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A near infrared electronic transition associated with conversion between S-states of the photosynthetic O_2-evolving complex

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A new electronic transition is observable in O_2-evolving photosynthetic membranes of spinach enriched in Photosystem II. The transition occurs predominantly in the oxidation state created after a single flash of light (S_2 state). It oscillates in yield with a period of flashes when exposed to a train of short light flashes. Conditions which abolish O_2 evolution and remove functional manganese destroy this effect. The spectrum and absorptivity of the oscillating component are similar to those found in synthetic manganese dimers poised in the mixed-valent oxidation state Mn_2(III,IV). A similar structural unit is thus implicated for the manganese site involved in O_2 evolution.

Photosynthetic oxygen evolution *Water oxidation* *Electronic spectroscopy* *Manganese*

1. INTRODUCTION

Electronic absorption spectroscopy is the most directed and powerful method for determining the electronic structural requirements for redox enzymes. Surprisingly, this technique has contributed very little to our understanding of the enzyme-catalyzed mechanism for the conversion of water to molecular oxygen during photosynthesis. This is due to the interfering absorption by chlorophyll and other pigments even in the simplest O_2-evolving samples. For example, in detergent-purified thylakoid membranes from spinach chloroplasts enriched in PS-II and the O_2-evolving complex there is a 200–300-fold excess of chlorophyll over the enzyme. Another reason is the emphasis on ultraviolet-visible spectral changes where many other absorbance charges predominate. The near infrared spectral region holds special promise in understanding the mechanism of this enzyme because of the absence of absorption by chlorophyll. Moreover, the clear involvement of manganese in this complex (review, [1]) in a binuclear, or possibly tetranuclear, form [2], and the observed near infrared electronic absorption in synthetic manganese dimers poised in the mixed-valent oxidation state, Mn_2 (III,IV) [3], are compelling reasons to search for near infrared absorption changes upon turn-over of the enzyme. Specifically, we searched for the anticipated intervalence electronic transition of the mixed-valent oxidation state of the manganese site in O_2-evolving samples from spinach. These samples are known to give rise to a light-induced multiline EPR signal [2] characteristic of the mixed-valent state of the manganese cluster [4]. Furthermore, it is established that the yield of this EPR signal oscillates

Abbreviations: ΔA, absorption change; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,3-dimethylbenzoquinone; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PPBQ, phenyl-*p*-benzoquinone; PS-II, Photosystem II; P700, photooxidizable chlorophyll of reaction center PS-I; P680, photooxidizable chlorophyll of reaction center PS-II

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with a period of 4 flashes when generated by a train of light pulses, parallel with the periodic release of O_2 [5].

2. EXPERIMENTAL

The samples used in this study are PS-II particles prepared as in [6]. These contain 3.5–4 Mn/PS-II after washing with 5 mM EDTA, evolve O_2 at rates of 500–800 μmol · mg $Chl^{-1} \cdot h^{-1}$ in SMN′ medium (0.4 M sucrose, 20 mM Mes (pH 6.0), 4 mM NaCl and 0.2 mM $MgCl_2$) containing recrystallized DMBQ and PPBQ as electron acceptors. $P700^+$ is absent, as indicated by EPR signal I.

Absorption measurements are conducted using a conventional single beam spectrophotometer [7] mechanically isolated to reduce noise traces to $\Delta A \leq 3 \times 10^{-5}$ in the 0.1–100 Hz frequency range. The detector bandpass is either 10–100 Hz or DC–100 Hz, as indicated. The dark-adapted sample is exposed to a train of actinic laser flashes (15 ns, 600 nm, >90% light saturation) spaced at 0.66 s. Signal averaging of 20–400 flash trains is performed on a new dark-adapted sample introduced between each train. New sample is introduced by means of a valve-controlled flow cuvette and measured at 7°C. The sample is circulated so that it is eventually flashed again after an average dark interval >15 min. For measurements above 750 nm there is no electron transfer photo-induced by the analyzing beam so that samples are indeed synchronized in a stable dark state.

