Biochimica et Biophysica Acta, 1144 (1993) 346–352

BBABIO 43892

Ultraviolet difference absorbance spectra of oxo-bridged manganese complexes: relationship to the photosynthetic Mn and Mn catalase

Warren Wells, Anthony Harton and John B. Vincent

Department of Chemistry, University of Alabama, Tuscaloosa, AL (USA)

(Received 8 March 1993)

Key words: Manganese; Difference spectroscopy; Manganese catalase; Catalase; Photosystem II

The ultraviolet difference spectra of isostructural multinuclear manganese carboxylate complexes which differ in manganese oxidation levels are described and compared against similar spectra reported for Mn catalase and reported to accompany the S-state transitions of the photosynthetic Mn. Analysis of the spectra suggest that previous assignments of specific Mn oxidation-state changes to the spectral features accompanying the S-state transitions are premature. The significance of the ultraviolet difference spectra associated with well-characterized Mn oxidation-state changes of Mn catalase are discussed in relation to the S-state difference spectra.

Introduction

In photosynthetic water oxidation, the oxygen-evolving complex cycles between five distinct oxidation levels, labelled S_0–S_4 in the formal scheme of Kok [1]. Experimental information from a wide variety of spectroscopic and magnetic techniques reveal that these oxidation levels reflect changes in the oxidation states of manganese ions essential for the four electron oxidation of water to dioxygen [2–4]. A number of studies have been directed at determining the oxidation states of each of the at least four (and perhaps six [5]) manganese ions in each of the S levels. One of the most commonly utilized techniques is difference ultraviolet spectroscopy, where the difference spectrum of a redox component (obtained after correcting for spectral changes due to other electron acceptors and donors) has been assigned to oxidation states changes of the multinuclear Mn center [6–20].

The oscillatory pattern of this ultraviolet spectral change, its shape and intensity, and the assignments of the change to particular Mn oxidation state transitions have all been under debate (reviewed in Ref. 9). At the center of the debate are problems in deconvoluting the spectral changes resulting from each S-level transition. As bioinorganic chemists, our role is not to address the complexities of this problem but to examine what the plausible shapes, positions and intensities of the ultraviolet difference absorption spectra associated with specific changes in manganese oxidation states are and then by extrapolation to address the native system, being careful that the proposals make both 'inorganic' sense and are consistent with available biochemical and biophysical data [20]. Herein are reported the ultraviolet difference spectra of a number of synthetic oxo (or hydroxo)-bridged multinuclear manganese complexes, containing Mn in a variety of oxidation states. Comparison of these spectra with those of Mn catalase, whose individual manganese oxidation states are well-characterized by independent techniques in a number of oxidation levels [22–24], reveals the potential of the method. Extension of the comparisons to the reported difference spectra of the Photosystem II S-state transitions will be discussed.

Materials and Methods

Synthetic manganese complexes were prepared as previously described (see Table I). All ultraviolet/visible measurements were performed on acetonitrile solutions (with the exception of compound *9*) using a Hewlett-Packard 8451A spectrophotometer. Because of solubility considerations, a methanol solution of *9*

Correspondence to: J.B. Vincent, Department of Chemistry, University of Alabama, P.O. Box 870336, Tuscaloosa, AL 35487, USA.
Abbreviations: Me_3TACN, *N,N′,N″*-trimethyl-1,4,7-triazacyclononane; TACN, 1,4,7-triazacyclononane; py, pyridine; ImH, imidazole; 3-Mepy, 3-methylpyridine; phen, 1,10-phenanthroline; bipy, 2,2′-bipyridine.

