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PHOTOACTIVATION OF THE MANGANESE CATALYST OF O_2 EVOLUTION II. A TWO-QUANTUM MECHANISM

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SUMMARY

Kinetic experiments described in a previous communication[1] suggested that photoactivation of the photosynthetic O_2-evolving site is a multi-quantum process. In the present report we present a kinetic analysis of these data. The results obtained from the computer simulation of the derived kinetic model compare favorably with the experimentally derived values. These studies suggest that photoactivation can be rationalized in terms of a two-quantum process.

INTRODUCTION

In a previous communication[1] kinetic experiments relating to the photoactivation of the Mn complex required for photosynthetic O_2 evolution were described. These experiments suggested that: (1) the rate of photoactivation in any given illumination is first order with respect to the number of inactive O_2-evolving centers; (2) the process occurs in the reaction centers of System II; and (3) photoactivation is a multiquantum process.

In this communication we will present the results of a computer study modelling these kinetic phenomena. These studies suggest that the experimental data obtained are consistent with a two-quantum process.

RESULTS AND DISCUSSION

There are several salient aspects of the data presented in Ref. 1 which might yield some insight concerning the mechanism of photoactivation. These aspects can be summarized as follows:

(1) There appear to be two forward reactions, one fast, with a half time on the order of 1–10 msec, and one slow, with a half time on the order of 1 sec.

(2) A back reaction exists with a half time on the order of 1 sec.

(3) Photoactivation is a low quantum-yield process (it appears that the quantum-yield is about 0.01)[1]. This suggests that either (a) a loss mechanism such as rapid cycling is present, or (b) at least one photoact has a very low quantum yield.

(4) Photoactivation is some function of Mn^{2+} concentration. (When $[Mn^{2+}] = 0$, the rate of photoactivation is zero.)

(5) There is no interaction between the reaction centers; *i.e.* the behavior of a given trap is independent of the state of neighboring traps.

(6) Since photoactivation is a first order process with respect to inactive O_2 evolving centers, this suggests that under the experimental conditions utilized, the concentration of manganese does not change appreciably during the course of the reaction.

These points suggest that any model presented to explain the observed phenomena must have the following characteristics:

(1) Since the two forward reactions have rate constants which differ by one or two orders of magnitude, photoactivation probably proceeds by a series rather than a parallel mechanism, *i.e.*

$$A \xrightarrow{k_1} B \xrightarrow{\phi_1} C \xrightarrow{k_2} D \xrightarrow{\phi_2} E$$

not

$$\begin{array}{l} A \xrightarrow{\phi_1} \xrightarrow{k_1} C \searrow \\ A \xrightarrow{\phi_1} \xrightarrow{k_1} C \nearrow \end{array} E$$

(A parallel mechanism would be expected to have two roughly equivalent rate constants).

(2) In the series model each light step must be separated by a dark forward step.

(3) The back reaction must originate between the two photoacts (*i.e.* C or D).

(4) The component which decays must be D. If C were to decay to a lower "state", this specie would be undergoing a forked reaction. In this case, the relative amount lost by this segment of the "reaction chain" would be time independent.

(5) D must decay to a state prior to the first rate limitation, k_1, (*i.e.* state A). If D were to decay to state B, which did not decay to A, the population of B would increase with the number of flashes whatever their spacing and would not be subjected to the rate limitation imposed by k_1.

(6) Since the "optimum quantum yield" of photoactivation is quite low compared to photosynthesis, a loss process must exist. This loss cannot be due to the observed back reaction, since its rate is quite low. One attractive hypothesis involves the rapid decay of the product of a dark reaction, giving the appearance of a partition or equilibrium constant. This possibility will be explored further in a later section.

If we accept for the moment that photoactivation proceeds according to the described model, then an analysis of the experimental data can yield some reasonable values for the proposed experimental constants. For example:

(1) The descending portion of the curves shown in Fig. 10, ref. 1, show a half-decay time of about 1.5 sec. This suggests that the magnitude of k_3 is about 0.5 sec^{-1}.

(2) The ascending portion of Curve 1, Fig. 10, ref. 1, shows a half-rise time of about 0.2 sec, while the latter portion of Fig. 11, ref. 1 (after about 50 msec), suggests a rate constant of about 1 per sec. Therefore, the low forward rate constant (k_1 or k_2) should be 1–3 sec^{-1}.

