and Shalani Wadwani for DNase footprint analysis. We gratefully acknowledge Dr. Stewart Noble for providing chiral intermediates from Glaxo Group Research.

Supplementary Material Available: Autoradiogram derived from DNase footprint (1 page). Ordering information is given on any current masthead page.

The O_2-Evolving Center of Photosystem II Is Diamagnetic in the S_1 Resting State

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Received June 15, 1992

The O_2-evolving center (OEC) of photosystem II (PSII) catalyzes the four-electron oxidation of water to O_2. Although four Mn ions per PSII are required for O_2-evolution activity, the nuclearity and structure of the Mn cluster constituting the OEC are currently under debate.

We previously used EPR spectroscopy and O_2-consumption measurements to probe the nature of the change in the OEC occurring during dark adaptation.[1] The Mn site was found to exist in resting and active S_1 states depending on whether it was subjected to long (4 h) or short (6 min) periods of dark adaptation at 0 °C, respectively.

In this report, we address the effect of dark adaptation on the magnetic properties of the Mn site in the OEC by using the pulsed EPR method of saturation recovery.[2] We have shown in ribonucleotide reductase from *Escherichia coli*[3] that the spin-lattice relaxation of its stable tyrosine radical could serve as a probe of the magnetic properties of the EPR-silent dinuclear Fe(III) center. In this study, we probe the magnetic properties of the Mn cluster in PSII by analyzing the spin-lattice relaxation behavior of the stable tyrosine radical, $Y_D^{\bullet}$.

PSII membranes were prepared by the procedure of Berthold et al.[4] as modified by Beck et al.[1] The S_1 resting and active states were prepared according to Beck et al.[1]

The technique of saturation-recovery EPR has been used[5] to probe the magnetic interaction between the tyrosine radical ($Y_D^{\bullet}$) of PSII and the non-heme Fe(II) in Mn-depleted PSII. Saturation-recovery traces are analyzed according to a model[5] that takes into account the scalar exchange (isotropic) and the dipole-dipole (orientation dependent) interactions between two paramagnetic species separated by a fixed distance but with a random orientation with respect to the external magnetic field. The previously measured[5] dipolar rate constants, $k_{1dipolar}$, for $Y_D^{\bullet}$ in Mn-depleted PSII membranes are shown in Figure 1. It is observed that the rates are 4-5-fold faster in the S_1 active state over the whole temperature range (4-57 K). This is an initial indication that the S_1 active state of the Mn cluster is paramagnetic. However, in short dark-adapted PSII membranes, about 25% of the centers are still in the S_0 state, which has also been shown to be a good relaxer of the $Y_D^{\bullet}$ radical.[6] The question of the contribution of the S_0 state to the relaxation enhancement of $Y_D^{\bullet}$ in short dark-adapted PSII will be addressed below.

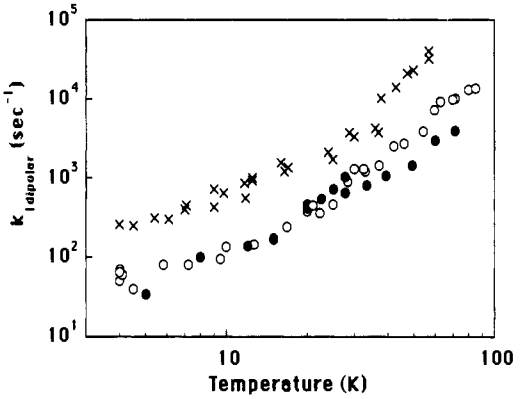

Figure 1. Dipolar spin-lattice relaxation rate constants ($k_{1dipolar}$) for $Y_D^{\bullet}$ obtained as a function of sample temperature in Mn-depleted PSII membranes (●) and in O_2-evolving PSII membranes in the S_1 active state (×) or in the S_1 resting state (○). Each data point is the average of three or four measurements with standard deviations typically 10-20% of the average value.

