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KINETIC OBSERVATION OF THE SYSTEM II ELECTRON ACCEPTOR POOL ISOLATED BY MERCURIC ION

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SUMMARY

Oxygen flash yields of spinach chloroplasts were measured in the presence and absence of Hg^{2+}. The observed flash yield patterns showed that Hg^{2+} abolishes the communication of the System II electron acceptor pool with System I and most of its associated redox pool. These experiments suggest that the primary electron acceptor Q reacts as one of about five kinetically identical equivalents.

In a previous communication [1] we concluded that the two pools of electron carriers between Photosystems I and II (A_2 and A_1) each contained about five electron equivalents. The A_2 pool behaved kinetically as if it were identical to Q, the primary electron acceptor of System II. From this and other evidence we suggested that the Q–A_2 complex consisted of two molecules of plastoquinone.

Our conclusions were based on observations with isolated chloroplasts without added electron acceptor. The interpretation was complicated by electron flow(s) from System II to the A_1 pool and to O_2 (either directly or indirectly via System I in the Mehler reaction [2]). Consequently, the data required extensive computer analysis.

In this communication, we report the use of a specific poison (Hg^{2+}) which completely abolishes the communication of System II with the A_1 pool, System I, and its leak path to O_2. Since the inhibitor did not affect System II or O_2 evolution, it allowed a more straightforward determination of the abundance of the acceptor pool, A_2.

The approach and general technique were the same as that used in ref. 1. After an appropriate dark "restoration" time (approx. 5 min) the analysis of the oxidizing and reducing sides of System II was made using sequences of short (< 5 μs) saturating light flashes. Since each saturating flash hits all traps in the system once, reactions close to the two photosystems proceed in single equivalent steps. By measuring the O_2 evolved by each flash in a series, it is possible to determine the momentary distribution of the "S states" of Photosystem II while (in the absence of electron acceptor), the endogenous electron acceptor pools are being reduced.

Abbreviations: A_1, Photosystem I; A_2, Photosystem II; Y_{ss}, steady-state yield.

Flash yields of O_2 were measured at room temperature (20–25 °C) using spinach chloroplasts in the absence of added electron acceptor. In these experiments we used a membrane-covered electrode which will be described elsewhere. With the bare electrode used in our earlier work, we did not observe any effect of Hg^{2+}, presumably because it was reduced on the electrode surface.

Treatment with Hg^{2+} was done by two different methods. In the first case chloroplasts were placed on the electrode and treated in situ by adding $HgCl_2$ to the flow medium. In the second case, the chloroplasts were treated before being deposited on the electrode; chloroplasts (1 mg chlorophyll) were incubated in 5 ml 50 mM Tris (pH 7.5)–1 mM $HgCl_2$ for 30 min at 0 °C. The rates of the $Fe(CN)_6{}^{-3}$ and methyl viologen–Hill reactions before and after incubation with $HgCl_2$ were determined using an O_2 concentration polarograph [3].

Kimimura and Katoh [4, 5] have reported that the incubation of chloroplasts with $HgCl_2$ results in the complete inhibition of the photoreduction of methyl viologen using either water or reduced dichlorophenolindophenol as the electron donor. They ascribe the inhibitory effect of Hg^{2+} to the inactivation of plastocyanin, an electron carrier close to P_{700}. Our results in the concentration polarograph assays confirmed their reports. We found that electron transport from water to low potential acceptors (e.g. methyl viologen) was completely inhibited ($< 1\,\%$ of V, the maximum rate in strong continuous light), while the electron transport to $Fe(CN)_6{}^{-3}$ had a residual rate of about 10 % of V, probably due to the photoreduction of these oxidants directly by the reducing side of System II [5, 6].

In the flash experiments a rate of $\leqslant$ 2 flashes/s had to used because of the limited time response of the covered electrode. Thus, the integrated rate did not exceed 2 equiv./s per trap and even slow leak paths of electrons to O_2 would be significant. Hg^{2+} proved to inhibit electron flow from System II to the required extent ($\ll$ 1 equiv./s per trap). Dibromothymoquinone [7] was found to inhibit electron transport to no less than approx. 20 % of V; KCN [8] had adverse effects on the O_2-evolving system, acting somewhat like 3(3,4-dichlorophenyl)-1,1-dimethylurea.

Fig. 1 illustrates the patterns of O_2 flash yields observed with ($\geqslant$ 5 min) dark-adapted chloroplasts in the absence of added electron acceptor. Fig. 1A, obtained without Hg^{2+} inhibition shows the typical flash pattern discussed and analyzed previously [1]. Fig. 1B shows the very different "abbreviated" flash pattern observed subsequently with the same chloroplast sample after $HgCl_2$ was added to the flowing buffer and allowed to equilibrate in the dark for 20 min. Addition of $Fe(CN)_6{}^{-3}$ to the flow medium (on top of the $HgCl_2$) restored the flash yield pattern to that of Fig. 1A, except for the fact that the yields now were sustained for many flashes. This is readily explained by a direct reduction of $Fe(CN)_6{}^{-3}$ by the reducing side of System II, compatible with the leak rate of 0.1 V seen in the concentration polarograph (see above). A pattern very similar to that of Fig. 1B was obtained when the chloroplasts were treated with $HgCl_2$ before being deposited on the electrode.

