D1-Ala344 ligation to Calcium in the Oxygen Evolving Complex of Photosystem II is consistent with FTIR Data: A First Principles study.

José A. Gascón, Eduardo M. Sproviero, James P. McEvoy, Gary W. Brudvig, and Victor S. Batista*

Department of Chemistry, Yale University, New Haven, Connecticut, 06520-8107

The oxygen-evolving complex (OEC) of photosystem II (PSII) is a manganese- and calcium-containing cofactor that catalyzes the oxidation of water to dioxygen according to the so-called 'S-state' catalytic cycle proposed by Joliot and Kok.[1,2] Several aspects of the OEC molecular structure have been elucidated by X-ray crystallography and Fourier-transform infrared (FTIR) spectroscopy. However, the results obtained from these two techniques have been judged to be contradictory with regards to the binding of the D1-subunit carboxyl terminus D1-Ala344 which has long been thought to bind to one of the metal ions in the OEC.[3,4] The most recent crystal structures of PSII, obtained at 3.5–3.0 Å resolution, suggest that D1-Ala344 is very close or directly ligated to Ca^{2+} in the OEC.[5,6] In contrast, results from several FTIR studies have been interpreted in terms of unidentate ligation of the C-terminus of D1-Ala344 to a Mn ion but certainly not to calcium.[7-9] It was observed that the frequency of the symmetric stretching mode of the D1-Ala344 carboxylate changes from 1356 cm^{-1}, in the S_1 state, to either 1337 cm^{-1} (a red shift of −19 cm^{-1}) or 1320 cm^{-1} (a red shift of −36 cm^{-1}) in the S_2 state,[9] likely due to a Mn^{3+} to Mn^{4+} transition.[10] The observed frequency shift is unaltered upon substitution of Ca^{2+} by Sr^{2+}, although the ionic radius increases from 0.99 Å to 1.12 Å, suggesting that D1-Ala344 is not directly ligated to calcium.[9] This communication addresses the apparent contradiction between FTIR and X-ray
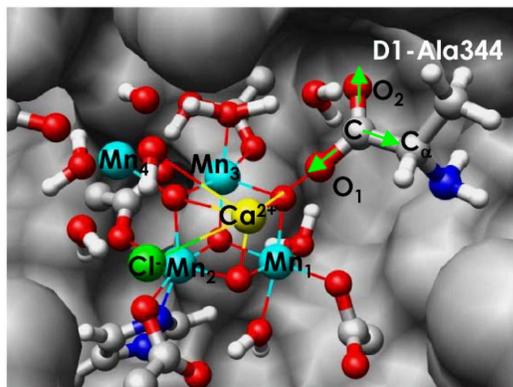

Figure 1: DFT QM/MM structural model of the OEC of PSII. Note that D1-Ala344 is unidentally ligated to Ca^{2+} which is linked to three Mn(1), Mn(2) and Mn(3) via μ-oxo bridges. The symmetric stretching mode of the D1-Ala344 carboxylate is indicated with arrows.

diffraction models, regarding D1-Ala344 ligation to calcium in the OEC, through the analysis of structural and vibrational properties of models of the OEC in the S_1 and S_2 states.

The structural models are constructed by using state-of-the-art quantum mechanics / molecular mechanics (QM/MM) hybrid methods in conjunction with the X-ray crystal structure of PSII from the cyanobacterium *Thermosynechococcus elongates*,[5] as recently described.[11] These density functional theory (DFT) QM/MM hybrid models suggest unidentate ligation of D1-Ala344 to calcium (Fig. 1), in partial agreement with X-ray diffraction models. However, the C-terminus of D1-Ala344 is not ligated to a Mn-ion. Therefore, these models offer a unique opportunity to analyze whether S-state transitions affect the vibrational frequencies of D1-Ala344, as observed in FTIR experiments, even

when such a ligand is not directly coordinated to the oxidized Mn ion. To this end, we have computed the symmetric-stretch vibrational frequency of the D1-Ala344 carboxylate, which is found to change from 1381 cm^{-1} to 1369 cm^{-1}, with a −12 cm^{-1} red shift, upon the S_1 to S_2 oxidation. These results indicate that a red shift of the same order of magnitude as the vibrational frequency shift reported by FTIR studies can be produced simply by the underlying redistribution of charge in the $S_1 \rightarrow S_2$ transition, even when D1-Ala344 is coordinated to Ca^{2+}.

