

Supplementary Material

Remarkable Affinity and Selectivity for Cs⁺ and Uranyl Binding to the Manganese Site of the apo-Water Oxidation Complex of Photosystem II.

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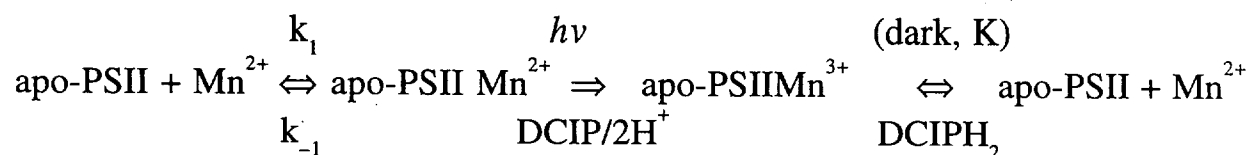
Supplementary Material Figure Legend.

Figure S1. Raw data demonstrating the effect of Cs⁺ on the rate of electron transfer to DCIP upon photoreduction by apo-WOC-PSII plus exogenous Mn²⁺. Panel (A) High affinity Mn²⁺ site: (a) no Mn²⁺, no Cs⁺; (b) 5 μM Mn²⁺, 5 μM Cs⁺; (c) 5 μM Mn²⁺, no Cs⁺. Panel (B) Low affinity Mn²⁺ site: (a) no Mn²⁺, no Cs⁺; (b) 100 μM Mn²⁺, 40 mM Cs⁺; (c) 100 μM Mn²⁺, no Cs⁺. 200 μL volume samples contained 100 μM DCIP as electron acceptor and 0.2 μM apo-WOC-PSII. Saturating illumination, at wavelength λ= 660 nm, was provided by a four ultra bright LED's positioned at 45° to the optical path of the 1 mm quartz cuvette. The sample was incubated in the dark for 5 minutes before measurements.

Analysis of Figure S1.

The initial rate was determined by extrapolation after subtraction of the control rate observed in the absence of cofactors. The observed decrease in the rate of DCIP photoreduction is not due to photoinhibition of apo-WOC-PSII centers. This was confirmed by examination of the effect of both preillumination of the samples in the absence of the cofactors, and by subsequent illumination after dark incubation. The decrease in rate with time arises from the effects of Mn²⁺ binding and photooxidation, as follows.

