Model complexes suggest certain S-state changes of the photosynthetic water-oxidation enzyme may involve an Mn(II)–Mn(III) transition

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The ultraviolet-visible absorbance differences spectra of Mn(II,III) and Mn(III,III) oxo-bridged carboxylate complexes are reported. The difference spectra are remarkably similar to those of the photosynthetic water-oxidation enzyme complex reported by Dekker et al. [(1984) Biochim. Biophys. Acta 764, 301–309] which were interpreted as being due exclusively to Mn(II→IV) transitions. This result indicates that certain S-state changes of the enzyme complex may instead involve Mn(II→III) transitions, and that difference spectra alone cannot be used with confidence to assign the Mn oxidation state changes during water oxidation.

Manganese *Photosynthesis* *Water oxidation* *Absorbance difference spectrum*

1. INTRODUCTION

Four atoms of Mn seem to be intimately involved in the four-electron oxidation of water to dioxygen by the enzyme in the photosynthetic apparatus of green plants [1]. This Mn complex is capable of cycling between five distinct oxidation levels, labelled S_0–S_4 in the pioneering work of Kok [2]. The UV-visible absorbance changes associated with the oxygen-evolution cycle have been thoroughly investigated [3–7]. The difference spectrum of a redox component of the enzyme (obtained after correcting for spectral changes due to electron acceptors Q and donors Z) has been assigned to oxidation state changes of the Mn complex. While the oscillatory pattern of this spectral change has been under debate [3,5–6], the oxidation state change assignment to an Mn(III)→Mn(IV) transition by Dekker et al. seems to have been well accepted. In addition, transitions $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ are now believed to involve the Mn oxidation pattern $+1:+1:+1:-3$ [7] which, together with the difference spectral conclusions, results in Mn oxidation state changes of 4 Mn^{3+}:3 Mn^{3+}, Mn^{4+}:2 Mn^{3+}, 2 Mn^{4+}:Mn^{3+}, 3 Mn^{4+}:4 Mn^{3+}. While this is reasonable, it would result in the S_2 state being EPR-silent, a conclusion inconsistent with EPR studies [8]. Since a tetranuclear site seems overall to be the more favored by the total available data, there is no unique pattern of Mn oxidation state changes which would rationalize all the apparently conflicting data. Since the results by Dekker et al. seem pivotal for specifying the Mn oxidation levels of a particular S-state, we decided to review the basis for their conclusions. Their difference spectra were compared with those of a binuclear Mn gluconate complex [9] which can be obtained with Mn in the +2, +3, and +4 oxidation states. The +3/+4 difference spectrum was indeed much more similar to the enzyme spectrum than the +2/+3 spectrum and this led to their conclusion that the S-state changes involve the +3/+4 transition. However, we felt that such an important conclusion should not be based on comparisons with only a single model system. We do not mean this

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as a criticism for there truly is a lack of isostructural Mn complexes in variable oxidation states available for such comparisons. However, our own work has recently provided isostructural Mn(II,III) and Mn(III,III) complexes and we have compared their UV-visible difference spectra with that of the enzyme. We herein report the result of this work.

2. MATERIALS AND METHODS

Reaction of $NBu^n_4MnO_4$ with $Mn(OAc)_2 \cdot 4H_2O$ and acetic or benzoic acids in pyridine or pyridine/ethanol mixtures yields a series of trinuclear complexes $[Mn_3O(O_2CR)_6(pyr)_{3-x}(H_2O)_x]^{0,+}$ ($x = 0$, R = Me; $x = 1$, R = Ph) which have been characterized by a variety of techniques including crystallography in some cases (submitted). Addition of 2,2′-bipyridine (bipy) to MeCN solutions of these trinuclear species yields a new class of tetranuclear Mn complexes $[Mn_4O_2(O_2CR)_7(bipy)_2]^Z$ ($Z = 0$, R = Ph, Mn^{2+}: 3 Mn^{3+}; $Z = 1$, R = Me, 4 Mn^{3+}) (submitted).

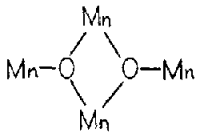
UV-visible spectra were recorded on ~5 mM MeCN or CH_2Cl_2 solutions using a Hewlett Packard model 4450A spectrophotometer.

3. RESULTS

Our research is seeking the synthesis of an inorganic model of the Mn site in the water-oxidation enzyme to assist in elucidating the nature and mode of action of this unit. Since no porphyrin rings have been detected and all quinones associated with the PS II reaction center have been accounted for in other functions [10], amino acid side group ligation to the Mn in the enzyme is suggested. Recent EXAFS results indicate bridging oxide (or hydroxide) and terminal O and/or N ligands [11], the latter presumably from tyrosine phenoxide, aspartic/glutamic acid carboxylate and histidine imidazole.