Remarkably, these observations regarding changes in vibrational frequencies of a ligand that is not directly coordinated to the oxidized metal-ion, apply not only to the complete DFT QM/MM models of the OEC of PSII, but also, more generally to reduced models with common structural features. In particular, the simplest possible model (model **I**) involves unidentate
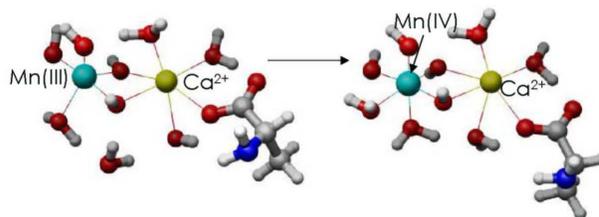

Figure 2: Model (**I**), in which Ala is coordinated to Ca^{2+} in close contact with a hydrated manganese ion undergoing $Mn^{3+} \rightarrow Mn^{4+}$

coordination of the carboxylate group of Ala to calcium in close contact with a hydrated manganese ion that undergoes $Mn^{3+} \rightarrow Mn^{4+}$ oxidation (see Fig. 2). Table 1 shows that the oxidation of manganese, in model **I**, induces a −40 cm^{-1} red-shift

Table 1. Vibrational frequency (in cm^{-1}) of the symmetric stretching mode of the Ala carboxylate in PSII, as described by FTIR (first row), and as computed for the computational structural models described in the text at the DFT (B3LYP/6-31G*) level (rows 2-4). Δ_{BO} is a measure of the change in the bond order for all bonds relevant to the symmetric stretching mode.

| Model | Oxidation State | | Freq. shift | Δ_{BO} |
	Mn^{3+}	Mn^{4+}		
Exp.	1356	1320, 1337	−19, −36	
QM/MM	1381	1369	−12	−0.017
I	1390	1350	−40	−0.024
II	1346	1297	−51	−0.050

in the symmetric-stretch of the Ala carboxylate, even when such a ligand is not directly ligated to the manganese ion. Instead, the Ala carboxylate moiety is monodentally coordinated to Ca^{2+} like in QM/MM structural models of the OEC of PSII. The underling origin of the red-shift is due to a partial injection of negative charge from the Ala carboxylate towards Ca^{2+} to compensate for the increase of positive charge on Mn and its coordination sphere. One effect of this is to decrease the bond order Δ_{BO} (see last column in Table 1) defined as the sum of bond orders for the C-O_1, C-O_2, and C-C_α bonds. These are the bonds that exhibit the largest displacement in the symmetric stretching mode (see arrows in Fig. 1), explaining the decrease in the force constant of this vibration mode.

Further agreement with the FTIR experiments is found by replacing, in model **I**, Ca^{2+} by Sr^{2+}. Such substitution produces only minor changes in the frequency of the symmetric stretching mode of Ala, shifting from 1390 cm⁻¹ to 1391 cm⁻¹ in the Mn^{3+} state and from 1350 cm⁻¹ to 1351 cm⁻¹ in the Mn^{4+} state. These results are consistent with the observation that substitution of Ca^{2+} by Sr^{2+} in PSII produces negligible changes in the S_2-*minus*-S_1 FTIR spectra.[9]

Next, we compare the frequency shift computed for model **I** to the corresponding frequency shift in a structural model where Ala is directly ligated to the Mn-ion. In order to carry out this analysis, we have investigated model **II** where Ala is unidentally ligated to a manganese ion that undergoes Mn^{3+} to Mn^{4+} oxidation (see Fig. 3).
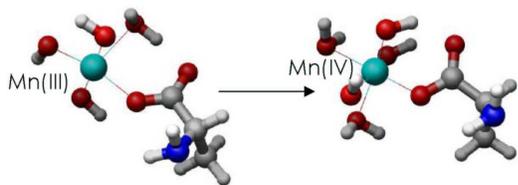

Figure 3: Model (**II**), described in the text, where Ala is coordinated to a hydrated manganese ion undergoing Mn^{3+} to Mn^{4+} oxidation.