3. RESULTS AND DISCUSSION

Near infrared absorption changes are produced by a train of flashes as shown by the data at 920 nm in fig.1A (top). Note absorbance increase on flashes 1, 2, 5 and 6 and bleaching on flashes 3 and 4. Note that for improved signal-to-noise ratio the detection system was AC-coupled; thus, the signals appear to decay between flashes. Thus, only the initial change is important in this experiment. This pattern of changes caused by the flash train persist for at least 10 flashes, but eventually decays in amplitude to zero and is absent for all flashes in samples illuminated with low intensity, continuous light at 715 nm (not shown). Subsequent dark adaptation restores the oscillations. Continuous illumination scrambles the synchronization of the occupancy of the 5 oxidation states of the O_2-evolving complex in different reaction centers. Thus, the oscillation can be attributed to differential absorbance in these states as they are advanced by single turn over flashes starting from an initially synchronized population (dark adapted).

Treatment with agents which abolish O_2 evolution and electron transport also abolish this oscillation pattern (10 μM DCMU; removal of electron acceptors; Cl^--depletion; 2 M NaCl (pH 6) (loss of 17- and 23-kDa proteins); (not shown). Incubation in 0.01–5 mM NH_2OH removes this oscillation pattern, converting it to positive absorbance changes on all flashes as shown in fig.1A (bottom). This treatment abolishes O_2 evolution by specifically removing 2 Mn atoms per PS-II center, a treatment which also eliminates the multiline EPR signal associated with the manganese complex in

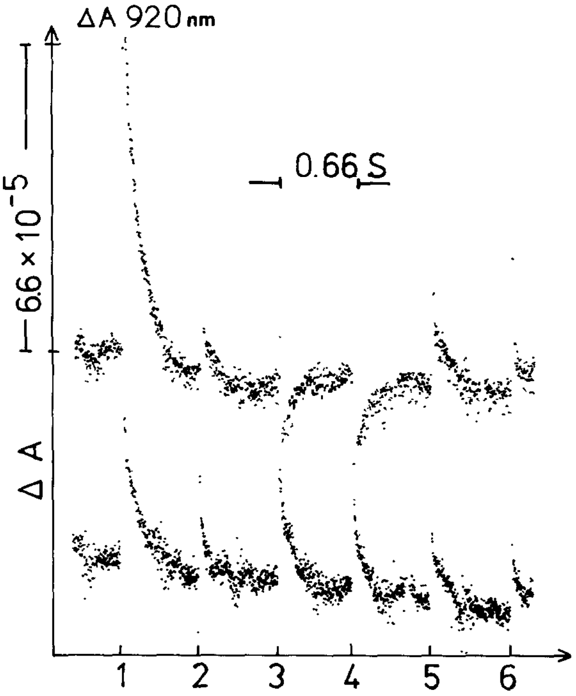

Fig.1. Absorption changes induced at 920 nm by a train of 6 saturating laser pulses. (600 nm; 15 ns) in a sample of dark-adapted PS-II particles (top trace) and after addition of 5 mM NH_2OH with a 10 min dark incubation period at 5°C (bottom trace). Each trace was an average of 20 trains each. The signals decay because of the mode of detection; this was AC-coupled with a bandwidth of 10–300 Hz. [Chl] = 0.1 mg/ml; acceptors: 0.4 mM DMBQ and 0.13 mM PPBQ; t = 7°C.

the so-called S_2 oxidation state, the state achieved after a single flash.

In order to obtain information about the lifetime of the states generated by the flashes the optical changes were measured with a wider bandpass ranging from DC to 100 Hz. Fig.2 shows that the absorbance changes do not decay between flashes corresponding to a stable state. Better S/N is achieved with the AC-coupled system, hence its use. The oscillation pattern and the decay kinetics for Y_1 are similar to those for the S_2 state in PS-II membranes [8], indicating that this state contains the predominant absorbing species on flashes 1 and 5.

