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## Heteronuclear Mn–Ca/Sr complexes, and Ca/Sr EXAFS spectral comparisons with the Oxygen-Evolving Complex of Photosystem II†

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### Abstract

Heterometallic Mn–Ca and Mn–Sr complexes have been prepared and employed as model complexes for Ca and Sr EXAFS spectral comparisons with the Oxygen-Evolving Complex (OEC) of Photosystem II (PS II); these have revealed similarities that support the presence of at least one O atom bridge between the Mn and Ca/Sr in the OEC.

The Oxygen-Evolving Complex (OEC) in Photosystem II (PS II) catalyzes the oxidation of H<sub>2</sub>O to O<sub>2</sub> in green plants, algae and cyanobacteria.<sup>1</sup> This four-electron light-driven process involves various oxidation levels of the OEC (the S<sub>i</sub> states, *i* = 0 to 4).<sup>2</sup> The complex is a tetranuclear, oxo-bridged Mn<sub>4</sub> cluster containing primarily Mn<sup>III</sup> and Mn<sup>IV</sup> ions. In addition, one Ca plays a crucial role in OEC activity; without Ca, the OEC does not advance to the S<sub>3</sub> state. This Ca can be substituted with major retention of activity (~40%) only with Sr, either chemically or biosynthetically.<sup>3</sup>

Several studies in the last few years have shown that Ca is an integral part of the OEC.<sup>1,3–7</sup> Ca/Sr EXAFS (extended X-ray absorption fine structure) studies of the OEC show a Mn···Ca(Sr) distance of 3.4(3.5) Å.<sup>4,5</sup> Recent crystallographic studies at 3.5 Å<sup>6</sup> and 3.0 Å<sup>7</sup> resolution have also detected the presence of Ca in the OEC using anomalous diffraction data. Although there is considerable uncertainty about the Mn<sub>4</sub>Ca structure obtained from crystallography (XRD) due to the current resolution and the radiation damage during X-ray data collection,<sup>8</sup> there is little doubt that the OEC is a heterometallic [Mn<sub>4</sub>CaO<sub>x</sub>] cluster on the basis of XRD<sup>6,7</sup> and EXAFS studies.<sup>4,5,9</sup>

To date, various multinuclear Mn complexes have been synthesized as models of the OEC.<sup>10</sup> In contrast, there have been very few synthetic Mn<sup>III/IV</sup>Ca<sup>11,12</sup> and no Mn<sup>III/IV</sup>Sr mixed-metal complexes. The Mn–Ca(Sr) distances of ~3.4(3.5) Å in the OEC detected by EXAFS studies<sup>4,5</sup> are rather short and are in the range expected for a Ca intimately

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associated with the Mn<sub>4</sub> portion of the OEC.<sup>11</sup> In fact, this short distance strongly suggests that the Ca/Sr atom is bridged to Mn *via* a single atom bridge, probably oxygen. Here we discuss two complexes that contain such single O-atom bridges between Mn<sup>III</sup>/Mn<sup>IV</sup> and Ca/Sr atoms, and present a comparison of their EXAFS spectra with that of the OEC; such model compound studies are important for better interpretation of data on the native system.<sup>5,9</sup> The complexes are the recently reported [Ca<sub>2</sub>Mn<sub>13</sub>O<sub>10</sub>(OH)<sub>2</sub>(OMe)<sub>2</sub>(O<sub>2</sub>CPh)<sub>18</sub>(H<sub>2</sub>O)<sub>4</sub>] (1·10MeCN; Mn<sup>IV</sup>Mn<sup>III</sup><sub>10</sub>Mn<sup>II</sup><sub>2</sub>) (Fig. 1A),<sup>11</sup> and the first MnSr molecular complex [SrMn<sub>14</sub>O<sub>11</sub>(OMe)<sub>3</sub>(O<sub>2</sub>CPh)<sub>18</sub>(MeCN)<sub>2</sub>] (2·12MeCN; Mn<sup>III</sup><sub>13</sub>Mn<sup>II</sup>) (Fig. 1B). The structures of **1** and **2** are similar but not identical, and both complexes contain single O-atom bridges between Mn and Ca(Sr).