TABLE I

Synthetic manganese complexes used in difference absorbance studies

Compound	Reference
1. $[Mn(III)_2O(O_2CCH_3)_2(Me_3TACN)_2](ClO_4)_2 \cdot H_2O$	25
2. $[Mn(III)Mn(IV)O(O_2CCH_3)_2(Me_3TACN)_2](ClO_4)_3$	26, 27
3. $[Mn(II)_2(OH)(O_2CCH_3)_2(Me_3TACN)_2](ClO_4)$	26
4. $[Mn(III)_2O(O_2CCH_3)_2(TACN)_2](ClO_4)_2$	25
5. $[Mn(III)Mn(IV)O_2(O_2CCH_3)(TACN)_2]$ $(BPh_4)_2 \cdot MeCN$	28
6. $[Mn(III)_3O(O_2CCH_3)_6(py)_3]ClO_4$	29
7. $[Mn(III)_2Mn(II)O(O_2CCH_3)_6(py)_3] \cdot py$	29
8. $[Mn(III)_3O(O_2CCH_2CH_3)_6(py)_3]ClO_4$	30
9. $[Mn(III)_3O(O_2CCH_3)_6(ImH)_3]O_2CCH_3$	29
10. $[Mn(III)_3O(O_2CCH_3)_6(3\text{-}Mepy)_3]ClO_4$	31
11. $[Mn(III)Mn(IV)O_2(phen)_4](ClO_4)_3 \cdot 4H_2O$	32
12. $[Mn(IV)_2O_2(phen)_4](ClO_4)_4 \cdot H_2O$	33

was used; a comparison between the spectra of saturated solutions of *9* in acetonitrile and those in methanol indicated that any solvent effects were minimal. The spectra of *5* were obtained vs. an acetonitrile reference containing appropriate concentrations of BPh_4^- to negate ultraviolet contributions from the anion. Similarly, spectra of *6* were collected with the presence of one equivalent of pyridine (for the comparison with the spectrum of *7*) and in the absence of pyridine (for other comparisons).

Results and Discussion

Current evidence suggests that the ligands to the photosynthetic Mn provided by the reaction center proteins are almost solely carboxylate functionalities from glutamate and/or asparate residues with perhaps a few imidazole moieties from histidine residues [21]. Site-directed mutagenesis studies have also implicated aspartate [34–36] and perhaps glutamate [36] residues as potential or probable Mn ligands. Electron spin echo envelope modulation studies suggest one histidine ligand might also be present [37]. As the ultraviolet spectra of manganese complexes are dominated by ligand-based transitions, ideally complexes of Mn with carboxylate and imidazole ligands should be probed by difference ultraviolet spectroscopy. Then, by careful variation in the ligands, the contribution of each type of ligand could be identified; analysis of the spectrum of the native system then could provide detailed information on the ligands of the photosynthetic Mn. While the last decade has witnessed an explosion in the syntheses of Mn carboxylate complexes, few complexes with imidazole-based ligation exist. Consequently, complexes with pyridine-based ligands were often examined in this study with the assumption that in many respects pyridine might serve as a reasonably conservative substitution for imidazole. In order to guarantee the identity of the synthetic species, only complexes characterized by X-ray crystallography or simple derivatives of such complexes (which have been extensively characterized by other techniques) have been utilized. Additionally (unless otherwise noted), only isostructural complexes with manganese ions in varying oxidation states are compared, such that analyses are not complicated by spectral features arising from or affected by structural changes unless these changes are inherent to the differences in oxidation state (i.e., changes in bond lengths, etc.). These limits severely restrict compounds currently available to study. Indeed, they are essentially exhausted by this work. It should also be stressed that only binuclear and trinuclear synthetic complexes are examined in this work while the photosynthetic Mn assembly is very likely tetranuclear; this unfortunately is a result of the lack of suitable synthetic materials in the literature. Unique features resulting from a special arrangement of four Mn ions in the photosynthetic system would not be observed with the models.

