Deprotonation of Water/Hydroxo Ligands in Clusters Mimicking the Water Oxidizing Complex of PSII and Its Effect on the Vibrational Frequencies of Ligated Carboxylate Groups

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S Supporting Information

ABSTRACT: The IR absorptions of several first-shell carboxylate ligands of the water oxidizing complex (WOC) have been experimentally shown to be unaffected by oxidation state changes in the WOC during its catalytic cycle. Several model clusters that mimic the $\text{Mn}_4\text{O}_5\text{Ca}$ core of the WOC in the S_1 state, with electronic configurations that correspond to both the so-called “high” and “low” oxidation paradigms, were investigated. Deprotonation at W2, W1, or O3 sites was found to strongly reduce carboxylate ligand frequency shifts on oxidation of the metal cluster. The frequency shifts were smallest in neutrally charged clusters where the initial mean Mn oxidation state was +3, with W2 as an hydroxide and O5 a water. Deprotonation also reduced and balanced the oxidation energy of all clusters in successive oxidations.

INTRODUCTION

Photosystem II (PSII) is a protein supercomplex found in all organisms that perform oxygenic photosynthesis. In PSII, light energy catalyzes the oxidation of water into protons and oxygen, which in turn enables the production of ATP and NADPH in the photosynthetic process.¹ The water oxidation reaction occurs at a catalytic site known as the water oxidizing complex (WOC), which is a metallorganic cluster containing a $\text{Mn}_4\text{O}_5\text{Ca}$ core. The WOC cycles through four metastable intermediate states (of increasing mean oxidation level), called S-states, labeled S_i ($i = 0-3$), and one transient state, S_4 . Each S-state is progressively generated by a single electron donation from the WOC, to the photo-oxidized reaction center, P680, via the intermediate electron transfer moiety, tyrosine Z (Y_Z). Oxygen is released in a concerted reaction on the S_4 to S_0 transition, while the dark stable state of the cluster is S_1 . The WOC has been investigated with a number of spectroscopic methods, such as extended X-ray absorption fine structure (EXAFS), X-ray diffraction (XRD), electron paramagnetic resonance (EPR), and Fourier transform infrared spectroscopy (FTIR) to name but a few, but the mechanism of water oxidation at the WOC is still a contentious topic, due to the wide range of possible interpretations of the spectroscopic data. For instance, the interpretation of X-ray spectroscopic data from measurements of the S_3 state is still being vigorously debated, with some researchers suggesting Mn-centered oxidation in the S_2 to S_3 transition, while others favor ligand-centered oxidation.²⁻⁴ However, it is well-established that the

S_0 and S_2 states have odd total Mn electron spin, while S_1 and S_3 are even spin.⁵⁻⁷

In 2011, Shen and co-workers obtained the first XRD structure of PSII capable of resolving the precise structure and ligation of the WOC, with a resolution of 1.9 Å. This structure indicated that the WOC has six direct “first-shell” carboxylate ligands, which are D1-Asp170, D1-Glu333, D1-Asp342, D1-Ala344, D1-Glu189, and CP43-Glu354.^{8,9} The most recent 1.95 Å XRD structure, obtained using femtosecond pulses so as to avoid radiation damage, was largely unchanged from the 1.9 Å structure.¹⁰ These first-shell ligands have been extensively studied using site-directed mutagenesis in combination with FTIR difference spectroscopy. Puzzlingly, mutations to Asp170, Glu333, Asp342, and Glu189 produced no significant changes in any of the S_{n+1} -minus- S_n FTIR difference spectra within the midfrequency region (1000–2000 cm^{-1}), while the Glu354Q mutant produces a very different S_2/S_1 spectrum from the wildtype.¹¹ Mutations to D1-Asp170 and D1-Glu189 do, however, cause pronounced differences in the low-frequency region that correspond to metal–metal and metal–oxo vibrations.^{12,13}

In a previous study of model compounds, we found that the deprotonation of terminal water/hydroxyl ligands during oxidation of a Mn center reduces frequency changes in the

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stretching modes of ligated carboxylates, and also results in a relatively constant oxidation energy (see eq 1) for the Mn(II)/Mn(III) and Mn(III)/Mn(IV) redox pairs.¹⁴ A recent study by Terrett et al. on a binuclear Mn–Ca complex analogous to the Asp170 region of the WOC further confirms those findings.¹⁵ Studies on synthetic carboxylate-bridged Mn dimer complexes by Eilers et al. also demonstrated that carboxylate stretching frequencies remain unchanged when deuterated water ligands are deprotonated.¹⁶