Treatment of (NBU<sup>n</sup>)<sub>4</sub>[Mn<sup>III</sup><sub>4</sub>O<sub>2</sub>(O<sub>2</sub>CPh)<sub>9</sub>(H<sub>2</sub>O)] with 1 equiv. of Sr(ClO<sub>4</sub>)<sub>2</sub>·xH<sub>2</sub>O in MeCN–MeOH (10 : 1 v/v) resulted in the subsequent isolation of 2·12MeCN in 55% yield; this procedure is very similar to that used for the synthesis of 1·10MeCN.<sup>11</sup> Complex 2·12MeCN crystallizes<sup>‡</sup> in the triclinic space group *P*1, and its structure consists of a [SrMn<sub>14</sub>O<sub>11</sub>(OMe)<sub>3</sub>]<sup>18+</sup> core whose Mn ions are mixed valent (Mn<sup>III</sup><sub>13</sub>Mn<sup>II</sup>) and bridged by six μ<sub>4</sub>-O<sup>2-</sup>, five μ<sub>3</sub>-O<sup>2-</sup>, two μ<sub>3</sub>-MeO<sup>-</sup>, and one μ<sub>2</sub>-MeO<sup>-</sup> ions. Peripheral ligation is provided by fourteen doubly- and four triplybridging benzoates, as well as two terminal MeCN molecules on the Sr<sup>2+</sup> ion. The core (Fig. 2B) can be described as two [Mn<sub>4</sub>O<sub>3</sub>(OMe)] cubanes attached, *via* oxide bridges, on either side of a central, near linear and planar [Mn<sub>3</sub>O<sub>4</sub>] unit. To the latter are also attached the Mn<sup>II</sup> (Mn8) and Sr<sup>II</sup> atoms above the central unit, and a [Mn<sub>2</sub>O(OMe)] rhombus below it. The core of **2** is thus overall fairly similar to the [Mn<sub>13</sub>Ca<sub>2</sub>O<sub>10</sub>(OH)<sub>2</sub>(OMe)<sub>2</sub>]<sup>18+</sup> core of **1** (Fig. 2A), the main difference being the single Sr<sup>II</sup> atom in **2**. The Ca<sup>2+</sup> atoms in **1** are eight-coordinate with the Ca–O bonds in the range 2.30–3.04 Å. The closest Ca···Mn separation is 3.51 Å to Mn5. The Sr in **2** is also eight-coordinate, with six Sr–O bonds in the range 2.50–2.77 Å and two Sr–N bonds of 2.71 and 2.76 Å, and the closest Sr···Mn separation is 3.29 Å to Mn3.

Mn<sub>4</sub>Ca and Mn<sub>4</sub>Sr structural sub-units present within complexes **1** and **2** have some similarity with the proposed structures of the OEC of PS II.<sup>4,5–7,9</sup> The Mn<sub>3</sub>Ca sub-units (also see Fig. 3) in the center of **1** (Mn1, Mn2, Mn3a, and Ca1) possess a Mn<sub>3</sub>O<sub>4</sub>Ca cubane-like topology as proposed by Ferreira *et al.*<sup>6</sup> Similarly, the distorted tetrahedral sub-unit topologies adopted by Ca1, Mn4, Mn5, Mn7 in complex **1**, and Sr, Mn1, Mn3, Mn4 in complex **2** are also present in one of the most recently proposed structures for the OEC Mn<sub>4</sub>Ca site on the basis of single-crystal EXAFS studies of PS II by Yano *et al.*<sup>9</sup> We therefore decided to carry out analogous Ca and Sr EXAFS studies on **1** and **2** to allow comparisons with the available Ca and Sr EXAFS spectra of the native Ca-containing and Sr-reconstituted OEC. The resulting comparative EXAFS spectra are presented in Fig. 4, and the Fourier transforms (FTs) of the EXAFS spectra are shown in Fig. 5. For all further discussions regarding EXAFS, we shall refer to Fig. 5.