$d \rightarrow d$ transitions of Mn(II) ions are spin forbidden, such that they have little intensity ($\epsilon < 10\ M^{-1}\ cm^{-1}$) and could not contribute appreciably to the ultraviolet spectra of their complexes, although some intensification can occur through exchange processes in mutinuclear systems. For Mn(III) ions in a ligand field comprised of the types of ligands likely to be bound to the PS II Mn, the $d \rightarrow d$ transitions have significantly more intensity ($\epsilon < 1000\ M^{-1}\ cm^{-1}$) but are lower in energy than the ultraviolet region. Of the three oxidation states of Mn readily accessible in a biological environment, only Mn(IV) might possess bands of appreciable intensity in the ultraviolet region resulting from $d \rightarrow d$ transitions; however, these transitions should be limited to the lower energy end of the ultraviolet. Consequently, the UV spectra of Mn(II), Mn(III) and Mn(IV) are expected to be dominated by charge transfer bands; for the expected ligands, these bands should be ligand to metal in origin. Unfortunately, no systematic studies of charge transfer bands of appropriate Mn(II), Mn(III) or Mn(IV) complexes have been reported.

Previously the spectra accompanying the S-state transitions have been compared to the difference spectra of four sets of Mn complexes with Mn in varying oxidation states [30,38–40]. The exact ligation of the Mn in two of these, Mn in dipalmitoyl-α-phosphatidylcholine vesicles [39] and Mn gluconate complexes [40], is unknown. The possible ligation of the Mn in these complexes should not resemble the supposed ligation of the photosynthetic Mn; consequently, the spectra of these synthetic complexes will not be considered further. One of these systems, for which the identity of the complexes has been established by X-ray crystallography, is comprised of complexes of the general formula $[Mn_3O(O_2CR)_6(py)_3]^{z+}$, where 3 Mn(III) are present if $z = 1$ and 2 Mn(III) and 1 Mn(II) are pre-

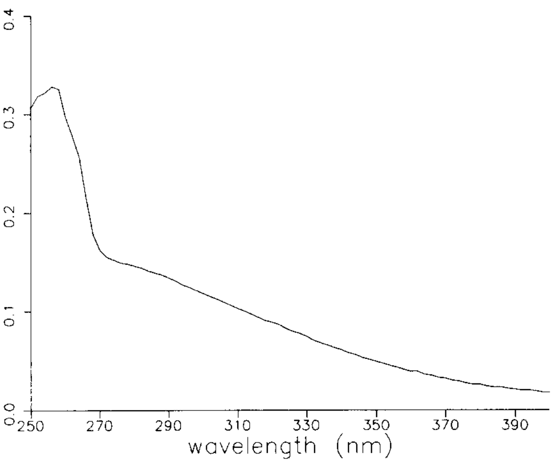

Fig. 1. Ultraviolet difference spectrum of (6 + pyridine) − 7. One absorbance unit corresponds to a molar absorptivity change of 10000 M^{-1} cm^{-1}.

sent if $z = 0$. Complexes can be synthesized with a number of different carboxylates, and the pyridine ligands can be replaced with derivatives of pyridine or with imidazole. The ability to vary the components of the complexes allows for the contributions of each component to the ultraviolet spectra of the complexes to be identified. Note also that these complexes possess an oxide ligand (O^{2-}); oxide has also been proposed as a constituent of the photosynthetic Mn complex [41,42].