In this study, we examine model clusters based on the 1.9 Å XRD structure of the WOC (see Figure 1), to assess whether

Figure 1. Geometry of the $\text{Mn}_4\text{O}_5\text{Ca}$ cluster in the 1.9 Å XRD structure of the WOC.⁹ The atoms are colored as follows: Mn, purple; Ca, yellow; C, green; O, red; N, light blue. The carboxylate ligands are shown as acetates, while all protons, and water molecules that do not directly bind to manganese centers, are omitted for clarity.

the results of our previous study can be scaled to WOC-like clusters. Scaling to WOC-like clusters is made more complicated by the likelihood of resonances between multiple carboxylate and water bending vibrations, and uncertainty in the hydrogenation of the heavy atoms visible to XRD. We suggest that the deprotonation of water/hydroxyl ligands may be responsible for the absence of oxidation-induced difference features in the carboxylate modes of the WOC's first-shell carboxylate ligands. Our results also show that the frequency shifts that still remain following oxidation with deprotonation are not independent of model geometry or electronic state; the lack of observable experimental FTIR shifts then implies that the effective suppression of oxidation-driven carboxylate frequency shifts through deprotonation may be a requirement for accurate models of the WOC.

METHODS

The $\text{Mn}_4\text{O}_5\text{Ca}$ clusters were constructed on the basis of the 1.9 Å XRD structure by Umena et al.,⁹ with the first-shell carboxylate ligands of the WOC being modeled by formate ligands. Only the core metal centers and the direct ligands to the Mn centers were included, with each model encompassing ~50 atoms. The initial oxidation states of the Mn ions within the clusters were selected to give an average of +3 or +3.5, to investigate both oxidation state schemes proposed for the S_1 state of the WOC.^{17–27} While the lower oxidation scheme has been argued against by Cox and co-workers,²⁸ it is quantitatively consistent with all published high-resolution crystal structures of the S_1 state, including the most recent at 1.95 Å.²⁵ For a further discussion on the validity of either

oxidation state scheme, please refer to refs 19, 25, 26, and 28–31. The nomenclature used in this study to refer to particular manganese and oxygen sites is the same as that used by Umena et al. in describing the 1.9 Å XRD structure of the WOC, and is illustrated in Figure 1.

Geometry optimization and harmonic vibrational frequency calculations were performed in the gas phase, using the M06L/6-31G(d,p) method in Gaussian09.³² All clusters were assigned a high-spin state to ease convergence, and the oxidation states were controlled by specifying the charge and electronic spin multiplicity of the cluster in question. The converged oxidation state of the metal centers and that of ligands was obtained from the calculated Mulliken spin density. We probed all oxidation pathways from the initial dark stable “ S_1 ” state of the cluster for up to three consecutive oxidations, with and without deprotonation (for up to two consecutive deprotonations). Oxidations that result in significant distortions of the geometry were considered unrepresentative of the WOC, and thus were excluded from the results.

The use of formate ligands and the M06L/6-31G(d,p) method were justified by results obtained from our previous study; increasing ligand chain length had very little effect on the response of carboxylate frequencies to oxidation, and M06L produced results similar to those obtained using CCSD, in terms of geometries, frequency shifts, and oxidation energies. Use of the 6-31G(d,p) basis set produced results similar to the larger SDD (Ca,Mn) + 6-31G(d,p) (C,O,N,H) mixed basis set regardless of the method employed.¹⁴ We also note that M06L has a harmonic frequency scaling factor of ~0.99 for most basis sets.³³

The carboxylate symmetric and asymmetric stretching modes were identified by inspection. The formate protons were deuterated to prevent nonphysical coupling of C–H wag vibrations with the carboxylate symmetric stretches.

The oxidation energy for an oxidation step is given by

$$E_{\text{oxidation}} = E_{\text{oxidized}} + nE_{\text{H}} - E_{\text{initial}} \quad (1)$$

where E_{Oxidized} , E_{H} , and E_{Initial} are the energies calculated for the oxidized cluster, the hydrogen atom, and the cluster before oxidation, respectively, and n is the number of protons removed ($n = 0$ or 1). Please note that the oxidation energies are not directly comparable with the physiological system and are meant for relative comparisons within this study only.

Exploratory calculations on one of the clusters used in the current work confirmed that the SDD+6-31G(d,p) mixed basis set produced results nearly identical to 6-31G(d,p), while setting the same cluster to the low-spin state had a negligible effect on the oxidation energies and the frequency differences caused by oxidation. ωB97XD , PBE0-D3, B3LYP-D3, and M11L (using the SDD + 6-31G(d,p) basis set) all produced very similar relative energies and frequency shifts to M06L, for this cluster. These results are provided in the Supporting Information.