Mn···Ca/Sr and Ca/Sr-ligand Fourier peaks (I and II) observed in the Ca/Sr EXAFS spectra of the model complexes and the OEC are in a similar distance range (Fig. 5). Peak I corresponds to the first shell of Ca/Sr-ligands: these are O for **1**, O and N for **2**, and most probably O and N for the native OEC of PS II and Sr-reconstituted PS II. Peak II is due to the Ca/Sr···Mn interactions. FT peak II from PS II was clearly identified with backscattering

<sup>‡</sup>Vacuum-dried solid analyzed as solvent-free. Elemental analysis (%) calcd. for 2 (C<sub>133</sub>H<sub>105</sub>Mn<sub>14</sub>Sr<sub>1</sub>N<sub>2</sub>O<sub>50</sub>): C, 47.15; H, 3.12; N, 0.83; found: C, 46.92; H, 2.92; N, 0.63%. Crystal structure data for complex 2·12MeCN: SrMn<sub>14</sub>C<sub>157</sub>H<sub>141</sub>N<sub>14</sub>O<sub>50</sub>, *M* = 3880.62, dark brown plates, triclinic, *P*1, *a* = 18.4417(16), *b* = 19.0430(16), *c* = 25.447(2) Å, *α* = 69.789(2), *β* = 103.6350(10), *γ* = 73.792(2)°, *V* = 8001.2(12) Å<sup>3</sup>, 52550 reflections collected; 1750 parameters were refined in the final cycle of refinement using 35042 reflections (*I* > 2σ(*I*)); *R*1 = 0.0896, *wR*2 = 0.2069 (based on *F*<sup>2</sup> and all data). CCDC 283564. Also see CCDC 249919 for complex 1·10MeCN. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b701355h

from Mn in both the Ca and Sr EXAFS because it was absent when the OEC was disrupted by treatment with  $\text{NH}_2\text{OH}$ . Peak II is at a similar position in both the PS II OEC and complexes **1** and **2** (Fig. 5, dashed line), indicating some similarity in the bridging environment around the metal atoms of the model complexes and the biological sites.

Fits of the data for complex **1** improve significantly when FT peak I (Fig. 5A) was fit with two Ca–O distances ( $R = \sim 2.4$  and  $\sim 2.8$  Å), in accord with the X-ray data (see Supplementary Material). One broad FT peak observed at the apparent distance  $R' = 3.0$  Å in the FT peak II region is primarily the mixture of three Ca···Mn distances in the range of 3.51–3.63 Å. For complex **2**, the FT peak I is best fit with  $7 \pm 2$  light atoms (O and N) at  $2.53 \pm 0.02$  and  $2.66 \pm 0.02$  Å, which is in good agreement with the X-ray structure (see Supplementary Material). Although there are three Sr···Mn separations (3.3, 3.7 and 3.8 Å; Fig. 5) in the  $\text{Mn}_{14}\text{Sr}$  structure, the shorter vector (3.3 Å) will be the dominant feature in the FT peak II region due to the weaker EXAFS oscillation of longer absorber–backscatterer interactions ( $>3.5$  Å). These short Mn···Ca/Sr distances are in contrast to the longer values expected for metal pairs bridged only by carboxylate ligands.<sup>10</sup>

The structural sub-units extracted from the cores of the Mn–Ca(Sr) complexes (Fig. 3) all contain a monoatomic O bridge between Mn and Ca/Sr relevant to the proposed OEC structure. In spite of the presence of a monoatomic O bridge, there is a distribution of Mn–Ca(Sr) distances in these complexes in the range of 3.3 to 4.0 Å, depending on the other bridging ligands. In fact, preliminary Ca and Sr EXAFS studies on the OEC of cyanobacteria grown on Ca- or Sr-containing media suggest such a Mn–Ca(Sr) distance heterogeneity in the range of 3.4 to 4.0 Å, as a function of the OEC S-state (unpublished data). It should also be noted that the similar overall structures of the Ca and Sr model complexes **1** and **2** support the fact that the Ca of the OEC can be replaced with Sr without a major perturbation of the OEC structure. However, the reduced activity of the Sr-substituted OEC ( $\sim 40\%$ ) may suggest that the small structural changes are nevertheless sufficient to affect the enzyme activity, possibly through the alteration of bridging interactions with Mn, the hydrogen-bonding network, redox potentials, the  $\text{p}K_a$  of bound water, and even the accessibility of water to the OEC.