The ultraviolet difference spectra between isostructural *6* and *7* is given in Fig. 1. The solvent for *6* contained one equivalent of pyridine to compensate for the solvate pyridine of *7*. The spectrum is very similar to that previously reported (over the range 270–410 nm) [38], except that the previously reported spectra possessed a maximum at circa 305 nm. This maximum results from not compensating for the pyridine solvate of *7*. The spectrum in Fig. 1 displays two dominate features: one which is maximum at circa 260 nm, assignable to a difference in enhancement of pyridine π to π^* transitions, and a broad shoulder at circa 280 nm. The second feature as discussed in Ref. 38 is similar in general profile to difference spectrum of the photosynthetic enzyme. In order to examine the origin of the second feature, the difference ultraviolet and visible spectra between complex *6* and its propionate and its 3-methyl pyridine derivatives (i.e., $[Mn_3O(O_2CCH_2CH_3)_6(py)_3]ClO_4$ (*8*) and $[Mn_3O(O_2CMe)_6(3\text{-}Mepy)_3]ClO_4$ (*10*), respectively) were measured (Fig. 2). The UV difference spectra of $6 - 10$ displays only one feature which results from a slight shift in the energy of pyridine-moiety-based $\pi \rightarrow \pi^*$ transitions; the visible difference spectrum is virtually featureless. The replacement of acetate by propionate results in a weak feature ($\epsilon < 500$ M^{-1} cm^{-1}) at high energy in the ultraviolet region and a derivative-type signal in the visible region. Given the lack of sensitivity of the $d \rightarrow d$ transitions in these trinuclear species to carboxylate substitutions [35], the visible feature may result from a low energy O_2CR to metal charge transfer band. Resonance Raman studies have previously suggested the possibility that an acetate to Mn(III) charge transfer band underlies the Mn(III) $d \rightarrow d$ transitions for some similar Mn(III)-oxide-carboxylate complexes [43]. A more informative substitution results from the replacement of the pyridine ligands with imidazole (Fig. 3). Comparing the difference between the UV spectra of *6* and *4*, $[Mn_3O(O_2CMe)_6(ImH)_3]^+$, reveals that the difference between them is extremely similar to the difference spectra of $6 - 7$. This suggests that both features of Fig. 1 arise from the presence of the pyridine; thus, the shoulder at circa 280 nm may arise from a high-energy pyridine to Mn(III) charge transfer band, which is relatively insensitive to methylation of the

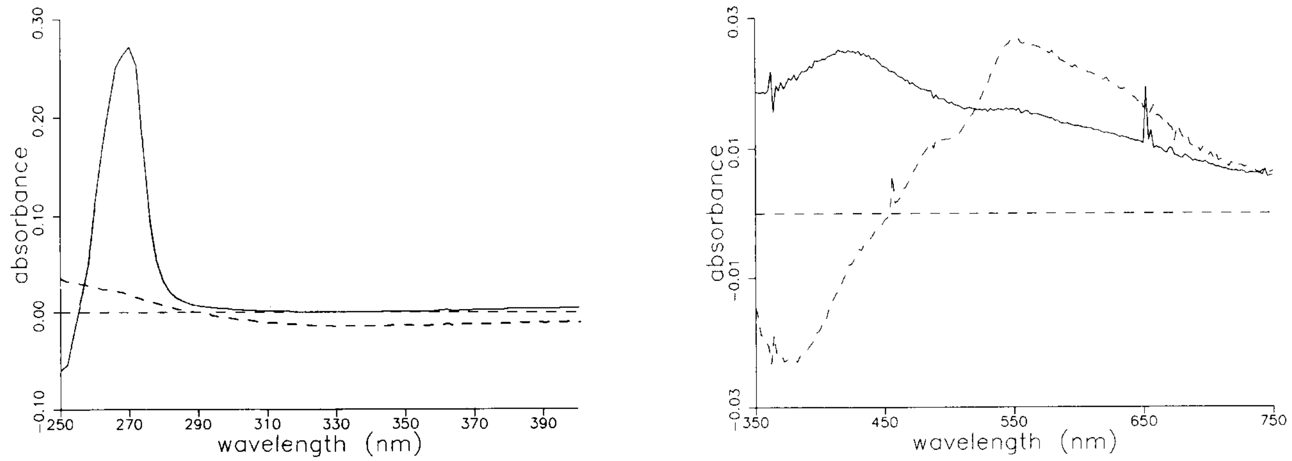

Fig. 2. (Left) Ultraviolet difference spectrum of $6 - 10$ (———) and $6 - 8$ (— — —). One absorbance unit corresponds to a molar absorptivity change of 10000 M^{-1} cm^{-1}. (Right) Visible difference spectra of $6 - 10$ (———) and $6 - 8$ (— — —). One absorbance unit corresponds to a molar absorptivity change of 1000 M^{-1} cm^{-1}.

pyridine in the 3-position. If this is the origin of the broad shoulder, it does not preclude the existence of underlying oxo to Mn(III) or carboxylate to Mn(III) charge transfer bands; these other contributions must be, however, small in intensity such that they could not account for the intensity generally associated with the S-state transitions (see, however, Ref. 9).