RESULTS

The six clusters selected in this study to model a range of initial protonations at O3, O5, and W2 in different metal oxidation states (as proposed by various authors^{21,27,34}) are shown in Figure 2. The protonation and electronic configuration of the clusters in their initial state are described in Table 1; calculations on E and F did not converge when their mean oxidations were set to +3 (with an overall charge of –2). Note that clusters A through D possess a mean oxidation of +3.5

Figure 2. Optimized structures of the model $\text{Mn}_4\text{O}_3\text{Ca}$ clusters investigated in this study, in their initial state. These clusters were obtained by performing geometry optimizations on the $\text{Mn}_4\text{O}_3\text{Ca}$ core of the 1.9 Å XRD structure, with varied protonation and oxidation states. The atoms are colored as follows: Mn, purple; Ca, yellow; C, green; O, red; N, light blue; H, gray. The oxidation state of each Mn center is labeled in roman numerals. See Figure 1 for the corresponding XRD structure.

Table 1. Oxidation State, Formal Charge, Multiplicity, and the Identity of O5, W2, and O3 in Each of the Clusters Investigated in This Study, in Their Initial State

cluster	lowest ox. state	charge	multiplicity (2S + 1)	O5	W2	O3
A	3, 4, 3, 2	0	17	H ₂ O	H ₂ O	O ²⁻
B	3, 3, 3, 3	0	17	H ₂ O	OH ⁻	OH ⁻
C	3, 4, 3, 2	-1	17	OH ⁻	H ₂ O	O ²⁻
D	3, 3, 4, 2	0	17	OH ⁻	H ₂ O	OH ⁻
E	3, 4, 4, 3	0	15	O ²⁻	H ₂ O	O ²⁻
F	3, 4, 4, 3	0	15	O ²⁻	OH ⁻	OH ⁻

after two oxidations, albeit with an overall charge of +2 if directly oxidized. All Mn Mulliken spin densities can be found in the [Supporting Information](#).

These clusters are reduced representations of the S_1 state of the WOC; see Figure 1 for the comparable 1.9 Å XRD structure. In Figure 2, these are arranged according to the protonation state of O5: O5 as H₂O in the first row, OH⁻ in the second row, and O²⁻ in the third row. Clusters A and E are tautomers to B and F, respectively, having a proton from W2 transferred to O3. This follows a suggestion by Gatt et al.,²¹ that a proton transfer involving W2 and O3 could rationalize the difference between the “short” Mn–Mn distances seen in EXAFS (~2.7 Å) and those seen in XRD (~2.8–2.9 Å), both

for the nominal S_1 state. D is not a tautomer of C, but is closely related as it has almost the same protonation pattern as C, except for an additional proton at O3. Setting W2 as OH in D caused the geometry to distort strongly. We found that A, B, and C have relatively long Mn–O5 distances (~2.4–2.5 Å), similar to those observed in the 1.9 Å XRD structure, which are largely retained in the latest 1.95 Å XRD structure.^{8–10}

The models here resemble some of the S_1 state models proposed by various authors, although it must be noted that these were never intended to represent fully mechanistic models of the WOC. A and B are similar to the models presented by Gatt et al., while C and D are reminiscent of the models proposed by Petrie and Shoji, with a hydroxide in the O5 position.^{25,35} Clusters E and F, which have an average Mn oxidation of +3.5 and a fully deprotonated O5 oxo-bridge, resemble the models of Amin, Pantazis, and Siegbahn.^{23,36,37} F in particular has a protonation pattern that is similar to the models found most compatible with EPR/ENDOR and EXAFS spectroscopic properties by Ames and co-workers, as W2 was also modeled as a hydroxide ligand.¹⁹

Nevertheless, the goal of this study is to evaluate the effectiveness of deprotonation in suppressing carboxylate frequency shifts, and how they depend on the geometry and electronic state of the model clusters. It is not to evaluate the accuracy of any of the aforementioned models in the literature, as we make no investigation of water oxidation with these small models.