Table 1 shows that the oxidation of the manganese ion in model **II** also induces a red-shift in the frequency of the symmetric-stretch of the Ala carboxylate. However, these results suggest that the frequency shift in such a model tends to overestimate the red shift (–51 cm⁻¹ versus –19 cm⁻¹ or –36 cm⁻¹). Therefore, although the observation of a frequency shift of –19 cm⁻¹ or –36 cm⁻¹ is consistent with the ligation schemes suggested by models **I** and **II**, the ligation schemes of model **I** compares more favorably with the FTIR experiments. Considering these results, in conjunction with the vibrational frequency shifts due to the S_1 to S_2 oxidation in the DFT QM/MM structural models, we conclude that the observed FTIR red-shift in the frequency of the carboxylate symmetric-stretch of D1-Ala344 is still consistent with the ligation scheme suggested by X-ray diffraction models, in which D1-Ala344 is very close or directly ligated to calcium.
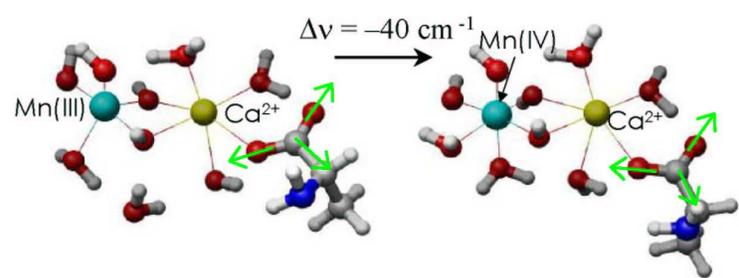
In summary, we have shown that quantum mechanical calculations of vibrational frequency shifts due to oxidation of a high-valent manganese-ion in DFT QM/MM structural models of the PSII's OEC, are consistent with FTIR measurements,[7-9] even when the C-terminus of the D1 polypeptide of PSII is directly ligated to calcium, as suggested by X-ray diffraction models. While the reported calculations do not rule out other possible ligation schemes, such as unidentate ligation of the carboxylate group of alanine to manganese, or the bridging of this group between manganese and calcium, we have shown that a frequency red shift of approximately -36 cm⁻¹, is consistent with the unidentate ligation of the carboxylate group of alanine to Ca^{2+} alone, which is linked in turn to the redox-active Mn ion *via* μ-oxo bridges. Furthermore, we have shown that *in silico* substitution of Ca^{2+} by Sr^{2+} predicts a negligible effect upon the calculated symmetric carboxylate stretching mode of the Ca-bound alanine, in line with experiment. Therefore, these results suggest that D1-Ala344 bound to calcium in the OEC of PSII is consistent with both X-ray diffraction models and FTIR data. The apparent disagreement between FTIR spectroscopy and crystallography on this point could be derived from the intrinsic difficulties associated with the interpretation of the FTIR data.

References

(1)   Joliot, P. J.; Trost, J. T.; Diner, B. A. *Photochemistry and Photobiology* **1969**, *10*, 309-29.

(2)   Kok, B.; Forbush, B.; McGloin, M. *Photochemistry and Photobiology* **1970**, *11*, 457-75.

(3)   Nixon, P. J.; Trost, J. T.; Diner, B. A. *Biochemistry* **1992**, *31*, 10859-71.

(4)   Diner, B. A. *Biochimica Et Biophysica Acta - Bioenergetics* **2001**, *1503*, 147-63.

(5)   Ferreira, K. N.; Iverson, T. M.; Maghlaoui, K.; Barber, J.; Iwata, S. *Science* **2004**, *303*, 1831-38.

(6)   Loll, B.; Kern, J.; Saenger, W.; Zouni, A.; Biesiadka, J. *Nature* **2005**, *438*, 1040-44.

(7)   Chu, H.; Hillier, W.; Debus, R. J. *Biochemistry* **2004**.

(8)   Kimura, Y.; Mizusawa, N.; Yamanari, T.; Ishii, A.; Ono, T. *J. Biol. Chem.* **2005**, *280*, 2078-83.

(9)   Strickler, M. A.; Walker, L. M.; Hillier, W.; Debus, R. J. *Biochemistry* **2005**, *44*, 8571-77.

(10)   Penner-Hahn, J. E. *Struc. Bond.* **1998**, *90*, 1-36.

(11)   Sproviero, E. M.; Gascon, J. A.; McEvoy, J. P.; Brudvig, G. W.; Batista, V. B. *J. Chem. Theor. Comput.* **2006**, *4*, 1119-34.

TOC.