Dekker [9] has criticized the presentation of the spectrum of *6 – 7* in Ref. 38 as it did not cover wavelengths below 290 nm (specifically over the range approx. 240–290 nm). While the spectrum did cover the range 270 nm to 410 nm, higher energies were not presented because any information of value was masked by changes in the pyridine $\pi \rightarrow \pi^*$ transitions not because of 'large absorbance changes' inherent to 'Mn(II) → Mn(III) oxidations.' This is particularly significant, as in the spectra used in Ref. 21 for comparison to the S-state spectra [39,40] the Mn(III) → Mn(IV) difference spectra are of equal or greater intensity when compared to the Mn(II) → Mn(III) spectra in this region, where the spectra of the biological system display little intensity [22].

A similar situation can be observed in the ultraviolet difference spectra associated with an oxidation of Mn(II) → Mn(III) in isostructural $[Mn_4O_2(O_2CPh)_7(bipy)_2]^{0,+}$ [30]. The spectrum presented in Ref. 30 is virtually identical in shape to that of 2,2′-bipyridinium [44]; a difference in the enhancement of the $\pi \rightarrow \pi^*$ transition of the bipy ligand completely dominants the spectrum. Little if any information on other features which might be significant for comparison to the photosynthetic spectra can be deciphered.

While the trinuclear complexes allow for examination of Mn(II) to Mn(III) transitions, this system is not amenable to probing Mn(III) to Mn(IV) transitions. The isostructural complexes $[Mn_2O_2(phen)_4]^{3+,4+}$ have been characterized by X-ray crystallography in both the $Mn(III)_2$ and Mn(III)Mn(IV) oxidation levels [45]. The ultraviolet spectra of these complexes are presented in Fig. 4. The spectra are dominated by $\pi \rightarrow \pi^*$ transitions, originating from the phenanthroline ligands. The difference spectrum of *12 – 11* (not presented) is complex and difficult to analyze. However, any other contributions to the spectra would appear to have little intensity. It becomes readily apparent that ligands based on pyridine such as py, bipy, and phen render the ultraviolet spectra of their complexes almost useless for detailed comparison to the S-state transitions.

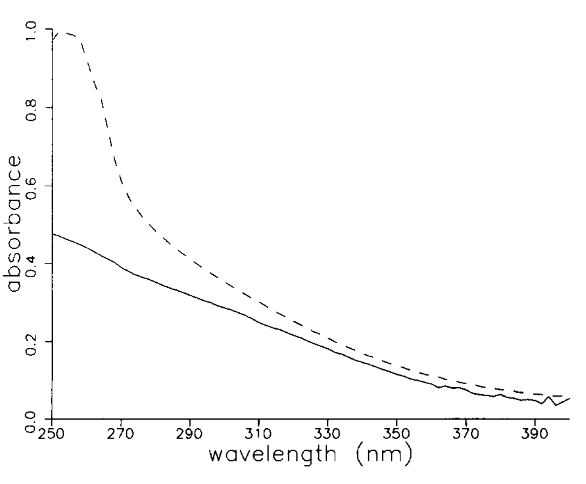

Fig. 3. Ultraviolet spectra of *6* (— — —) and *9* (———). One absorbance unit corresponds to a molar absorptivity change of 10000 M^{-1} cm^{-1}.