The relative energy levels of each cluster on oxidation, both direct and with deprotonation, are shown in Figure 3. The manganese oxidation states are also indicated. In all clusters except C, which is negatively charged in its lowest oxidation state, deprotonation lowers the oxidation energy of the cluster. Deprotonation also maintains the oxidation energies of successive oxidations to be within 1 eV of each other, whereas oxidizing the clusters without deprotonation causes rapid increases in the oxidation energy. However, we note that the oxidation energies associated with oxidation with deprotonation are ~3 eV, which is too high compared to the likely potential of P680⁺.^{38–40}

Figures 4 and 5 summarize the gross effect of deprotonation on carboxylate frequency shifts. When the mean Mn oxidation is +3 (Figure 4), the deprotonation of W1 and W2 significantly suppresses frequency shifts in all clusters compared to oxidation without deprotonation, except the negatively charged C whose average shift does not appear to vary, regardless of deprotonation. O3 deprotonation significantly suppresses shifts for B, but is less effective for D, whose shifts with O3 deprotonation are twice those recorded with W1 or W2 deprotonation. O5 deprotonation with oxidation, however, causes large shifts comparable to those caused by direct oxidation.

When the mean Mn oxidation level is +3.5 (Figure 5), the deprotonation of W1 suppresses oxidation-related frequency shifts, with the exception of E. O3 deprotonation, on the other hand, was generally ineffective, except for the neutrally charged F. At this level of mean oxidation, oxidation with W2 deprotonation consistently resulted in Mn1 being oxidized instead of Mn4 (which W2 ligates), and resulted in relatively large frequency shifts. Note that this figure includes data from clusters E and F which have an initial mean Mn oxidation of +3.5, as well as the doubly oxidized clusters A–D.

Tables 2–5 describe the frequency shifts associated with manganese-centered oxidation, from Mn1 to Mn4, respectively.

Figure 3. Oxidations undertaken by each cluster, shown with the Mn oxidation states, deprotonated species, and relative energies. The initial mean Mn oxidation state is +3 for clusters A–D, and +3.5 for clusters E and F. The lower x-axis gives the number of relative oxidations, while the upper x-axis gives the mean Mn oxidation. The oxidation state is given in the order of Mn1–Mn4, while the types of oxidation are differentiated by the color of the lines. Single and repeated oxidation with deprotonation are indicated by blue and red lines, respectively, while direct oxidations are indicated by black lines. The species deprotonated in each step is shown in brackets, in the order in which the deprotonations were undertaken.

Figure 4. Average frequency shifts versus the deprotonated species, for all clusters with an initial mean Mn oxidation of +3.

Figure 5. Average frequency shifts versus the deprotonated species, for all clusters with an initial mean Mn oxidation of +3.5.

Table 2. Frequency Changes Associated with Oxidation of Mn1

	charge (i/f)	final ox. state	final mult (2S + 1)	deprot species	Asp170		Glu354		Glu189		Glu333		Asp342		Ala344	
					sym	asym	sym	asym	sym	asym	sym	asym	sym	asym	sym	asym
A	+2/+3	4, 4, 4, 3	14		-26	+3	-29	-28	-29	-57	-3	-21	-68	+4	-37	-3
	+2/+2	4, 4, 4, 3	14	W2	-32	-10	+3	-34	-32	-36	+5	+5	-58	+25	-13	+1
	+2/+2	4, 4, 4, 3	14	O5	-22	+1	+8	-51	-31	+17	-1	+10	-31	+8	-12	+2
B	0/0	4, 4, 3, 3	15	O3, O5	-10	+1	0	-5	-53	+67	+6	0	-22	-8	-27	-7
	+2/+2	4, 4, 4, 3	14	O3	-12	-3	-13	-17	-31	-49	+6	+1	-58	+28	+12	-6
	+2/+2	4, 4, 4, 3	14	O5	-8, -13	0	+4	-47	-96, -101	+60	0	+7	-39	+3	-29	+8
C	-1/-1	4, 4, 3, 3	15	O5, W2	+10	-5	+7	-15	-16	-9	+2	+7	-8	-19	-7	-2
	+1/+1	4, 4, 4, 3	14	W2	+19	+1	+2	-20	-36	0	+6	-6	-34	-22	-13	-2
	+1/+2	4, 4, 4, 3	14		-25	-5	-17	-31	-49	-6	-13	-20	-38	-35	-30	-8
D	+2/+2	4, 4, 4, 3	14	O3	-10	+19	-25	+3	-13	-11	-9	+8	-43	-30	+18	-39
E	0/+1	4, 4, 4, 3	14		-24	-3	-5	-15	-21	-12	+1	-20	-4	-68	-25	-2
	0/0	4, 4, 4, 3	14	W2	-57	-48	-4	+2	-22	+20	-4	-18	+4	-51	-21	+2
F	0/0	4, 4, 4, 3	14	O3	-8	+2	+4	-3	-37	+6	-3	-2	-3	0	+23	-3
	+1/+2	4, 4, 4, 4	13		-25	-2	-14	-34	-90	+23	-7	-24	-50	-26	-28	-14
	+1/+1	4, 4, 4, 4	13	W1	+2	-11	-9	-15	-64	+26	+9	+2	-25	-16	-12	-10
	+1/+1	4, 4, 4, 4	13	O3	-6	-4	-4	+1	-31	+14	-4	+9	-34	-7	+23	-21

