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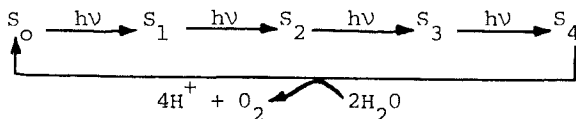
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AN NMR STUDY OF MANGANESE IN CHLOROPLAST MEMBRANES

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I. INTRODUCTION

The photo-induced oxidation of water during photosynthesis is of major importance in the bioconversion of solar energy; however, relatively little is known of the reactions involved. Most of the information has been gleaned from oxygen yield measurements made as a function of brief saturating flashes of light on aqueous suspensions of chloroplasts (1). Kok et al. (2) have postulated a species S which is generated by light flashes and involves four intermediate states. According to this model, upon reaching its most oxidized state (S₄) the intermediate undergoes a concerted reaction with water as is indicated schematically below:



Since the peak oxygen yield occurs after the third flash, Kok postulated that most of the photosynthetic oxygen evolving centers are initially in the S₁ state in the dark. A miss parameter (i.e., the fraction of oxygen evolving centers which are not advanced to the next S state after a given flash) has been invoked to account for the damping of the oxygen yield flash pattern. Initially Kok et al. also invoked a double hit parameter β which

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was related to the absorption of two photons per photosynthetic unit, however, under the experimental conditions employed here (flashes $< 1 \mu\text{sec}$) β is effectively zero.

Manganese has been shown to be necessary for the evolution of oxygen by water oxidation (3) and has usually been linked to the cycling of the intermediate S states; however, there has been no direct evidence for the involvement of manganese until recently. Since the oxygen evolving apparatus is highly unstable outside of the chloroplast membrane an *in situ* experimental approach which is sensitive to manganese is necessary.

It is well known that Mn(II) has a large effect on the NMR relaxation rates (i.e., T_1^{-1} and T_2^{-1}) of water protons (4). Using a pulsed NMR method, it has recently been shown (5,6,7) that dark-adapted aqueous buffered chloroplast suspensions show enhanced water proton T_1^{-1} and T_2^{-1} relaxation rates. An appropriate analysis of the frequency dependence of the proton relaxation rates (PRR) by means of the Solomon-Bloembergen-Morgan equations (see reference 4 for an excellent exposition) coupled with the observed dependence of the NMR data on experimentally determined manganese concentrations has shown that the PRR are governed by the presence of membrane bound Mn(II). A detailed description of these results will be published elsewhere (8).

II. RESULTS AND DISCUSSION

Figure 1a shows the T_2^{-1} and O_2 yield for a sample of pea chloroplasts at pH 6.7 measured as a function of brief ($< 1 \mu\text{s}$) 590 nm laser flashes. Both graphs show an oscillating pattern indicating that the PRR can be correlated with the oxygen evolving system. Inhibition of oxygen evolution either by means of the herbicide 3-(3,4 dichlorophenyl)-1,1 dimethylurea (DCMU) or manganese extraction results in loss of T_2^{-1} oscillation as can be seen in Figures 1b and 1c.

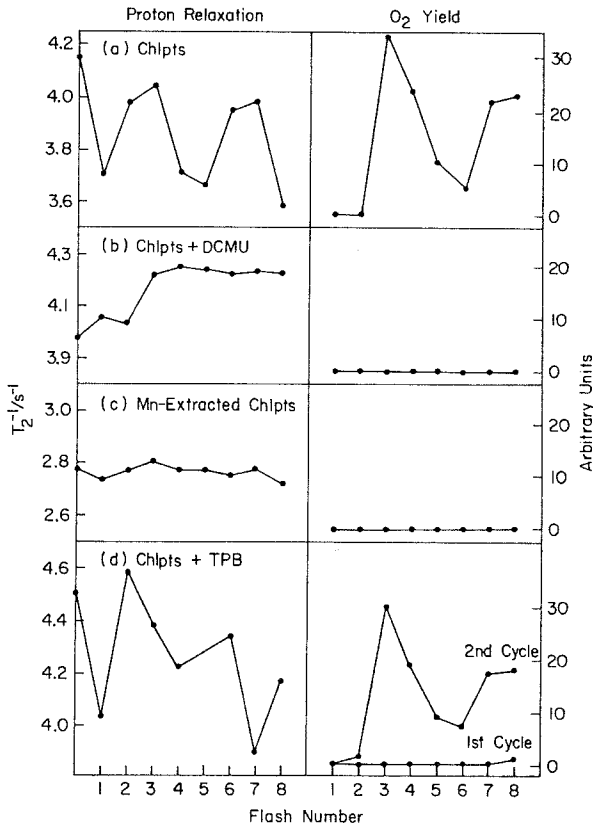


Fig. 1. Water proton relaxation rates T_2^{-1} and O_2 yield measurements vs light flashes under various conditions. For details of the light flashing see the text.