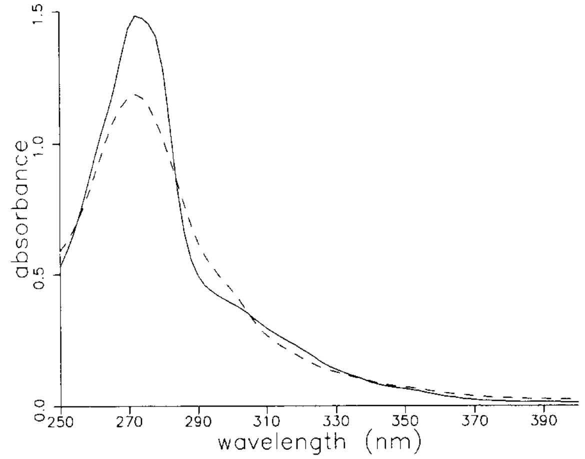

Fig. 4. Ultraviolet spectrum of *11* (———) and *12* (— — —). One absorbance unit corresponds to a molar absorptivity of 40000 M^{-1} cm^{-1}.

This begs the question of what type of complexes might be suitable. The enzyme Mn catalase, which catalyzes the two-electron disproportionation of peroxide to water and dioxygen, contains a dinuclear active site. A combination of X-ray absorbance, electronic, and electron spin resonance spectroscopic studies indicate the Mn can exist in four oxidation levels: $Mn(II)_2$, Mn(II)Mn(III), $Mn(III)_2$ and Mn(III)Mn(IV) (see Refs. 22–24 and references therein). EXAFS studies indicate the active site of the Mn(III)Mn(IV) form possesses a $Mn_2(\mu\text{-}O)_2$ core [41]; a comparison of the visible spectrum of the enzyme with that of $Mn(III)_2(\mu\text{-}O)(\mu\text{-}O_2CR)_2$ model complexes suggests the $Mn(III)_2$ form may contain a similar unit [26]. All evidence to date suggests that the probable Mn ligands are asparate and glutamate carboxylate functionalities and the imidazole moieties of histidines. Thus, difference UV spectra between the different oxidation levels of Mn catalase could provide insight into interpreting the S-state UV spectra. Recently, Khangulov et al. have reported the electronic spectra of the $Mn(II)_2$, $Mn(III)_2$ and Mn (III)Mn(IV) forms of the enzyme [24], which allow for the calculation of difference ultraviolet spectra (see inset to Fig. 5).

The difference absorbance spectra of the $Mn(III)_2$ form minus the $Mn(II)_2$ form (intensity divided by two

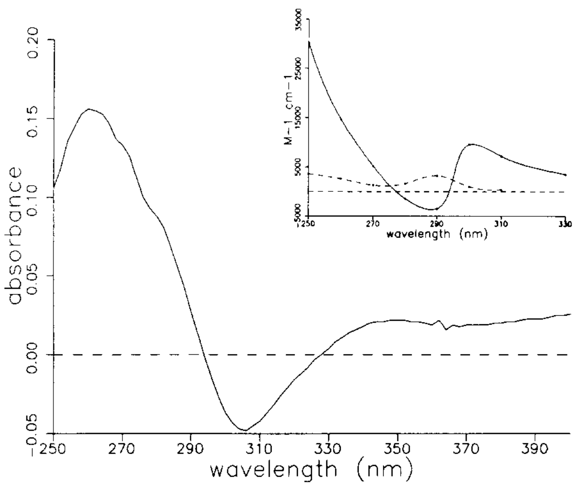

Fig. 5. Ultraviolet difference spectrum of *2* – *1*. One absorbance unit corresponds to a molar absorptivity change of 10000 M^{-1} cm^{-1}. Inset: Ultraviolet difference spectrum of the Mn_2(III,IV) form – Mn_2(III,III) form (———) and Mn_2(III,III) form – Mn_2(II,II) form (— — —) of Mn catalase. The intensity of the latter has been reduced by one half. Catalase spectra calculated from data in Ref. 23.