Table 3. Frequency Changes Associated with Oxidation of Mn2

	charge (i/f)	final ox. state	final mult (2S + 1)	deprot species	Asp170		Glu354		Glu189		Glu333		Asp342		Ala344	
					sym	asym	sym	asym	sym	asym	sym	asym	sym	asym	sym	asym
B	0/+1	3, 4, 3, 3	16		-5	+5	+3	-30	-11	-4	-2	-10	+1	-17	-51	+4
	0/0	3, 4, 3, 3	16	O3	-2	-2	-12	-1	-1	+1	-4	-5	-4	0	-12	0
	0/0	3, 4, 3, 4	15	W1, O3	-1	-3	-12	-14	0	+9	-2	-6	-3	-1	-10	+3
D	0/0	3, 4, 4, 2	16	O3	+6	-12	+5	+7	-2	-20	-1	-2	-4	+2	-28	+32
	0/0	3, 4, 4, 3	15	O5, W2	-24	-32	+1	-9	-3	+28	+7	-17	-7	-11	-50	+29
	0/0	3, 4, 4, 3	15	O5, O3	+2	-32	+6	-9	-4	+28	-1	-17	-1	-11	-28	+29
	0/0	3, 4, 4, 3	15	W2, O3	+6	-5	+3	+1	-1	-3	-2	-4	-4	-1	-28	+9
	+1/+2	3, 4, 4, 3	15		-15	-20	+17	-41	-37	+5	+1	-33	+2	+16	-87	+52
	+1/+1	3, 4, 4, 3	15	O3	+10	-3	+13	-23	-3	-2	+1	-3	-4	+21	-38	+21
	+1/+1	3, 4, 4, 3	15	W2	+11	-18	+17	-39	-9	-6	+4	+3	-9	+5	-73	+51
	+1/+1	3, 4, 4, 3	15	O5	-1	-29	+12	-54	+22	-46	+5	-11	-19	-6	-58	+42

Table 4. Frequency Changes Associated with Oxidation of Mn3

	charge (i/f)	final ox. state	final mult (2S + 1)	deprot species	Asp170		Glu354		Glu189		Glu333		Asp342		Ala344	
					sym	asym	sym	asym	sym	asym	sym	asym	sym	asym	sym	asym
A	+1/+2	3, 4, 4, 3	15		-7	-3	-48	+3	+3	+1	-3	-30	-8	-55	-12	-13
	+1/+1	3, 4, 4, 3	15	O5	+6	-5	-15	-15	+1	+43	+2	-7	-10	-13	+3	-7
	+1/+1	3, 4, 4, 3	15	W2	+24	-7	-31	+6	+2	+12	-2	+4	-7	-35	+3	-5
B	0/0	3, 3, 4, 3	16	O5	-25	+6	-22	-14	-25	+77	+1	-4	-3	-2	-12	-18
	0/0	3, 3, 4, 4	15	W1, O5	+4	-4	-27	-46	+5	+17	0	+4	-7	+10	0	0
	+1/+2	3, 4, 4, 3	15		-41	-7	-44	-22	+15	-16	-12	-39	-5	-29	-19	-7
	+1/+1	3, 4, 4, 3	15	O3	-9	-6	-42	-3	+15	-5	-9	-24	-3	-6	+22	-12
	+1/+1	3, 4, 4, 3	15	O5	-36	-2	-27	-36	-22	+72	-2	-10	-11	+7	-36	-7
	0/0	3, 4, 4, 3	15	O5	+15	+41	-8	-38	+15	-40	-6	+4	-9	+4	-7	-2
C	0/0	3, 4, 4, 3	15	W2	+2	+27	-4	-28	-1	+8	+1	-17	-5	+6	-9	0
	0/+1	3, 4, 4, 3	15		+2	-2	-13	-45	0	-9	0	-31	-4	-8	-19	-11

