Understanding Two Different Structures in the Dark Stable State of the Oxygen-Evolving Complex of Photosystem II: Applicability of the Jahn–Teller Deformation Formula


Tanaka et al. (U. Am. Chem. Soc., 2017, 139, 1718) recently reported the three-dimensional (3D) structure of the oxygen evolving complex (OEC) of photosystem II (PSII) by X-ray diffraction (XRD) using extremely low X-ray doses of 0.03 and 0.12 MGy. They observed two different 3D structures of the CaMn$_5$O$_{6}$ cluster with different hydrogen-bonding interactions in the S$_1$ state of OEC keeping the surrounding polypeptide frameworks of PSII the same. Our Jahn–Teller (JT) deformation formula based on large-scale quantum mechanics/molecular mechanics (QM/MM) was applied for these low-dose XRD structures, elucidating important roles of JT effects of the Mn$^{IV}$ ion for subtle geometric distortions of the CaMn$_5$O$_{6}$ cluster in OEC of PSII. The JT deformation formula revealed the similarity between the low-dose XRD and damage-free serial femtosecond X-ray diffraction (SFX) structures of the CaMn$_5$O$_{6}$ cluster in the dark stable state. The extremely low-dose XRD structures were not damaged by X-ray irradiation. Implications of the present results are discussed in relation to recent SFX results and a blue print for the design of artificial photocatalysts for water oxidation.

1. Introduction

A number of experimental studies for oxygen evolving complex (OEC) of photosystem II (PSII) have been performed using several kinds of experimental techniques.[1, 2] Structural parameters of the CaMn$_5$O$_{6}$ cluster included in OEC of PSII have been investigated by the extended X-ray absorption fine structure (EXAFS) [3–10]. On the other hand, X-ray diffraction (XRD) experiments[11–19] play an important role for elucidation of complex, three-dimensional (3D) structures of transition metal-containing enzymes such as OEC of PSII, providing structural bases for successive investigations by spectroscopic methods such as EPR and FTIR.[1] However a critical issue of the XRD for redox-active OEC of PSII is the radiation damage with intense synchrotron radiation as compared with EXAFS.[3–10] In the past six years, serial femtosecond X-ray (SFX) diffraction method, known as “diffraction-before-degradation”, using the X-ray free-electron laser (XFEL)[20–29] have been developed to obtain damage-free XRD structures of redox-active enzymes such as OEC of PSII. On the other hand, low dose XRD experiments have also been desired for suppression of the X-ray damage.[6, 7] Tanaka et al.[30] recently reported the 3D structures of the CaMn$_5$O$_{6}$ cluster in OEC of PSII by XRD using extremely low X-ray doses of 0.03 and 0.12 MGy, for which the external Mn reductions were estimated to be less than 1 and 3.5 (%), respectively.[6, 7, 30] They observed that geometrical structures of the A-monomer were different from those of the B-monomer in the dimer units of both S85E with 0.03 MGy and S86G with 0.12 MGy XRD results even in the S$_1$ state of the Kok cycle,[31, 32] although the surrounding polypeptide frameworks of PSII were the same.[30]

In the past decade we have performed broken-symmetry (BS) hybrid DFT (UB3LYP) calculations[33–36] of the CaMn$_5$O$_{6}$ cluster in OEC of PSII starting from the 3D XRD (0.43 MG) structure[19] for theoretical investigation of geometrical, electronic and spin structures of OEC of PSII. The UB3LYP calculations...
were performed for total 48 (−8×6) valence configurations obtained by 8 spin states for 6 mixed valence structures (see SV)\cite{37,38,39}. The energy diagrams for all the configurations elucidated that the ground valence configuration of the cluster in the dark stable S1 state was the CaMn44Mn55Mn33Mn22Mn11Mn00 structure. This was abbreviated as (3443). Moreover the DFT calculations elucidated that the nature of the Mn4−O5−−Mn bond of the cluster was labile\cite{40,41} indicating structural symmetry breaking (SSB)\cite{35,36} because of the Jahn–Teller (JT) effects of the Mn4+44 ion. Full geometry optimizations of OEC of PSII by large-scale QM/MM methods\cite{37,38} indeed elucidated four different topological structures based on the JT effects as illustrated in the Supporting Information Figure S1 (see SI 1). An estimation formula\cite{42} of the JT deformations of the cluster also emerged on the basis of a number of the optimized geometries by QM and QM/MM\cite{33–39}–structures together with available experimental geometrical parameters of manganese oxides clusters. In this paper, we apply our JT deformation formula for two different structures by the low-dose XRD experiments of Tanaka et al.,\cite{43} proposing a unified view of the EXAFS\cite{44–46,47} XRD\cite{11–16,47–49} and XFEL\cite{47–49} structures and theoretical models\cite{33–44,46} of the S1 state of OEC of PSII. Implication of present results is discussed in relation to recent SFX results and a blue print for the design of artificial photo-catalysts using abundant 3d-transition metals.