The spectrum of the oscillating component, as represented by Y_5–Y_4, is given in fig.3. It exhibits a broad absorption peaking near 780 nm and extends at least to 1050 nm. The difference extinction coefficient of 125 $M^{-1} \cdot cm^{-1}$ at 780 nm shows this to be truly a weak band that is easily missed. A value of 400–600 $M^{-1} \cdot cm^{-1}$ for this band is arrived at by correcting for the estimated decay at flash 5 and the fraction of centers which do not advance past the S_2 state. These spectral properties are unlike those for all other previously observed bands in this region, including $P680^+$, $P700^+$ and the carotenoid cation radical. However, they are strikingly similar to those observed for the Mn_2 (III,IV) mixed valence state in the synthetic dimers of $L_2Mn \genfrac{\langle}{\rangle}{0pt}{}{O}{X} MnL_2$; L = 2,2′-bipyridine, X = O [3] and L = benzimidazole, X = Br ([9], and unpublished). The oxidation state assignment here correlates nicely with the findings on the S_2 multiline EPR signal, establishing the presence of at least one pair of electronically interacting manganese ions capable of stable one-electron oxidation. These ions are believed to constitute the active site of the water oxidizing enzyme.

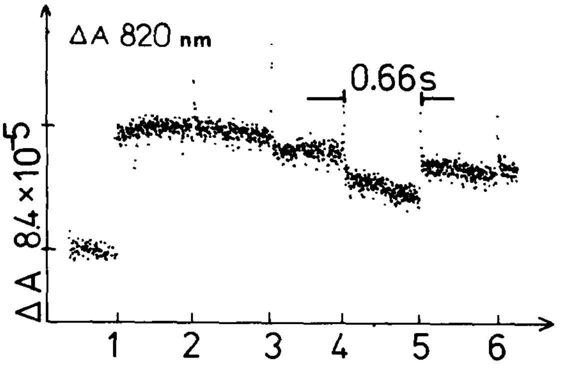

Fig.2. Absorption changes induced at 820 nm by a train of 6 saturating laser pulses (600 nm; 15 ns) in a sample of dark-adapted PS-II particles. Average of 40 trains each on a dark-adapted sample; bandwidth DC–100 Hz; Δt = 0.66 s; [Chl] = 0.06 mg/ml; acceptors: 0.4 mM DMBQ and 0.13 mM PPBQ; t = 7°C.

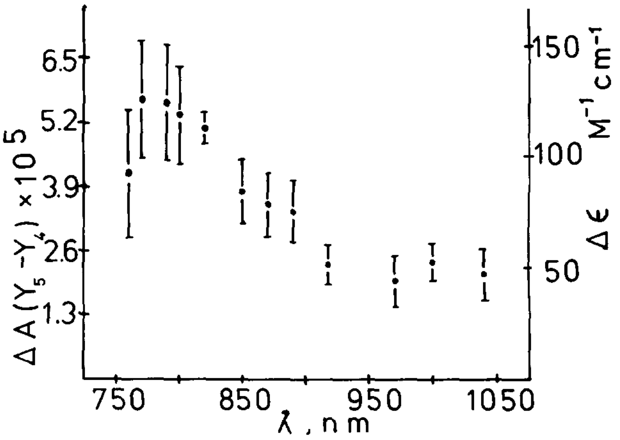

Fig.3. Spectrum of near-infrared absorption changes in PS-II particles, Y_5–Y_4, which oscillate with period 4 as in fig.1. Conditions as in fig.1. Average of 20 or 40 laser trains. $\Delta\epsilon$ is calculated assuming 224 Chl/PS-II. Probe beam filtering as follows: for $\lambda > 820$ nm, $\Delta\lambda$ = 6 nm at the detector and 780 nm cut-off at the cuvette; for $\lambda \leq 820$ nm; $\Delta\lambda$ = 3 nm filtering at the cuvette and at the detector.

Further characterization of this new electronic transition will provide more evidence to test provisional assignment, as well as new insights into the electronic structure of this site.

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