Table 5. Frequency Changes Associated with Oxidation of Mn4

	charge (i/f)	final ox. state	mult (2S + 1)	deprot species	Asp170		Glu354		Glu189		Glu333		Asp342		Ala344	
					sym	asym	sym	asym	sym	asym	sym	asym	sym	asym	sym	asym
A	0/+1	3, 4, 3, 3	16		-41	+15	+8	-36	+4	-58	-12	-49	+1	-3	-10	-16
	0/0	3, 4, 3, 3	16	W2	-20	-10	+5	+1	0	+10	-3	-30	+1	+1	+5	-3
	0/0	3, 4, 3, 3	16	W1	0	-14	+6	-9	-1	-15	0	-22	+1	-10	+1	0
	0/0	3, 4, 3, 3	16	O5	+24	+35	+5	-4	-21	+28	6	-7	-6	-6	-12	-12
	0/0	3, 4, 3, 4	15	W2, W1	-6	-4	+2	-9	+3	-19	-4	-9	+1	-13	0	-1
	0/0	3, 4, 3, 4	15	W1, W2	-26	0	1	+1	4	+6	-7	-17	+1	-2	+4	-4
	0/0	3, 4, 3, 4	15	O5, W1	-17	+4	+9	-4	-3	-8	+1	-13	-5	-3	+2	+7
	0/0	3, 4, 3, 4	15	W1, O5	-73	-35	-1	-5	-17	-2	-10	-27	-6	-6	-13	+19
	+1/+1	3, 4, 3, 4	15	W1	-37	-3	-1	-3	-1	-7	-11	-7	-10	-5	+4	+1
	+2/+2	3, 4, 4, 4	14	W1	-44	-1	-12	-2	0	-10	-8	-8	0	-8	-5	+1
B	0/0	3, 4, 3, 4	16	W1	-1	0	+1	+2	+1	+5	-7	-9	+1	+1	+1	-4
	0/0	3, 4, 3, 4	15	O3, W1	-1	-1	+1	-1	+3	+4	-6	-10	+2	+1	+3	-1
	0/0	3, 3, 4, 4	15	O5, W1	-16	-2	-2	-2, -2	0	-6	-6	-2	+2	-2, -2	+1	-2
	+2/+2	3, 4, 4, 4	14	W1	-8	-11	+9	+9	+4	+3, -3	-4	-2	-10	+3, -3	+11	-5
	-1/-1	3, 4, 3, 3	16	W2	+5	+61	+8	-11	-8	+5	+1	+23	-6	-3	0	+3
C	-1/-1	3, 4, 3, 3	16	O5	-21	+31	+1	+1	-1	+14	+2	-2	-4	-1	-3	+21
	-1/0	3, 4, 3, 3	16		+2	-1	+9	-29	-4	+14	+3	+2	+2	-22	-1	-13
	0/0	3, 4, 3, 4	15	W1	-17	+1	0	0	0	+1	-12	-33	+2	+8	-3	0
	0/0	3, 4, 4, 4	14	W2, W1	-12	+34	+2	-1	-2	-14	+2	-3	+4	0	-2	+2
	+1/+1	3, 4, 4, 4	14	W1	-19	+1	-6	-5	0	-1	-8	-17	+1	-2	-1	-1
	0/+1	3, 3, 4, 3	16		-72	+10	-21	+6	0	-22	-5	-31	+2	-39	-11	-20
	0/0	3, 3, 4, 3	16	W2	-47	-22	0	0	-12	+1	+2	-16	0	0	-1	+1
	0/0	3, 3, 4, 3	16	W1	-16	-11	-1	-4	-4	-6	-9	-3	+1	-3	-2	-7
D	0/0	3, 3, 4, 3	16	O5	-9	+4	-6	-14	+18	-56	-1	-4	-8	-12	-1	-1
	0/0	3, 3, 4, 4	15	W2, W1	-22	+3	-3	-2	+1	-3	-4	-3	+2	-3	+1	-6
	0/0	3, 4, 4, 3	15	O3, W1	-18	-12	+1	-8	-3	-1	-11	-2	+3	-6	-5	-3
	0/0	3, 4, 4, 3	15	O3, W2	-27	-4	-2	-6	-3	+17	+1	-14	0	-4	0	-23
	0/0	3, 3, 4, 4	15	O5, W1	-40	-9	+7	+1	+2	+10	-4	-13	0	0	+4	-1
	+2/+2	3, 4, 4, 4	14	W1	-39	-1	-6	-3	-4	0	-4	-3	0	-4	-1	-2
	+2/+2	3, 4, 4, 4	14	O5	-52	+17	-5	-18	+24	-18	-12	+6	-8	-27	+5	-19
	0/0	3, 4, 4, 4	14	W1	-102	-40	-1	-4	+20	-18	-16	-27	+8	-10	-11	+9
	0/0	4, 4, 4, 4	13	W2, W1	-42	+1	+3	-18	-5	+1	+3	-2	-8	+22	0	+1
	+1/+1	4, 4, 4, 4	13	W1	-102	-30	-2	-22	-2	0	-16	-25	-8	+20	-9	+12
	+1/+2	4, 4, 4, 4	13		-55	+6	-12	-35	-28	-7	-27	-6	-33	+10	-15	-2
F	0/+1	3, 4, 4, 4	14		-68	+11	+1	-16	-33	+5	-13	-26	+6	-11	-10	-3
	0/0	3, 4, 4, 4	14	W1	-36	+3	+1	+4	-8	+5	-1	-3	+3	+2	+6	0
	0/0	4, 4, 4, 4	13	O3, W1	-24	0	-1	+2	-4	-3	+2	-2	-7	-4	+1	-2