2. Theoretical Background

2.1. Structural Symmetry Breaking of the CaMn4O5 Cluster in the OEC of PSII

The high-resolution XRD\cite{18} experiments first elucidated the 3D structure of the CaMn4O5 cluster with almost central (C) conformation, as illustrated in Figure S1. Our QM and QM/MM computations\cite{33–39} revealed slightly right (C4) and left (C4)-elongated quasi-central structures as well as right (R) and left (L)-openened structures of the CaMn4O5 cluster of OEC (see Figure S1). The structural symmetry breaking (SSB) parameter defined by the distances of the Mn4−O5−−Mn4 and Mn4−O5−−Mn3 bonds is as follows (Eq. (1))\cite{35–39}:

$$\delta = \left| R(\text{Mn}_{4}) - O(5) \right| - \left| R(\text{Mn}_{4}) - O(5) \right| / 2 \tag{1}$$

The \(\delta\) value was 0.05 Å for the 3ARC XRD central (C) structure\cite{18} of the CaMn4O5 cluster. The \(\delta\) values were 0.12 and 0.16 (Å) for A- and B-monomers of 3WU2\cite{18} namely refined 3ARC structure, indicating the C4 structure. The \(\delta\) values for 4UB6A, 4UB86, 4UB8A and 4UB8B by the damage-free XFEL method\cite{27} were 0.20, 0.23, 0.15 and 0.20 (Å) respectively, exhibiting the C4 structure in Figure S1. Thus the SSB parameters for 3WU2, 4UB6 and 4UB8B are smaller than 0.25 Å.

The SSB (\(\delta\)) parameter was 0.71 Å for the full-optimized low-spin (LS) S4 structure of the CaMn4O5 cluster by large-scale QM/MM method\cite{28,30}–structure on the assumption that the O5− site was oxygen dianion (O2−), indicating the R-structure in Figure S1. The optimized S4 structures obtained by other and our groups under the same assumption of O5−=O2− were also the R-structure.\cite{28,30}–The large \(\delta\) value (> 0.5 Å) obtained by the theoretical calculations is one of the reasons for the claim\cite{44,46} that the XFEL structure with small \(\delta\)-value (< 0.25 Å)\cite{33} might be the S4 structure induced by the radical addition to the CaMn4O5 cluster. On the other hand, we have shown that the C4 structure for the S1 state can be reproduced under the assumption of the protonation of the O5− site, namely O5−=OH−\cite{35–36} and/or the rotation of the JT deformation axis (dR−dL) by O5−=O2−\cite{39}. Therefore, theoretical analysis of the damage-free low dose XRD structure\cite{46} is very important for elucidation of the most plausible S1 structure and scope and reliability of the XFEL\cite{27} and SFX structures.\cite{28,29}

2.2. Theoretical Modeling of Structural Symmetry Breaking in the CaMn4O5 Cluster

Our QM and QM/MM calculations\cite{33–39} of OEC in PSII revealed that the Mn4−Mn3−−O5− distance was correlated with Mn4−O5−−Mn3− distance in the CaMn4O5 cluster of OEC of PSII. We have already presented a practical estimation equation\cite{33–39} of the Mn4−Mn3−−O5− distance by the use of the Mn4−O5−−Mn3− distance (see Figure S2 in SI 1) as follows (Eq. (2)):

$$R(\text{Mn}_{4}−\text{Mn}_{3}) = 2.80 + x / 2n \tag{2}$$

where the deformation parameter \(x\) is defined by (Eqs. (3a) and (3b)):

$$R(\text{Mn}_{4}−\text{O}_{5}) = 2.18 + x \tag{3a}$$

$$R(\text{Mn}_{4}−\text{O}_{5}) = 2.88−x \text{ (Å unit).} \tag{3b}$$

The \(n\) values were taken to be 1 for O5−=OH− and 2 for O5−=O2− respectively, depending on the strength of the Mn4−O5− bond (see Figure S2 in the Supporting Information). The \(x\)-value was determined using the calculated Mn4−O5−−Mn3− distance, R(Mn4−O−5−−Mn3−), by QM and QM/MM methods. The SSB parameter was defined by Equation (1). The \(x\) value and Mn4−O5−−Mn3− distance were in turn estimated using R(Mn4−Mn3−−O5−) values by the EXAFS\cite{18,27} XRD\cite{18,39} XFEL\cite{27–29}– and computational methods\cite{33–47}.