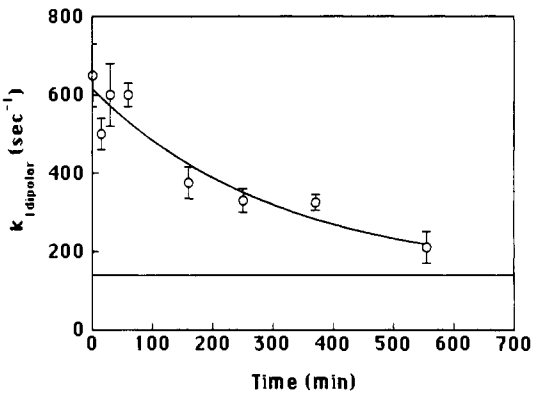

Figure 2. Change of the dipolar spin-lattice relaxation rate constant for $Y_D^{\bullet}$ (measured at 12 K) as a function of dark adaptation time at 0 °C. The sample is initially in the S_1 active state, and the exponential decrease of $k_{1dipolar}$ to its S_1 resting state value ($k_{1dipolar}$ = 140 s^{-1}) takes place with a half-time of 3.5 ± 0.7 h. The solid horizontal line is drawn at the value of $k_{1dipolar}$ at 12 K in the S_1 resting state.

In contrast, the dipolar spin-lattice relaxation rate constants of $Y_D^{\bullet}$ in S_1 resting state samples are indistinguishable from those of Mn-depleted PSII membranes below 30 K. This result shows that the ground spin state of the Mn cluster in the S_1 resting state does not contribute a relaxation enhancement pathway more efficient than that provided by the non-heme Fe(II). The fact that the dipolar rate constant increases with temperature shows that the system is in the slow relaxation limit, as discussed by Hirsh et al.[5] Previous results[7,8] show that, in this limit, even the slowly-relaxing $S = {}^1/_2$ multiline EPR signal form of the S_2 state enhances the spin-lattice relaxation of $Y_D^{\bullet}$. Therefore, we conclude that the ground spin state of the Mn cluster in the S_1 resting state is diamagnetic.

In both the S_1 active and resting states, a break is observed in the temperature dependence of the dipolar rate constants at around 30 K. The steeper temperature dependence for $T > 30$ K is probably an indication of a higher spin state of the Mn cluster being populated[3] and causing an additional contribution to the spin-lattice relaxation kinetics of $Y_D^{\bullet}$.

One can follow the change of the Mn cluster from the short dark-adapted state to the diamagnetic S_1 resting state. The dipolar rate constants were determined after successive dark incubations

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of a 20-min dark-adapted PSII sample (Figure 2). The active to resting state transition has a half-time of 3.5 ± 0.7 h at 0 °C, a rate which is consistent with the results of Beck et al.;[1] in that case, the half-time was determined to be 30 ± 2 min at 25 °C.

We now consider the contributions of S states other than the S_1 active state to the relaxation enhancement of $Y_D^•$ in short dark-adapted PSII. The long time course of the change (Figure 2) shows that there are no significant contributions from the short-lived S_2 and S_3 states to the time-dependent change of the spin-lattice relaxation behavior of $Y_D^•$. The S_0 state is longer-lived and has been found to be slowly oxidized by $Y_D^•$ to the S_1 state during dark incubation.[9] The conversion of S_0 to S_1 has been followed[9b] by measuring the fast phase of decay of $Y_D^•$ ($t_{1/2}$ = 14 min at pH 6.0 and 21 °C). We have measured the decrease of the $Y_D^•$ EPR signal as a function of dark incubation time at 0 °C in order to assess the rate of the $S_0 \rightarrow S_1$ conversion (data not shown). The fast phase of $Y_D^•$ decay is over within 1 h and involves 20-30% of $Y_D^•$. These results are consistent with earlier measurements[9] and indicate that the S_0 to S_1 conversion occurs much more rapidly than the changes shown in Figure 2. Therefore, we conclude that the time-dependent change in spin-lattice relaxation of $Y_D^•$ is due mainly to the conversion of the S_1 active state, which has a paramagnetic ground state, to the S_1 resting state, which has a diamagnetic ground state.