This "abbreviated" flash yield pattern resembles those observed with uninhibited chloroplasts after a short dark period (30–60 s during which most of the A_2 pool but very little of the A_1 pool is oxidized) or with Scenedesmus Mutant 8 [9] which lacks System I (c.f. Fig. 3 of ref. 1). However, in Hg^{2+}-treated chloroplasts the observed pattern is "more abbreviated" than that of the mutant or non-inhibited chloroplasts after short dark: yields 7 and 8 are very low and, most notable, the

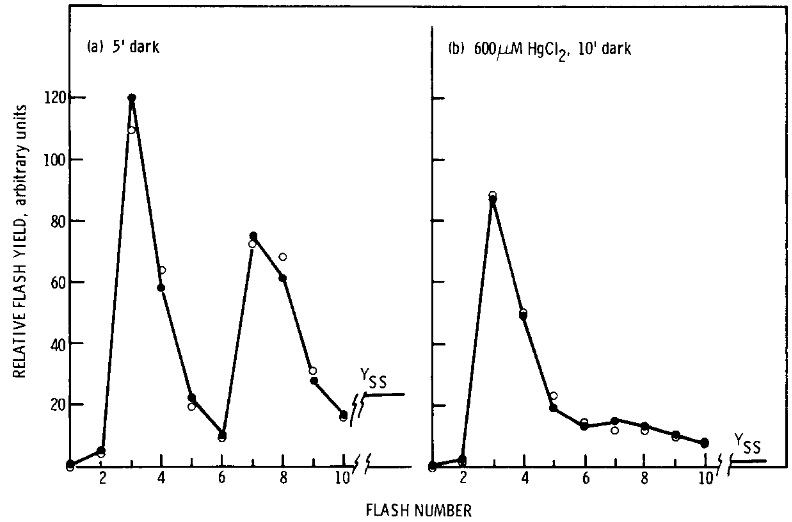

Fig. 1. Comparison of observed (●–●) and computed (○–○) O_2 flash yields (2 flashes/s) given after: (a) 5 min darkness; (b) 10 min darkness with 600 μM $HgCl_2$ in the flow medium. Y_{ss} is the flash yield obtained in the steady state. The basic flow medium contained 0.4 M sucrose, 0.1 M KCl, 0.05 M Tricine buffer (pH 7.0), and 6 mM $MgCl_2$. The computed values shown in a were obtained using the parameters given in ref. 1 with the exception of: $\alpha = 0.10$, $\beta = 0.02$ (these deviations are probably due to differences in the optical configuration in the two experiments). The computed values shown in b were obtained by setting $A_1 = 1$, and $k_3 = 0$ (cf. ref. 1). Computationally, this had the effect of allowing System II access to one equivalent between the photoacts and no access to system I.

steady-state yield (Y_{ss}) is negligible. Presumably, the inhibitor effectively blocks any leak of electrons out of System II. The observation that Hg^{2+} inhibits Y_{ss} some 20-fold (c.f. Figs 1A and 1B) suggests that the reoxidation of Q by O_2 is quite slow ($\ll$ 1 equiv./s). Therefore, under normal conditions approx. 95 % of the leak path must be through System I, a conclusion we were unable to draw in our earlier paper.

In Fig. 1B (as in the experiments of Figs 3 and 11 of ref. 1) the O_2 flash yield oscillation is rapidly damped. We ascribe this to the flash-induced reduction of Q, the primary acceptor of System II, and depletion of its associated pool A_2.

The observation that $Fe(CN)_6^{-3}$ restored the O_2 flash yield pattern to normal suggests (1) that the O_2-evolving system is not inhibited by Hg^{2+} and (2) that Hg^{2+} specifically blocks the reoxidation of the System II acceptor via the reaction chain to System I, but not other oxidation paths. We have also observed that in the absence of $Fe(CN)_6^{-3}$ the Hg^{2+}-inhibited chloroplasts "restore" in the dark after a series of flashes, so that the flash yield pattern of Fig. 1B can be repeatedly obtained if sufficient dark time separates the light periods. This again points to a locus of Hg^{2+} inhibition on the reducing side of System II. On the basis of these two observations we conclude that System II and its O_2-evolving apparatus are unaffected by Hg^{2+} under the conditions used. Therefore, a comparative determination of the pool sizes via O_2 flash yields in the presence and absence of Hg^{2+} is probably valid.

A comparison of the observed O_2 flash yield pattern shown in Fig. 1 (dots) with a pattern which was computed according to the reaction scheme presented in ref. 1 (open circles) suggests (1) that an A_2 pool of about five equivalents is operative in both cases and (2) that in the presence of Hg^{2+} only about one equivalent of the A_1 pool is accessible to System II (while without Hg^{2+} approx. 5 equivalents are available). The calculated abundance of the Q–A_2 complex (5 equivalents) and the computed equilibrium constant for the Q–A_2 reaction $K_{eq} = 1$ both agree with the values obtained in our earlier work using uninhibited chloroplasts. These observations are thus again consistent with our earlier [1] idea that primary electron acceptor Q acts as one of five kinetically identical equivalents. Although in the presence of Hg^{2+} the Q–A_2 system is better isolated kinetically, and thus its observation "cleaner", other factors (principally the quantitative interpretation of the signal obtained from the membrane-covered electrode) tend to counteract this advantage. Consequently, the uncertainty in this analysis comparable to that in our previous report; i.e. $A_2 = 4$–6, and $K_{eq} = 0.5$–2.0.

If we accept the hypothesis that the inhibitory behavior of Hg^{2+} is due to the inactivation of plastocyanin [4], our data suggest that active plastocyanin is required for the reduction of all of the A_1 pool by System II except for one equivalent which is accessible to Q directly, either on the way to plastocyanin or on a side path.

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