The Mn4−O5−−Mn3− distances were estimated to be 2.00 and 1.82 (Å) for O5−=OH− and O5−=O2− respectively, assuming the short Mn4−Mn3− distance (2.71 Å by EXAFS\cite{18}) of the CaMn4O5 cluster (see Eq. (3a)). The optimized Mn4−O5−−Mn3− distance by low-spin QM/MM under the assumption of O5−=O2−\cite{27,38,39}–was equivalent to the latter value (1.82 Å), confirming the reliability of Equations (2) and (3a) for estimation based on the QM/MM results. The optimized Mn4−Mn3−−O5− distances obtained by QM calculations by ours and other groups\cite{33–39}–also provided 1.8–1.9 (Å) for the Mn4−O5−−Mn3− distance in accord with the assumption of O5−=O2− as shown in Figure 1 A. These short Mn4−O5−−Mn3− distances in turn were considered to support the assumption of O5−=O2− in the geometry optimizations by QM since the Mn4−Mn3−−O5− distance of EXAFS by Glöckner et al.\cite{52} was about 2.7 Å (see Table S6). However, the observed Mn−O distances of the CaMn4O5 cluster by the EXAFS\cite{33–47}–are classified into two groups with about 1.8 and 2.0 (Å), respectively, indicating the
difficulty for discrimination between R(Mn_{44u}=O_{43b}) ≈ 1.8 Å and R(Mn_{44u}=Mn_{43b}) ≈ 2.7 Å for O_{43b} = O^{2−} and R(Mn_{44u}=O_{43b}) ≈ 2.0 Å and R(Mn_{44u}=Mn_{43b}) ≈ 2.7 Å for O_{43b} = O^{−}. Therefore, precise determination of R(Mn_{44u}=O_{43b}) by other experimental methods such as the low dose XRD[35,36] is first crucial for discrimination between O_{43b} = O^{2−} and O_{43b} = O^{−} in the CaMn_{43}O_{5} cluster of OEC of PSII to elucidate the possibility of the X-ray damage of the XFEL[29] and SFX structures[28,29]. Furthermore, it is noteworthy that discrimination between O^{−} and O^{2−} at the O_{43b} site in the S_{3} state is not at all trivial because possible mechanisms for water oxidation may be different by protonation of the site.

2.3. Jahn–Teller Effect of the Mn^{III} Ion

The high-resolution XRD[18] experiment revealed that ligand fields of Mn ions are essentially octahedral in the CaMn_{43}O_{5} cluster in OEC of PSII[1,2]. The first QM computation[33,34] of the S_{3} structure of the CaMn_{43}O_{5} cluster by 3WU2[18] elucidated the (3443) valence configuration of the CaMn_{43}O_{5} cluster as mentioned above. Therefore, the Jahn–Teller (JT) effect of the Mn^{III}_{44u} ion plays important roles for subtle deformations of the CaMn_{43}O_{5} cluster[37–39]. The JT elongation axis responsible for the d_{y^2} orbital was vertical (v) to (W_{1})=−O^{−}−Mn^{III}_{44u}−O_{43b} bond for the short Mn_{44u}=Mn_{43b} distance (2.7 Å) as shown in Figure 1A, suggesting the Mn_{44u}=O_{43b} distance with about 2.0 Å for O_{43b} = O^{−} or about 1.8 Å for O_{43b} = O^{2−}. The intermediate Mn_{44u}=Mn_{43b} distance (2.75 Å) provided the Mn_{44u}=O_{43b} distances with about 2.1 and 2.0 (Å) for O_{43b} = O^{−} and O_{43b} = O^{2−}, respectively, because the JT deformation was the d_{y^2} type, as shown in Figure 1B. On the other hand, the JT axis for the d_{x^2} orbital becomes almost parallel to the (W_{1})=−O^{−}−Mn^{III}_{44u}−O_{43b} bond, namely horizontal (h), as shown in Figure 1C. In this case the Mn_{44u}=Mn_{43b} distance is 2.80 Å for which the Mn_{44u}=O_{43b} distances are about 2.2 Å for both O_{43b} = O^{−} and O_{43b} = O^{2−} (see Figure S2 in SI.1). The horizontal JT (d_{x^2}) distortion is also operative for R(Mn_{44u}=Mn_{43b}) = 2.85 Å, although the Mn_{44u}=O_{43b} distances are about 2.3 and 2.4 (Å) for O_{43b} = O^{−} and O_{43b} = O^{2−}, respectively.

