to the increased chemical stability of $TMQH_2$ at low pH, or due to the low steady-state rate of O_2^- production, or both.

Fig. 5 (A,B) illustrates that the kinetics of ATP formation follow very closely the kinetics of O_2 uptake at all $TMQH_2$ concentration ranges — from the zero-order kinetics range ($\geqslant$ 0.5 mM) to the first order range ($\leqslant$ 50 μM). The P/e_2 (P/O_2) ratio remains constant throughout the course of the reaction. These data strongly suggest a direct coupling between ATP formation and the electron transport observed as O_2 consumption. There was no sign of concurrent cyclic photophosphorylation at any stage of the reaction. It seems that the induction of cyclic electron flow by accumulating TMQ (oxidation product of $TMQH_2$) is effectively prevented by the presence of the efficient acceptor MV in our aerobic reaction system. The data of Fig. 5B also show that the exhaustion of 50 nmol of $TMQH_2$ (per ml reaction mixture, or 50 μM) is accompanied by an uptake of 50 nmol of O_2 as predicted by Eqn. 1.

DISCUSSION

The experiments described above demonstrate that the flow of electrons from the plastoquinone region to PS I can be observed in the presence of DCMU using $TMQH_2$ as the electron donor and MV/O_2 as the acceptor system. Although the susceptibility of $TMQH_2$ to O_2^- poses some problem in computing electron flow

rates from O_2 uptake rates, the problem can be overcome by the use of SOD as documented above. However, under routine conditions ($TMQH_2 \leqslant 0.5$ mM, pH $\leqslant 8$) the SOD-sensitive part of O_2 uptake, that is the "extra" O_2 consumption due to the oxidation of $TMQH_2$ by O_2^-, does not exceed 40% of the total O_2 uptake rate (Figs. 3,4). This would mean that even if the relation $2e^- \equiv O_2$ (Eqn. 1) was applied to O_2 data obtained in the absence of added SOD, the resultant overestimation of electron flow would not exceed 40%.

The ability of $TMQH_2$ to donate electrons to plastoquinone ($E_{m7} \sim +0.1$ V) is not surprising in view of the lipophilicity and the standard redox potential of $TMQH_2$ (E_{m7} +0.07 V), and also in view of the structural kinship between the two substances. The near-complete inhibition (>95%) of the $TMQH_2$ reaction by DBMIB, which indicates that $TMQH_2$ is virtually unable to donate electrons to cytochrome f or plastocyanin, was somewhat unexpected. Qualitatively, however, this is in agreement with in vitro experiments (10, 11) which show that electron transfer from p-hydroquinone to these metalloproteins in solution is quite slow. The slowness of this in vitro electron transfer has been ascribed to the thermodynamic barrier inherent in the removal of 1 e^- from the hydroquinone (11).

The phosphorylation efficiency (P/e_2) of the $TMQH_2 \rightarrow MV$ reaction, which presumably represents the enrgy coupling efficiency of the electron transfer step plastoquinone $\rightarrow$ cytochrome f, is 0.6 to 0.65 — that is almost exactly one half of the efficiency of standard noncyclic photophosphorylation in the chloroplast preparations used in this study (P/e_2 1.2). Clearly this is in line with the currently accepted two-site model of photosynthetic phosphorylation. Furthermore, the magnitude of the stimulation of electron flow by the addition of ADP/phosphate or of uncouplers (Fig. 1), the absolute rate of electron flow (e.g., 500-700 μequiv.hr^{-1}.mg $chlorophyll^{-1}$ under phosphorylating conditions), and the effects of pH on electron flow and phosphorylation (Fig. 4), are all quite similar to those regularly observed for the whole chain electron transport system (e.g., $H_2O \rightarrow MV$). Thus, it would appear that the energy coupling and the rate-controlling mechanisms associated with the plastoquinone $\rightarrow$ cytochrome f step still operate normally when $TMQH_2$ substitutes for PS II as the supplier of electrons to the plastoquinone.

The P/e_2 value of 0.5 reported by Hauska et al. (5) for the thymohydroquinone $\rightarrow$ NADP system is somewhat lower than the value we found for the $TMQH_2 \rightarrow MV$ reaction. This difference is probably not significant considering the differences in experimental conditions. It is interesting, however, that the DBMIB-insensitive DAD $\rightarrow$ MV reaction run in parallel experiments (cf. Fig. 2) consistently showed a lower P/e_2 value of 0.45 to 0.5. The P/e_2 values previously found for various other DBMIB-insensitive PS I reactions (4,9) are also

slightly lower (0.5-0.6). However, the reduced 2,6-dichlorophenolindophenol → MV system not only did consistently show a P/e_2 ratio of 0.65 (4,9) but also exhibited characteristics (e.g., pH optima, absolute electron flow rates, and the energy control of electron flow) which are rather similar to those of the $TMQH_2$ → MV system we have documented above (12). The implications of these and other differences and similarities between the $TQMH_2$ reaction and DBMIB-insensitive PS I reactions have yet to be explored.

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ADDENDUM

After the submission of this communication, a paper by White et al. (White, C.C., Chain, R.K., and Malkin, R. (1978) Biochim. Biophys Acta 502, 127-137) has become available which deals with the same reaction system as we have described. Some of the results shown in the paper are very similar to ours.

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