to give change per oxidizing equivalant) reveals a gradual increase in intensity from low energy to high energy with a distinct feature at circa 290 nm. In contrast, the Mn(III)Mn(IV) form minus the $Mn(III)_2$ form spectrum reveals a dramatic increase in intensity from low energy to high energy; a derivative-type feature (maximum at approx. 300 nm, minimum approx. 290 nm) is superimposed on the high-energy feature. The maximum in the former spectrum probably arises from a ligand to Mn(III) charge transfer band; in the later, the minimum then represents the loss of this transition with the maximum arising from a new ligand to Mn(IV) charge transfer band, shifted to lower energy. Both charge transfer bands probably originate from the same ligand(s); for ligand to metal charge transfer bands, a shift to lower energy is expected with an increase in the reducibility of the metal [47]. Given the results with the synthetic trinuclear species, a μ-oxide to metal charge transfer band would seem the likely origin.

If the Mn catalase difference UV spectra should more closely approach those of the S-states than those of any synthetic system available, just how do these two sets of spectra compare? Unfortunately, three qualitatively different sets of S-state spectra have been reported. In the most recent report, Dekker indicates that the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions are accompanied by an identical spectral change that is maximal at circa 300 nm (ϵ approx. 6000 M^{-1} cm^{-1}) with a small shoulder at circa 350 nm [9]. The features of the $S_0 \rightarrow S_1$ transition spectrum are similar in shape while significantly weaker in intensity ($\epsilon_{300} < 5000$ M^{-1} cm^{-1}). The spectra of Lavergne are somewhat similar for the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions [19]. The former has a peak at circa 320 nm (ϵ approx. 8000 M^{-1} cm^{-1}) with a shoulder at circa 350 nm; the latter possesses a maximum at approx. 300 nm (ϵ approx. 6000 M^{-1} cm^{-1}) with a shoulder at circa 350 nm. In contrast, the $S_0 \rightarrow S_1$ transition is accompanied by an essentially featureless spectrum. Witt, whose work was performed in the presence of hydroxylamine, reported that the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions were accompanied by identical difference spectra with a maximum at circa 340 nm (ϵ approx. 2500 M^{-1} cm^{-1}) [13]. The $S_0 \rightarrow S_1$ spectra, in contrast, possessed a maximum at 310 nm (ϵ approx. 2000 M^{-1} cm^{-1}). The discrepancies in the difference extinction coefficients of these transitions is becoming an area of intense study [20]. None of these spectra has features with large negative intensity similar to that arising from the Mn(III) → Mn(IV) oxidation of Mn catalase, nor do they possess the enormous increase in intensity at higher energies. While significantly less intense, the Mn catalase Mn(II) → Mn(III) spectrum resembles in position of the maximum all the spectra of Dekker, the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions of Lavergne, and the $S_0 \rightarrow S_1$ transition of Witt; however, the feature in the Mn catalase spectrum is not as broad as those of the photosynthetic Mn. This does not indicate that all these S-state spectra should be assigned to Mn(II) → Mn(III) oxidations, especially as this would be contradictory to the results on PS II using other spectroscopic and magnetic techniques [48].

To examine the origin of the features in the Mn catalase spectra, a remarkable series of synthetic dinuclear manganese complexes is available from the laboratory of Wieghardt and co-workers [25–28]. These complexes, which have all been characterized by X-ray diffraction studies, contain $Mn(II)_2(OH)$, $Mn(III)_2$ O, Mn(III)Mn(IV)O and Mn(III)Mn(IV) O_2 cores. All possess two bridging acetate ligands, except the last which has one. The remaining three coordination sites of each Mn are filled by tridentate 1,4,7-triazacyclononane (TACN) or *N*,*N*′,*N*″-trimethyl-1,4,7-triazacyclo-nonane (Me_3TACN) ligands. These ligands based on primary amine functionalities avoid the complications in the ultraviolet region which were found with the pyridine-based ligands.