The estimation formula (2) and (3) are not effective for discrimination between O_{43b} = O^{−} and O_{43b} = O^{2−}[37–39] near the crossing region, R(Mn_{44u}=O_{43b}) = 2.2 Å in Figure S2 (see Tables 1 and 5). The Mn_{43b}=O_{43b} distance can be employed as second JT deformation index in the region. Thus, the orbital degree of freedom at the Mn_{43b} site is one of the important factors for subtle geometrical deformation of the CaMn_{43}O_{5} cluster that is regarded as a characteristic property of strongly correlated electron system (SCES)[35,39,45].

3. Theoretical Studies on the Low-Dose XRD Structures of the CaMn_{43}O_{5} Cluster

3.1. Structural Symmetry Breaking of the Low-Dose XRD Structures

After the discovery[18] of the high resolution XRD structure of the CaMn_{43}O_{5} cluster in OEC of PSII, X-ray damage[26,27] of the

| Table 1. The Mn−Mn distances (Å) of the CaMn_{43}O_{5} cluster in the S_{3} state of OEC of PSII by the low-dose (LD) XRD[35] and the estimation procedure [Eqs. (2) and (3)]. |
|---|---|---|---|---|---|---|
| Structures | Mn_{3}−Mn_{5} | Mn_{4}−Mn_{5} | Mn_{1}−O_{43} | Mn_{1}−O_{43} | O_{43} | SSB |
| SB5EA | 2.82 | (2.82)[a] | 2.24 | 2.09 | O_{43} = O^{−} | 0.29 |
| SB5EB | 2.82 | (2.82)[a] | 2.24 | 2.09 | O_{43} = O^{−} | 0.29 |
| SB66A | 2.75 | (2.79)[a] | 2.17 | 2.07 | O_{43} = O^{−} | 0.36 |
| SB66B | 2.75 | (2.79)[a] | 2.17 | 2.07 | O_{43} = O^{−} | 0.36 |
| [a] The Mn_{3}−Mn_{5} distances were estimated by using the experimental Mn_{44u}=O_{43b} distance in Equation (2) and (3) under the assumption of (a1) O_{43} = O^{−} and (a2) O^{−}. | [a] The Mn_{4}−Mn_{5} distances were estimated by using the experimental Mn_{44u}=Mn_{43b} distance in Equation (2) and (3) under the assumption of (b1) O_{43} = O^{−} and (b2) O^{−}. | [c] The Mn_{1}−O_{43} distances were estimated to be 2.0 and 1.8 for (c1) O_{43} = O^{−} and (c2) O^{−}, respectively. | [d] Assignment of the O_{43} site. | [e] Structural symmetry breaking (SSB) parameter. | [f] Topology. |
high-valent Mn^{IV} ions under the high dose conditions such as 0.43 MGy\textsuperscript{18} was pointed out by several groups.\textsuperscript{40–47} Tanaka et al.\textsuperscript{30} recently performed the XRD experiments using low-doses of 0.03 (SB5E(A)) and 0.12 MGy (SB66A(B)) for OEC of PSII. Therefore, the S_{1} structure by their XRD experiments is considered to be almost X-ray-damage free (1–3\%). Table 1 summarizes the observed and calculated Mn\textsubscript{4A}–Mn\textsubscript{3bA} and Mn\textsubscript{4A}–O\textsubscript{5B} distances and SSB parameters for the low dose XRD structures.\textsuperscript{30} From Table 1, the \( \delta \)-values were 0.29 and 0.25 (Å) respectively, for A-monomers of SB5E and SB66,\textsuperscript{30} exhibiting the C\textsubscript{S} structure in our terminology (see Figure S1).\textsuperscript{35–39} On the other hand, the \( \delta \)-values were 0.36 and 0.41 for SB5E and SB66 respectively, showing the R-opened (R) structure near C\textsubscript{S}. The SSB for the B-monomers were a little larger than those of the A-monomers in the low dose XRD structure.\textsuperscript{30} However, the \( \delta \)-values of the B-monomers were only one half of the optimized value (about 0.7) of the CaMn\textsubscript{5}O\textsubscript{3} cluster by QM and QM/MM under the assumptions of O\textsubscript{5B}=:O\textsuperscript{2−} and the vertical JT (d\textsubscript{yz}) distortion (Figure 1A).\textsuperscript{28,34} Thus, the \( \delta \)-values of the low dose XRD structures\textsuperscript{30} are rather consistent with those of the damage-free XFEL structures\textsuperscript{27} in contradiction to the claim based on the R-structure (Figure 1A).\textsuperscript{28,46}