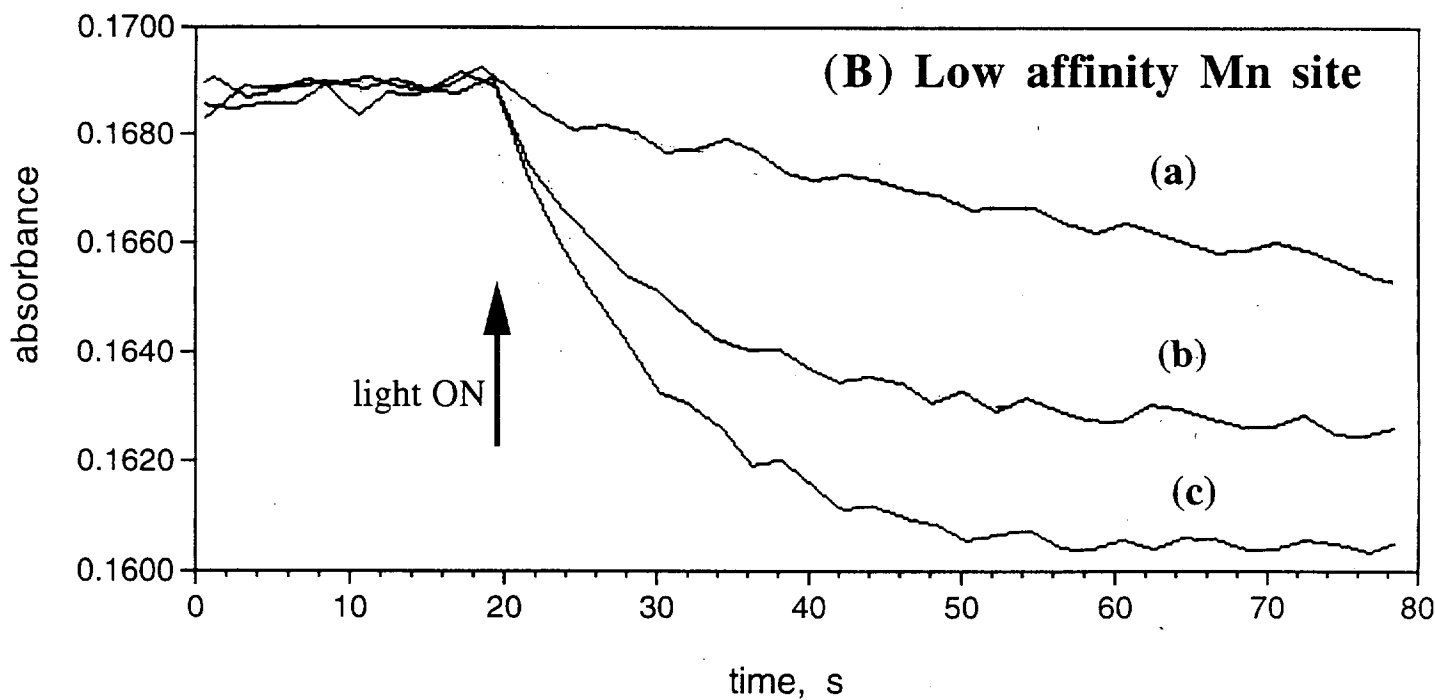
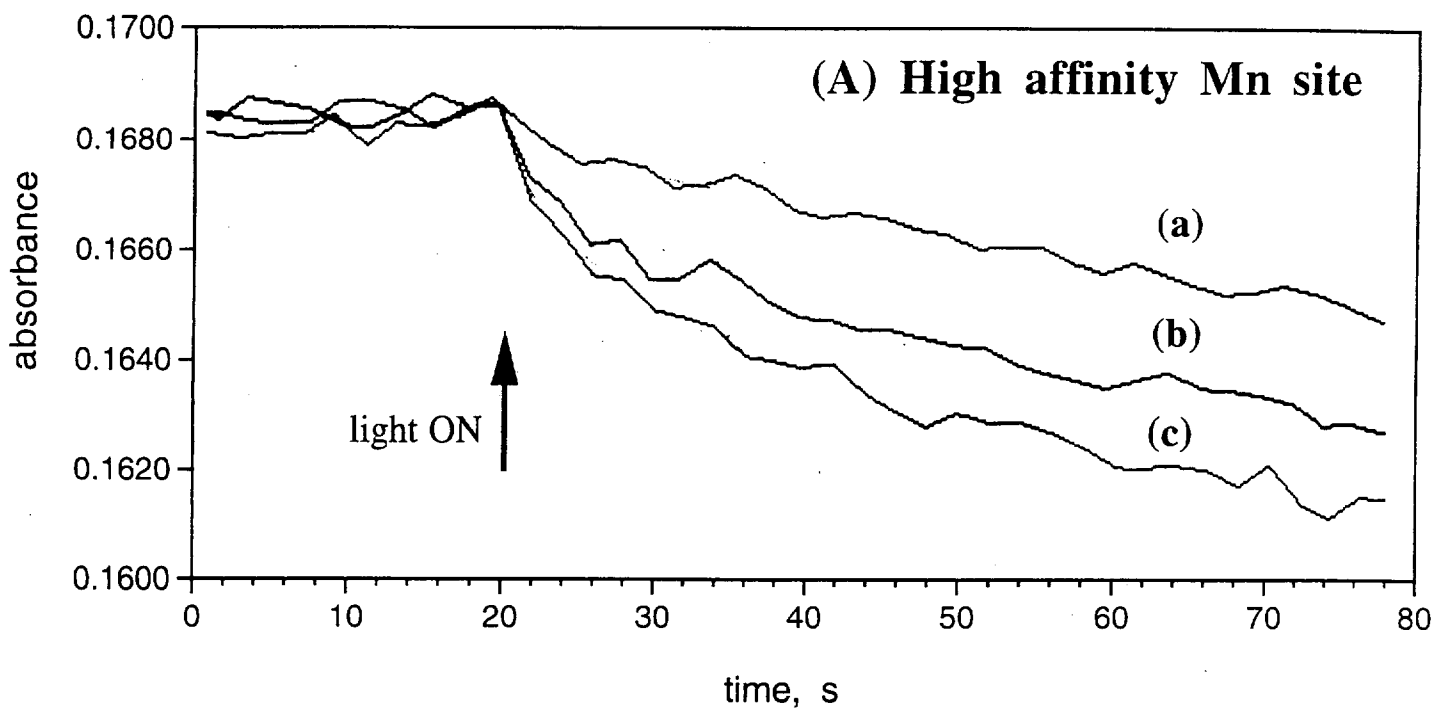
When an electron transfer occurs catalytically in the absence of CaCl₂, the oxidized Mn³⁺ will eventually be reduced by DCIPH₂ and dissociate from apo-PSII according to the eq.:



At the high affinity Mn site, the 5 μM Mn^{2+} that is added is photooxidized completely to Mn^{3+} after 60 second illumination time, based on the known number of DCIP molecules that are photoreduced. Therefore, the method of initial rates can provide only a lower estimate of the DCIP reduction rate, with the limited sensitivity of the instrument. From a titration of the Mn^{2+} dependence this yielded an upper limit for the dissociation constant of 5 μM for Mn^{2+} , in agreement with a more accurate literature value of 1 μM . Addition of more Mn^{2+} after illumination stopped followed by dark incubation restored the kinetic curve.

At the low affinity Mn site, only 15% of the added Mn^{2+} is converted to Mn^{3+} after 60 s. Here the method of initial rates is accurate. The decrease in rate is not due to consumption of Mn^{2+} or DCIP. Rather it is due to the accumulation of a species which photoreduces DCIP more slowly. This species disappears after several minutes incubation in the dark. It is attributed to the accumulation of apo-WOC-PSIIMn³⁺ the first photoactivation intermediate IM_1 . This can be understood if the binding constant for apo-WOC-PSIIMn³⁺ ($1/K$) is larger than that for apo-PSIIMn²⁺ (k_1/k_{-1}) at the low affinity site. Since the addition of more Mn^{2+} after 60 s restored the light-induced decrease of the absorbance, this drop is not due to photoinhibition but to the (slightly) large binding constant for apo-WOC-PSIIMn³⁺ compared to that for apo-WOC-PSIIMn²⁺.

Figure S2. Analysis of the Dixon plot to obtain the Mn^{2+} binding constant.

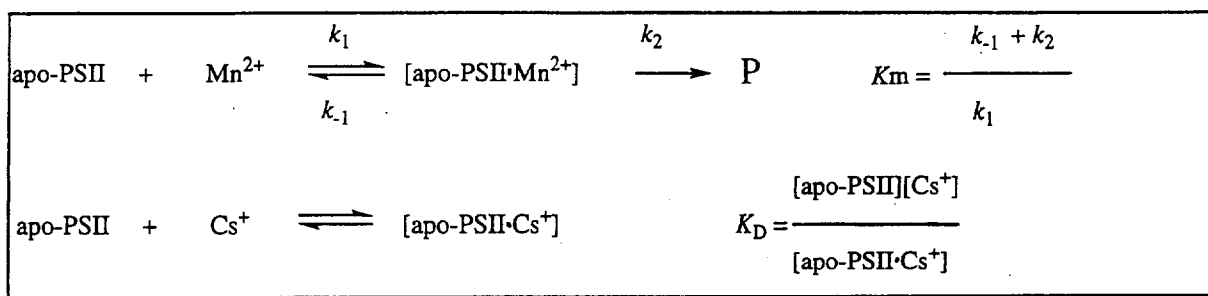


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Figure S1, Ananyev et al.

The determination of the dissociation constant of the apo-PSII-Cs⁺ complex

Reaction



Reaction rate

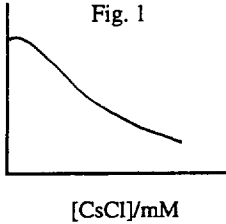
$$v = \frac{V[\text{Mn}^{2+}]}{K_m (1 + [\text{Cs}^+]/K_D) + [\text{Mn}^{2+}]} \quad V = k_2[\text{apo-PSII}]$$


Fig. 1

$$\frac{1}{v} = \left[\frac{K_m + [\text{Mn}^{2+}]}{V[\text{Mn}^{2+}]} \right] + \left[\frac{K_m}{VK_D[\text{Mn}^{2+}]} \right] [\text{Cs}^+]$$

\downarrow intercept \downarrow slope

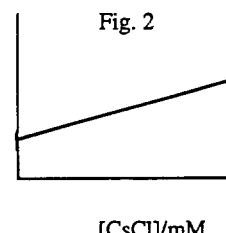


Fig. 2

Results

$$\begin{array}{l}
 \text{intercept} = \frac{K_m + [\text{Mn}^{2+}]}{V[\text{Mn}^{2+}]} \quad \text{slope} = \frac{K_m}{VK_D[\text{Mn}^{2+}]} \\
 \\
 \frac{\text{slope}}{\text{intercept}} = \frac{K_m}{K_D(K_m + [\text{Mn}^{2+}])} \quad \Rightarrow \quad K_D
 \end{array}$$

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Figure S2, Ananyev et al.