Our efforts in this area have led us to high yield syntheses of isostructural $[Mn_3O(O_2CMe)_6(pyr)_3](pyr)$ and $[Mn_3O(O_2CMe)_6(pyr)_3](ClO_4)$ possessing Mn(II,III,III) and Mn(III,III,III) oxidation levels, respectively. Since these also contain oxide bridges and biologically relevant ligands (the pyridine is a conservative replacement for imidazole), we decided their difference spectrum might allow informative comparisons with the previous work by Dekker. The difference spectrum in the range 270–400 nm is shown in fig.1 where we have also reproduced the difference spectrum of the enzyme from fig.10 of [4]. As can be seen, the general profiles are very similar in the two plots; a maximum at 303 nm in the model spectrum is very close to that of the native Mn center, ~305 nm. Indeed, the difference spectrum of the former is closer to that of the latter than found when the Mn(III)/Mn(IV) gluconate system is used for comparison.

An additional comparison can be made if the difference spectrum of $[Mn_4O_2(O_2CMe)_7(bipy)_2]^+$ and $[Mn_4O_2(O_2CPh)_7(bipy)_2]$ is employed. The X-ray structure of the former has been obtained and shown to contain the bridged unit shown below:



The two complexes contain different carboxylates, i.e. Me vs Ph, but this is unlikely to have a major effect on the difference spectrum. With this proviso specified, we offer for inspection in fig.2 the corresponding difference spectrum. Again, the general appearance of the spectrum is similar to that of the enzyme and has a maximum at ~308 nm.

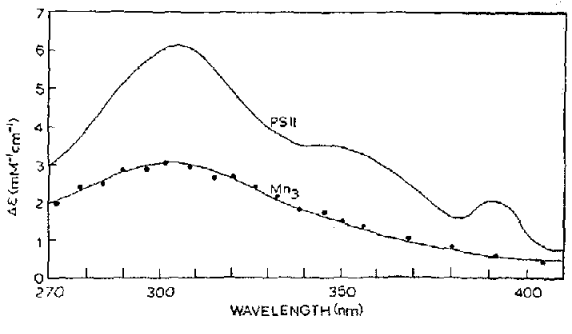


Fig.1. Difference absorbance spectra for $[Mn_3O(O_2CMe)_6(pyr)_3]^{0,+}$ and the enzyme site, labelled Mn_3 and PS II, respectively.

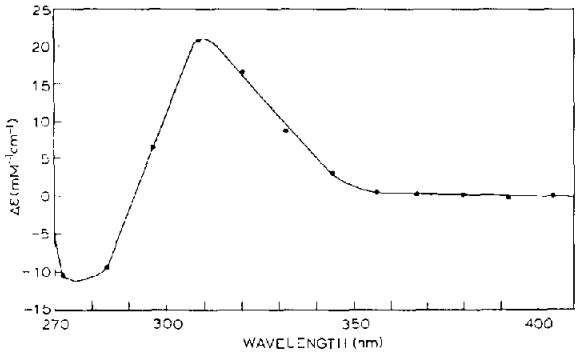


Fig.2. Difference absorbance spectrum for $[Mn_4O_2(O_2CR)_7(bipy)_2]^Z$ (Z = 0, R = Ph; Z = 1, R = Me).

4. DISCUSSION

It should be stated immediately that we do not possess complexes of formulation $[Mn_3O(O_2CR)_6(pyr)_3]^{2+}$ or $[Mn_4O_2(O_2CR)_7(bipy)_2]^{2+}$, containing (2 Mn^{3+}, Mn^{4+}) and (3 Mn^{3+}, Mn^{4+}), respectively, and cannot therefore ascertain the corresponding difference spectra for the Mn(III→IV) transitions. Nor do we claim that the above results indicate that S-state changes involve the Mn(II→III) transition exclusively. Indeed, enough evidence is available from EPR and EXAFS data supporting the S_2 state as being (3 Mn^{3+}, Mn^{4+}) rather than (Mn^{2+}, 3 Mn^{3+}). Our results do suggest, however, that difference spectra alone cannot be used with confidence to assign metal oxidation states, and that the possibility of some S-state changes involving the Mn(II→III) transition should not be ruled out. Entertaining this hypothesis further, an alternative pattern for the Mn oxidation states for a tetranuclear enzyme complex can be drawn up which is still in accord with both the oxidation state transitions +1: +1: +1: −3 and with the EPR and EXAFS data, i.e. S_0(4 Mn^{3+}), S_1(3 Mn^{3+}, Mn^{4+}), S_2(2 Mn^{3+}, 2 Mn^{4+}), S_3(1 Mn^{3+}, 3 Mn^{4+}), with the latter on further oxidation rapidly reverting to S_0 via S_4 with evolution of O_2. Such a scheme would also imply that $Mn_4O_2(O_2CPh)_7(bipy)_2$ and $[Mn_4O_2(O_2CMe)_7(bipy)_2]^+$ represent potential models of the S_0 and S_1 states, respectively.

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