Our conclusion that the ground spin state of the S_1 resting state is diamagnetic and that the ground spin state of the S_1 active state is paramagnetic is also consistent with several previous experimental results. The noncyclical behavior in the magnetic susceptibility measurements at room temperature[10] can be explained by the generation of the paramagnetic S_1 active state after four laser flashes, while the initial S_1 resting state has a lower level of magnetism since it has a diamagnetic ground state. Variable temperature magnetic susceptibility measurements[11] showed that "the net paramagnetism of the OEC in the dark-adapted state is low and may be zero." Finally, EPR studies of the variation of $P_{1/2}$ of $Y_D^•$ with S state[6] showed a larger value of $P_{1/2}$ when the S_1 state was produced on the fourth flash compared to the initial dark-adapted sample.

A new EPR signal from the S_1 state in long dark-adapted PSII has been detected[12] at $g = 4.8$ in the parallel mode. The signal was very weak, which could be explained if it arises from the S_1 active state. However, we have not been able to detect a perpendicular-mode EPR signal in the difference spectrum of the S_1 active minus the S_1 resting state.

Our results have significant implications on the structure of the Mn cluster. In particular, the arrangement of a manganese trimer plus a mononuclear manganese center is ruled out because a single Mn ion is expected to be paramagnetic in all of its possible oxidation states. Our results, together with past studies,[10,13-16] point to a tetrameric Mn cluster as the most probable structure. We account for the different magnetic properties of the S_1 active and resting states by a change in the ferro- and antiferromagnetic couplings between the four Mn ions due to a structural change in the cluster.

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Comparison of Experimental and Theoretical Absolute Rates for Intervalence Electron Transfer

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Received June 12, 1992

Recently, direct time-resolved measurements on optical metal-metal charge-transfer (MMCT) bands have been performed, yielding the first kinetic data and other dynamical information on this important class of electron-transfer (ET) reaction.[1,2] In this paper we report ultrafast pump-probe measurements on the MMCT band of reaction 1 in various solvents over a range of temperatures.[3]

$$(NH_3)_5Ru^{III}NCRu^{II}(CN)_5^- \underset{k_{ET}}{\overset{h\nu}{\rightleftharpoons}} (NH_3)_5Ru^{II}NCRu^{III}(CN)_5^- \quad (1)$$

Typical pump-probe transient signals for reaction 1 pumped at ~800 nm and probed at ~800 or 700 nm are shown in Figure 1. The data are well described by a biexponential decay function convoluted with the instrument response function (see Table I). There is an initial transient bleach, which recovers in ~0.1-0.7 ps depending on solvent and temperature,[4] that we assign to ground-state recovery, i.e., reverse ET.

Subsequent to the initial bleach there is a slower kinetic component, which is either a bleach or increased absorption, depending on the wavelength of the probe beam relative to the maximum of the MMCT band. The MMCT band immediately after ground-state recovery is apparently shifted toward longer wavelength, relative to the equilibrated band, as a result of excess energy content in the solute and, potentially, the solvent.[7] The second component is assigned to relaxation in the electronic ground state. An analogous effect has been reported for the betaines, an organic compound class that exhibits intervalence electron transfer.[5] Furthermore, this assignment is consistent with the visible pump/IR probe measurements of Doorn et al., who concluded that the reverse ET of reaction 1 takes place in <0.5 ps followed by vibrational relaxation of the solute in the ground state.[2]

Interestingly, the ET rate shows a pronounced, temperature-dependent hydrogen/deuterium solvent isotope effect in glycerol, indicating that hydrogenic vibrational modes of the solvent, such as librations, are significantly involved in the ET reaction, especially in frozen media.[6]

One of the main goals of this work is to evaluate absolute rate models for ET. The comparison is limited to water, the only solvent for which the necessary parameters are available.[6,7] The input for the theoretical calculations was obtained by combining MMCT resonance Raman data[8] with an MMCT band shape analysis, and in some cases only two representative solute vibrational modes were employed to limit the size of the rate calculation. Further detail will be given in a future paper. The parameters

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