The charge column describes the initial and final charge of the clusters; oxidation with deprotonation of a water or hydroxyl ligand conserves the charge, while direct oxidation increases the positive charge on the cluster. The multiplicity is tied to the

oxidation state of the cluster since each Mn center is assumed to be high spin. The oxidation state is written in the order of Mn1, Mn2, Mn3, Mn4. Some carboxylate stretching modes are strongly coupled to each other and cannot be resolved by

inspection, so these ligands would appear to have two frequencies for some vibrational mode. The shifts of these coupled modes are separated by a comma in the tables. The species deprotonated in each step is shown in brackets, in the order in which the deprotonations were undertaken.

Mn1 oxidation (Table 2) causes large, widespread shifts in all clusters. The shift of the Glu189 symmetric mode roughly decreases by a factor of 3 with O3 deprotonation in **B** and **D**. No corresponding decrease in frequency shifts was observed with other deprotonations, however.

Mn2 oxidation (Table 3) has only been observed in clusters **B** and **D**. In cluster **B**, Mn2 oxidation downshifts the symmetric stretch of Ala344, and mildly downshifts the asymmetric stretches of Glu354, Glu333, and Asp342; O3 deprotonation suppresses these shifts, and decreases the maximum shift at Ala344 from -51 to -12 cm^{-1} . In **D**, oxidation with O3 deprotonation suppresses shifts relative to direct oxidation, whereas W2 and O5 deprotonations do not suppress shifts.

Mn3 oxidation (Table 4) also causes widespread frequency shifts which are not strongly affected by deprotonation. O5 deprotonation in **A** reduces the shifts somewhat relative to direct oxidation, but does not have a clear-cut effect on the shifts of **B** or **C**.

Mn4 is the most frequently oxidized manganese center in this study (Table 5). Direct oxidation of Mn4 without deprotonation results in widespread shifts similar to those observed in the oxidation of Mn1 and Mn3. With deprotonation of W2 or W1, however, the shifts are significantly decreased in all clusters except **E**. A comparison between (O5, W1) and (W2, W1) in **A**, **B**, and **D** demonstrates that larger shifts occur if O5 is deprotonated, as (W2, W1) has smaller shifts than (O5, W1) in these clusters. **A** and **B** which have O5 as H_2O generally display smaller shifts than the other clusters in this study, especially when they are neutrally charged.

We also observed that frequency shift suppression via W2 or W1 deprotonation is most effective when the charge of the cluster is zero. **C** is negatively charged in its lowest oxidation state, and has smaller shifts without deprotonation of W2 when oxidized in that negatively charged state, while the shifts in other clusters progressively increase as the charge increases (from neutral charge). The protonation state of W2 also appears to have an effect, as **B** and **F** show smaller frequency shifts than their tautomers **A** and **E**, respectively, following deprotonation.

■ DISCUSSION AND CONCLUSION

Deprotonation of O3 and terminal waters on Mn4 suppresses frequency shifts caused by oxidation, as shown in Figures 4 and 5, provided that the deprotonated species is directly ligated to the oxidized manganese center. Deprotonation also curbs frequency shifts in ligands not directly ligated to the metal center oxidized. For example, in **D**, the direct oxidation of Mn4 caused pervasive frequency shifts, consistent with a change in the conformation of the cluster; the oxidation of Mn4 with deprotonation of W1 or W2, however, suppresses these shifts, leaving only a moderate shift in the Asp170 frequencies. Thus, deprotonation also serves to minimize conformational changes within the cluster. The deprotonation of O5, however, does not reduce shifts and appears to cause additional frequency shifts on its own. This can be attributed to the conformational changes that follow a change in the protonation state of O5,

which is unsurprising as O5 occupies a central position in these clusters.