(3) The initial ascending portion of Fig. 11, ref. 1, suggests that the magnitude of the high forward rate constant should be on the order of 100 sec^{-1}.

(4) The choice of appropriate quantum yields for this model presents some difficulty. We make the assumption that Mn^{2+} photooxidation[2-4] is an integral part

of the photoactivation process. This assumption is partly justified from unpublished experiments showing the reversible inhibition of photoactivation by compounds known to either donate electrons to the System II trap or to chemically reduce valency states of Mn $>^{2+}$. Since the optimal quantum yield of Mn^{2+} oxidation to Mn^{3+} by System II is 0.3–0.5 (ref. 2 and G. M. CHENIAE AND I. F. MARTIN, unpublished results), this suggests that $\emptyset_1$ is of this order of magnitude. The choice of the magnitude of $\emptyset_2$ is not quite so simple. For want of a better choice, we will assume $\emptyset_2$ equal to 1.

(5) It is possible to determine from the available data which forward rate constant (k_1 or k_2) is greater. The ascending portions of Curve 1 and Curve 2 in Fig. 10, ref. 1, illustrate that there is not much difference between the 1 and 2 flashes with short delay times of the primary flash, while with longer delay times the effect of the second flash is appreciable. This suggests that the short delay flash is effective only when each pair of flashes is preceded by a long "priming time". This can only be explained if k_1 is slow and k_2 is fast.

(6) Since k_2 is a fast reaction, its upper limit is determined by the magnitude of the product of the single delay flash at long "priming times", *e.g.* Curve 2, Fig. 10, ref. 1, at 8 sec. This suggests that when the system is fully "primed" roughly one-half of the maximal double flash yield is attained, and that this maximal yield is comprised of roughly equal single and double flash contributions. Since the delay flash was presented 15 msec after the primary flash in these experiments, this suggests that $k_2 \leqslant 50$.

(7) The data presented in Fig. 11, ref. 1, give some indication of the cause of the low quantum yield of photoactivation. These data suggest that a small pool (< 10 % of an intermediate) is depleted rapidly (about 10 msec), and that the subsequent rate of photoactivation is limited by another kinetic step. This could come about, for example, if one of the steps in the kinetic mechanism has a "finite" equilibrium constant. The measured pool would then be the fraction of intermediates which are in the photoactive state. For example, if this reaction were of the type:

$$x + T \xrightarrow{K_{eq}} xT$$

where xT is photoactive, then $K_{eq}[x] < 0.1$. Since we initially measure the fast phase, this limitation and pool must be associated with a slow forward step.

The above considerations suggest the following series mechanism and representative kinetic values:

$$\mathrm{A} \xrightarrow{k_1} \mathrm{B} \xrightarrow{\phi_1} \mathrm{C} \xrightarrow{k_2} \mathrm{D} \xrightarrow{\phi_2} \mathrm{E}$$

(f); return arrow from D to A: k_3

where, k_1 is about 2 per sec; k_2 about 50 per sec; k_3 about 0.5 per sec; f_1, the fraction of the A–B pair in the B state at equilibrium about 0.05; $\emptyset_1$ about 0.5; $\emptyset_2 = 1$.

This model was simulated and tested using a digital computer. In these studies, we have attempted to simulate only the "flashing light" experiments presented earlier[1]. By using saturating flashes of light every trap is hit by every flash and secondary corrections for possible photon transfer[5] are not necessary.

In addition to this series mechanism, we have also studied other mechanisms which might be consistent with the observed kinetic phenomenon. In particular, we have spent some time testing parallel mechanisms of the type described earlier.

However, these mechanisms have never been found to fit the data. Kinetic schemes with fewer dark steps have also been tested and have been found not to satisfy the experimental data obtained. We thus feel that the kinetic model presented is at least the minimal requirement necessary and consistent with the kinetic phenomena observed[1].

Fig. 1 illustrates experimental and calculated results of an experiment in which a repetitive regime of flashes of variable primary dark time with delay flashes (15 msec) was imposed on Mn-deficient Anacystis cells and "model cells". These data show that a reasonable agreement between the theoretically and experimentally derived values was obtained. It should be pointed out that the experimental data presented are slightly extreme in one aspect; the ratio between the double and single flash maximum values is usually not a factor of two, as presented here, but closer to 1.6. Thus the theoretically predicted value generally would more closely approach the experimentally derived data. This particular set of data is presented because "three flash" data were also obtained in the same experiment.