This work has provided the first Ca and Sr EXAFS data on mixed-metal model complexes and their comparison with similar data from the OEC. Note that although the overall metal nuclearities of the model complexes are much higher than the OEC, the presence of two well-separated, symmetry-equivalent Ca atoms in **1** and a single Sr atom in **2** permit a ‘single Ca(Sr) site’ analysis. This allows comparisons of their Ca and Sr EXAFS data with those of the corresponding Ca and Sr OEC. The work has provided an important yardstick for assisting in the interpretation of data from the native site.<sup>4,5</sup> Synthetic efforts in progress are providing additional examples of mixed-metal Mn–Ca complexes,<sup>11–13</sup> with different structural topologies, and these are allowing an extension of such comparative studies. Detailed X-ray absorption spectroscopy on these and other complexes synthesized by our group will be reported in a full paper.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

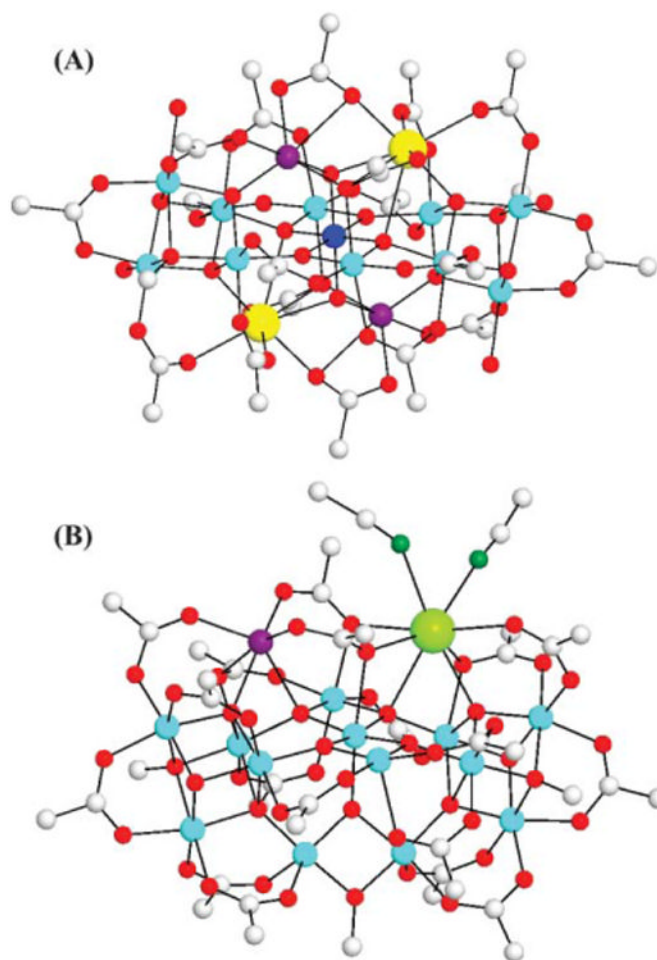
## Acknowledgments

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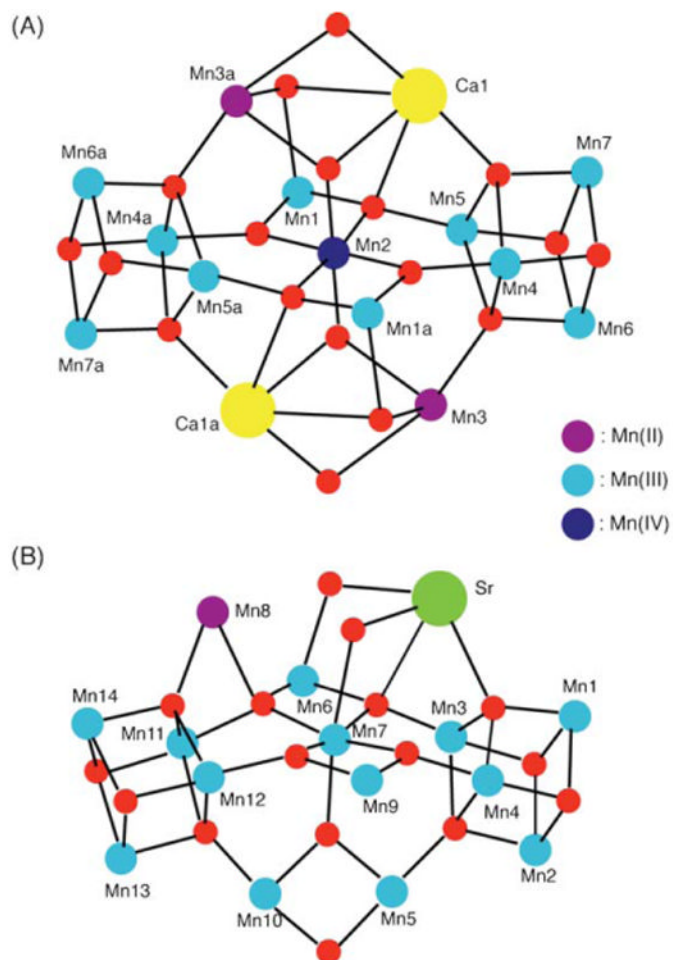
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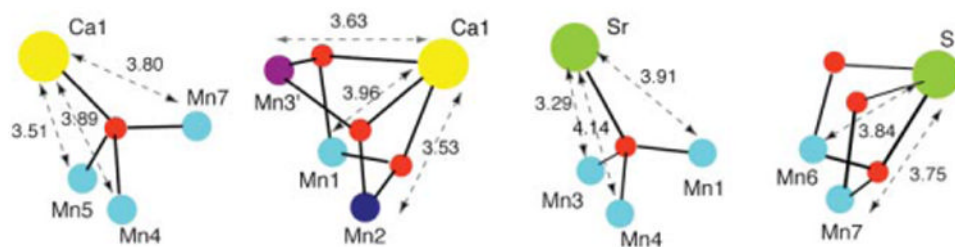
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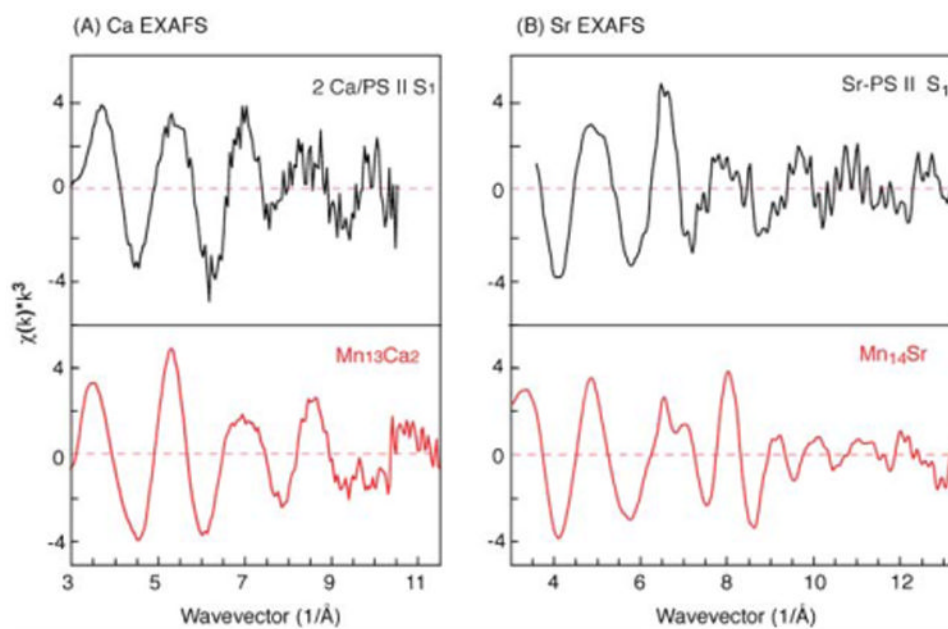
**Fig. 1.** The structures of complexes **1** (A)<sup>11</sup> and **2** (B), with only the *ipso* C atoms of benzoate rings shown for clarity. Color code: blue, Mn<sup>IV</sup>; cyan, Mn<sup>III</sup>; purple, Mn<sup>II</sup>; yellow, Ca; green, Sr; red, O; dark green, N; gray, C. H atoms have been omitted for clarity.