At low concentrations tetraphenylboron (TPB) is believed to act as a competitive electron donor to the oxygen evolving system (9). Figure 1d shows that a low concentration of TPB (40 μ M) initially alters the T_2^{-1} pattern (note that these data correspond to the O_2 yield data labelled "1st cycle" in Figure 1d) while the O_2 yield is completely inhibited. However, after at least fifteen consecutive flashes the TPB is used up and, after a even minute dark adaptation period, the normal O_2 yield flash pattern is observed (Figure 1d - second cycle).

Using Kok's model the initial dark adapted populations of the S_0 and S_1 states and the miss parameter α can be calculated. For the O_2 evolution data shown in Figure 1a $S_0 = 0.30$, $S_1 = 0.70$ and $\alpha = 0.10$. It is reasonable to assume that oxidation states higher than Mn(II) do not contribute significantly to the T_2 relaxation rate so that the T_2^{-1} level in the flash pattern is related to the amount of Mn(II) in the chloroplast suspensions. Therefore, from the T_2^{-1} data in Figure 1a relative weights, which are proportional to the Mn(II) contribution to the relaxation, can be assigned to S_0 , S_1 , S_2 and S_3 . The lifetime of S_4 is known to be short compared to the time required to measure T_2^{-1} or O_2 yield (1) and hence it is not included in the scheme. In addition, it was found necessary to assign a relative Mn(II) concentration weight of 4 to the dark adapted chloroplasts (i.e. the zero flash level in the figures). Thus, by using the values for the initial population of S_0 and S_1 and α calculated from the O_2 yield data and assigning weights during the light flashes 2,1,1 and 3 to S_0 , S_1 , S_2 and S_3 respectively, the theoretical flash pattern shown in Figure 2a was generated. The T_2^{-1} flash pattern for chloroplast suspensions containing TPB (Figure 1d) can also be fitted by the model by assuming that the weighting factor for S_0 is zero and all the other parameters are the same (Figure 2b).

The proposed modification of Kok's model indicates that S_3 is highly reduced (i.e. high concentration of Mn(II)) and not highly oxidized as postulated by Kok's model. However, this result implies that the water molecule(s) undergoing oxidation give(s) up its (their) electrons during the approximately milli-second time lag between the light flash and the T_2 measurement. In addition, the model suggests that the water molecules transfer electrons to the S intermediate prior to the release of oxygen. This is consistent with recent results which show that protons are released stepwise during the flashing cycle (10).

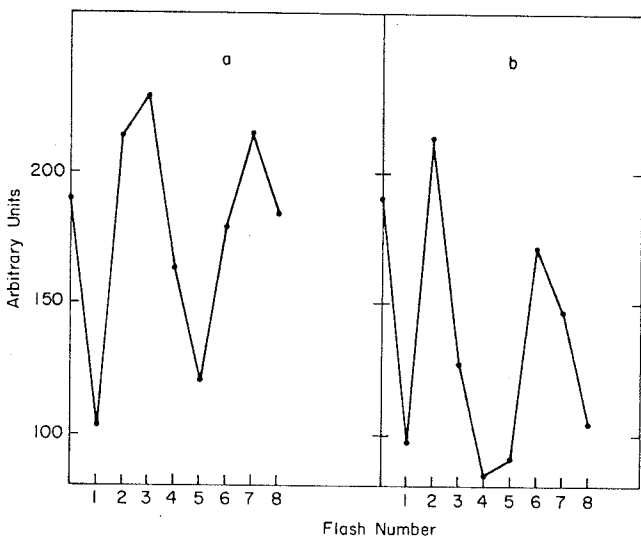


Fig. 2. Theoretical fits of T_2^{-1} as a function of light flashes using a modified version of Kok's model for O_2 evolution. The initial distribution of $S_0 = 0.30$, $S_1 = 0.70$ and $\alpha = 0.10$ were calculated from the O_2 yield data of Figure 1a. Figure a assigns a weight of 4 to the dark adapted chloroplasts and a weight of 2, 1, 1, 3 to S_0 , S_1 , S_2 and S_3 , respectively. Figure b has the identical parameters except that S_0 is assigned a weight of zero. See text for details.

In conclusion, the theoretical fit to the experimental T_2^{-1} data suggests that: (1) the cycling of the intermediate states is correlated with the oxidation state of manganese in the chloroplast membrane, (2) there exists a special dark-adapted state of the chloroplast suspensions (11), (3) water donates electrons to the intermediate S states prior to O_2 release in the final $S_4 \rightarrow S_0$ transition and (4) the intermediate S states undergo an altered cycling in the presence of various chemical modifiers.

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