Fig. 2 compares the effect of a delay flash (delay flash Δt variable; primary flash $\Delta t = 2$ sec) on the biological and theoretical systems. Again, the computed data yield the same conclusions as the experimental data, although some discrepancies are apparent. Inasmuch as the experimental data are only good to about 10 %, it is difficult to ascertain whether the observed discrepancies, particularly at long Δt, are real. Some data obtained in experiments suggest that the long tail may indeed be flat as shown by the theoretical curve.

We might, for a moment, speculate on the possible significance of these experiments and calculations. The main point of this study is the demonstration that two

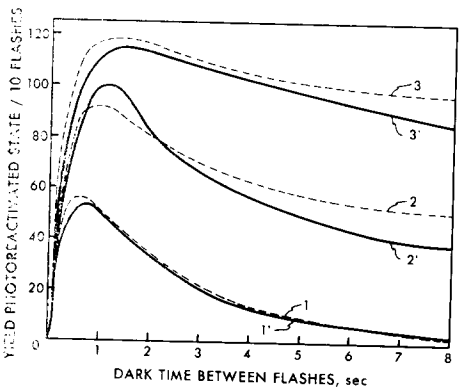

Fig. 1. A comparison of computed and experimentally derived data showing the effect of dark time between flashes on the yield of photoactivation. The experimental data are presented as solid lines and the computer-generated data as dashed lines. Curves 1 and 1′ show the calculated and experimentally obtained yields induced by 10 saturating flashes separated by dark times shown on the abscissa. Curves 2 and 2′ present similar data except that paired flashes were used ($\Delta t = 15$ msec for the delay flash). Curves 3 and 3′ present similar data except that 2 delay flashes were used ($\Delta t = 15$ msec each).

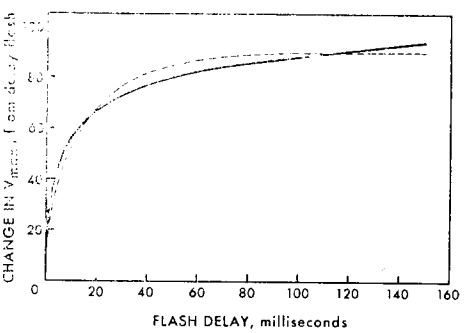

Fig. 2. A comparison of computed and experimentally derived data showing the effect of the length of the dark interval between the primary and delay flash on the yield of photoactivation. The experimental data are presented as a solid line and the computer-generated data as a dashed line. The difference in yield between 20 single flashes and 20 paired flashes (40 total flashes) of 2 sec periodicity is plotted on the ordinate versus the dark time between the paired flashes.

quanta are sufficient to give the observed experimental results. If photoactivation is, indeed, a two-quantum, two-electron oxidation of a manganese atom, then the kinetic constants and other constants could be rationalized as follows:

(1) k_1, the first constant, might be a measure of diffusion of manganese or one of its complexes to the reaction site. The rate constant of this reaction is of the same order of magnitude that would be expected from simple diffusion. If this were true, then the "equilibrium constant" would be a measure of the number of sites occupied at any one time; (2) the second rate constant, k_2, might be a measure of the time it takes to transfer an electron from the primary electron acceptor of System II to System I[6]; k_3 then would be a measure of stability of Mn^{3+}. Again, this number is in rough agreement with values[2] estimated for Mn^{3+} stability in photosynthetic tissue.

The scheme shown in Fig. 3 represents an attempt to incorporate the kinetic interpretation of photoactivation into current concepts of System II quantum trapping and O_2-evolving centers.

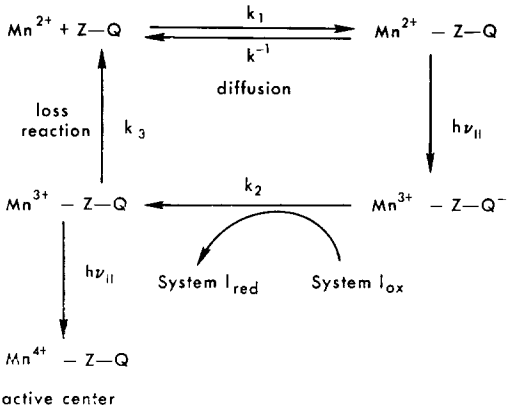

Fig. 3. Proposed mechanism of photoactivation. In this scheme Z is the primary donor and Q the primary acceptor of System II.

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