**Fig. 2.** (A) The centrosymmetric core structure of complex **1**. (B) The core structure of complex **2**. Color code: blue, Mn<sup>IV</sup>; cyan, Mn<sup>III</sup>; purple, Mn<sup>II</sup>; yellow, Ca; green, Sr; red, O.

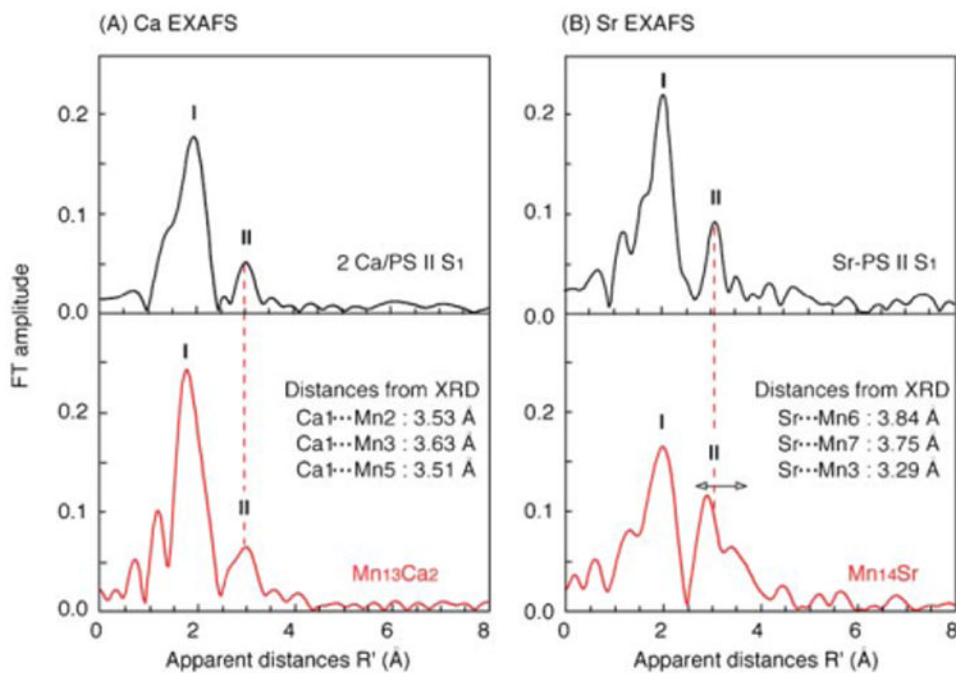


**Fig. 3.**  
Selected Mn–Ca and Mn–Sr sub-units present within the structures of complexes **1** and **2**.



**Fig. 4.** (A) Ca EXAFS spectra of the Mn<sub>13</sub>Ca<sub>2</sub> complex **1** (red) and PS II S<sub>1</sub> state (black),<sup>4</sup> and (B) Sr EXAFS spectra of the Mn<sub>14</sub>Sr complex **2** (red) and Sr-reconstituted PS II S<sub>1</sub> state (black).<sup>5</sup>





**Fig. 5.** (A) Fourier transforms of the Ca EXAFS spectra of the Mn<sub>13</sub>Ca<sub>2</sub> complex **1** (red) and PS II S<sub>1</sub> state (black),<sup>4</sup> and (B) Fourier transforms of the Sr EXAFS spectra of the Mn<sub>14</sub>Sr complex **2** (red) and Sr-reconstituted PS II S<sub>1</sub> state (black).<sup>5</sup> Note that there are two Ca per PS II, including one Ca extraneous to the OEC. Also, note that the apparent distances ( $R'$ ) of the FT peaks are shorter by  $\sim 0.5$  Å compared to the actual distances due to a phase shift induced by the potentials of the absorber–backscatterer pair on the photo-electron. The distances in the inset are from the X-ray crystal structures (XRD).