The factors responsible for variances in the efficacy of frequency shift suppression in these clusters appear to be the Mn–O5 distances, the charge of the cluster, and the protonation state of W2. **A** and **B** have long Mn–O5 distances owing to O5 being fully protonated, and display very small frequency shifts when oxidized with deprotonation in their neutral states. We observed a reduction in the frequency suppression effect as the clusters take on shorter Mn–O5 distances (due to O5 being OH or O) or deviate from neutral charge (i.e., **C**, and all clusters in positively charged states). Frequency shift suppression via deprotonation is also more effective when W2 is OH, since **B** and **F** display smaller shifts than **A** and **E**, respectively, following deprotonation; **A** and **E** also display smaller shifts after W2 is deprotonated [see (W2, W1) in Table 5]. The reason W2 protonation has such an effect is as yet unclear.

In **B**, the shifts are usually less than 10 cm^{-1} when deprotonation has occurred at the center of oxidation (e.g., O3 deprotonation when Mn2 is oxidized, and W1 deprotonation when Mn4 is oxidized); the largest shifts were only 12 cm^{-1} at Glu354 and Ala344 with O3 deprotonation. If we take the frequency shifts of our gas phase clusters to be representative of what might be expected in PSII, then these shifts are small enough that they may be obscured by the minor changes that occur in the protein backbone due to ligand mutation. The Mn4 oxidation-induced shifts at Asp170, in particular, are almost completely nullified by W1 deprotonation. This result indicates that any oxidation-driven frequency shift in Asp170 carboxylate modes would be very difficult to observe in midfrequency FTIR difference spectra, which would explain the experimental results obtained from Asp170 mutants.⁴¹

It is also known that no deprotonation occurs in the S_1/S_2 transition,^{42–44} and a 17 – 36 cm^{-1} downshift has been experimentally assigned to the symmetric mode of the D1 protein C-terminus in that transition, with that mode being at ~ 1356 cm^{-1} in the S_1 state.^{11,45} The calculated frequency of the corresponding ligand in the nominal S_1 state of **B** has a frequency of 1389 cm^{-1} , and it is shifted by -51 and -12 cm^{-1} when **B** is oxidized without deprotonation and with O3 deprotonation, respectively (see Table 3 for the shifts; absolute frequencies are available in the Supporting Information). The experimentally observed shifts appear to fit within that calculated range of frequency shifts (17 – 36 versus 12 – 51 cm^{-1}). It is also notable that the next largest symmetric mode shift in the direct oxidation of **B** was only -11 cm^{-1} for Glu189, which is compatible with the difficulty in assigning FTIR difference features to other first-shell ligands in that transition experimentally.

Deprotonation coupled with oxidation generally reduces the oxidation energy, and maintains it to within 1 eV for successive oxidations (see Figure 3), consistent with our previous findings and also experimental results obtained with synthetic manganese complexes.^{14,46,47} Oxidation with O5 deprotonation appears to be very thermodynamically favorable, and is slightly more so than oxidation with W2 or W1 deprotonation. However, it is important to note that the dielectric and conformational effects of protein ligation have not been accounted for in these gas phase calculations, which will affect these oxidation energy calculations.^{48,49} In particular, the hydrogen bond network that surrounds the WOC could favor

the deprotonation of the terminal waters over the deprotonation of O5, as W1 and W2 are directly linked to this network.^{8,9,11,50} Furthermore, a recent study by Saito and co-workers has shown that a corresponding hydrogen bond network is absent for O5 in the S₁ state, which greatly increases the activation barrier for O5 deprotonation.⁵¹

In 2008, Sproviero and co-workers performed DFT-QM/MM vibrational calculations on their model of the WOC, and found that carboxylate ligand frequencies were generally insensitive to manganese oxidation states, unless a carboxylate was ligated along the Jahn–Teller axis of a Mn(III) center.⁵² Our results are not consistent with those findings, as we found that large frequency shifts can be induced in equatorial carboxylate ligands; Berggren et al. also found that carboxylate stretching shifts are easily observed even in carboxylate ligands that do not ligate a Jahn–Teller axis, in synthetic manganese dimers.⁵³ We conclude that the insensitivity of FTIR S-state difference spectra to carboxylate ligand mutation is due to water/hydroxyl ligand deprotonation at the metal center undergoing oxidation in the WOC, rather than an inherent insensitivity of carboxylate ligand stretching frequencies to manganese oxidation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.5b09987.

Results of exploratory calculations on cluster B, as well as details on the absolute energies in Hartrees, carboxylate stretching frequencies, Mn spin densities, and coordinates of the selected models (PDF)

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Notes

The authors declare no competing financial interest.

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