The difference spectra of isostructural *2* – *1* is shown in Fig. 5. This spectrum represents a Mn(III) to Mn(IV) oxidation; while the shape resembles the difference spectrum of the Mn(III)Mn(IV)–$Mn(III)_2$ forms of Mn catalase, the intensity is only a small fraction of that of the enzyme's spectrum. This renders interpretation of the spectrum of the synthetic species difficult. In contrast, the difference spectra of *1* – *3* and *5* – *4* are similar in shape and intensity to those of Mn catalase for the corresponding oxidation state changes (Fig. 6). The change in the oxide/hydroxide content associated with the Mn oxidation state changes in the synthetic complexes are believed to mirror the changes associ-

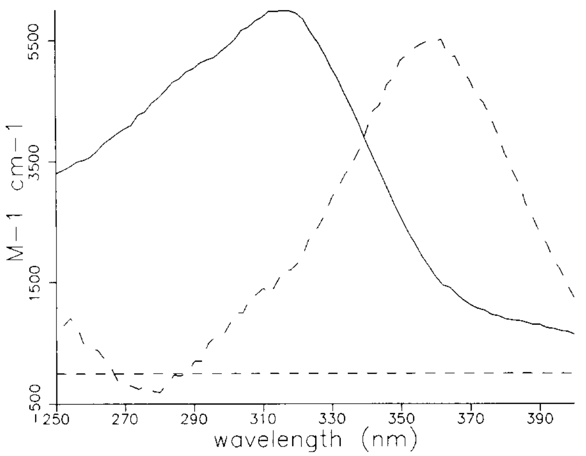

Fig. 6. Ultraviolet difference spectrum of *1* − *3* (— — —) (the intensity has been reduced by one half) and the ultraviolet difference spectrum of *5* − *4*, (———).

ated with the oxidation state changes of Mn catalase. The spectrum of *1* − *3* displays an intense maximum at circa 310 nm [22–24]; this maximum appears to shift to circa 360 nm in the spectrum of *5* − *4*. This shift would be consistent with both features having a similar origin with the shift to lower energy resulting from the increase in Mn oxidation level. The most likely origin of these bands given the associated changes in oxide content are oxide to Mn(III) and oxide to Mn(IV) transitions. The much less intense features of the spectrum of *2* − *1* could result from gain and loss of similar transitions; the lower intensity would result from no associated change in the number of oxide ligands in this spectrum. The lack of an intense higher energy feature in any of the difference spectra of the synthetic complexes may suggest the high energy feature in the difference spectrum resulting from oxidation of the $Mn(III)_2$ form of Mn catalase to the $Mn_2(III,IV)$ form arises from a histidine to Mn(IV) transition. Yet, while the difference spectra associated with the TACN and MeTACN complexes support the proposed Mn oxidation states and oxide contents of the various forms of Mn catalase, the spectra provide little insight on the photosynthetic Mn assembly, especially given the discrepancies in the spectra reported for the photosynthetic assembly.

In summary, the results of this study indicate that the difference ultraviolet absorbance spectra of synthetic multinuclear Mn complexes in varying oxidation levels and of the different forms of Mn catalase are significantly different than those reported for the photosynthetic Mn. This perhaps suggests that none of the synthetic models has structures and electronic properties sufficiently close to the photosynthetic Mn or the photosynthetic difference spectra are more complicated than currently believed. The results certainly reveal that assignment of the photosynthetic difference spectra to specific Mn oxidation state changes is premature (Renger has also stressed the premature nature of these comparisons but was focussing on ambiguities in the spectra of the native system [49]). Indeed, the energies of features associated with Mn(II) to Mn(III) oxidations in the synthetic complexes' spectra (and in the spectra of Mn catalase) are closer in energy to features assigned to Mn(III) to Mn(IV) oxidations in the photosynthetic system [9] than for features associated with Mn(III) to Mn(IV) oxidations of the synthetic compounds; yet, other spectroscopic studies (X-ray absorption, etc.) clearly indicate that the photosynthetic Mn is being oxidized from Mn(III) to Mn(IV) during these S-state transitions. Clearly at this time, difference ultraviolet spectra alone cannot be used to assign specific Mn oxidation state changes.

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