The SB5E structure by low dose XRD in Figure 2A(C) indicated that the Mn\textsubscript{40b}–Mn\textsubscript{4A}, Mn\textsubscript{26A}–Mn\textsubscript{3bA}, Mn\textsubscript{16d}–Mn\textsubscript{26A}, Mn\textsubscript{16d}–Mn\textsubscript{3bA} and Mn\textsubscript{16d}–Mn\textsubscript{4A} distances were 2.82(2.85), 2.76(2.78), 2.74(2.72), 3.22(3.22) and 4.91(4.92) (Å) respectively, where the corresponding distances for SB66A structure\textsuperscript{30} (see also Figure 1C) are given in parentheses. The observed Mn–Mn distances for A monomers indicated the following trend (Eq. (4a)):

\[
R(\text{Mn}_{1}–\text{Mn}_{2}) < R(\text{Mn}_{2}–\text{Mn}_{3}) < R(\text{Mn}_{3}–\text{Mn}_{4}) < R(\text{Mn}_{1}–\text{Mn}_{3}) \quad \text{<} \quad R(\text{Mn}_{1}–\text{Mn}_{4}).
\] (4a)

The trend, R(\text{Mn}_{1}–\text{Mn}_{2}) < R(\text{Mn}_{2}–\text{Mn}_{3}) was also observed for 3WU2\textsuperscript{18} and XFEL\textsuperscript{27} structures.

On the other hand, the corresponding Mn–Mn distances for 3WU2 were 2.75(2.77), 2.77(2.82), 2.65(2.72), 3.22(3.24) and 4.88(4.89) (Å) respectively, for the SB5E (SB66B) structures as shown in Figure 2B(D), showing a different trend [Eq. (4b)]:

\[
R(\text{Mn}_{1}–\text{Mn}_{2}) < R(\text{Mn}_{2}–\text{Mn}_{3}) < R(\text{Mn}_{3}–\text{Mn}_{4}) < R(\text{Mn}_{1}–\text{Mn}_{3}) < R(\text{Mn}_{1}–\text{Mn}_{4}).
\] (4b)

The reverse trend, R(\text{Mn}_{1}–\text{Mn}_{2}) < R(\text{Mn}_{2}–\text{Mn}_{3}), was also observed for the EXAFS structure reported by Yano and co-workers.\textsuperscript{8,10} The average Mn–Mn distances of Mn\textsubscript{40b}–Mn\textsubscript{4A}, Mn\textsubscript{26A}–Mn\textsubscript{3bA} and Mn\textsubscript{16d}–Mn\textsubscript{26A} are 2.77, 2.78, 2.72 and 2.77 (Å) respectively for SB5E, SB66A, SB5E and SB66B\textsuperscript{30} in agreement with the average Mn–Mn distances revealed by the damage-free XFEL,\textsuperscript{27} namely 2.72 Å for 4UB6, and 2.78 Å for 4UB8, and 2.73 Å for EXAFS.\textsuperscript{8,10} Thus, there is no serious differences (namely within the experimental uncertainty) of the average Mn–Mn distance among the low dose XRD,\textsuperscript{30} XFEL\textsuperscript{27} and EXAFS\textsuperscript{18} structures. On the other hand, the corresponding average Mn–Mn distances were 2.91 and 2.86 (Å) for 3WU2A and 3WU2B,\textsuperscript{18} respectively, indicating non-negligible elongations (0.1–0.2 Å) because of the X-ray damage.\textsuperscript{27,30,45} However, the topological structure of 3WU2 [see Eq. (4a)] is similar to

![Figure 2. Three dimensional (3D) structures and Mn–Mn and Ca–Mn distances of the CaMn\textsubscript{5}O\textsubscript{3} cluster in oxygen evolving complex (OEC) determined by an extremely low dose XRD experiment by Tanaka et al.\textsuperscript{30} A) SB5EA, B) SB5EB, C) SB66A and D) SB66B.](image-url)

\[\text{ChemPhotoChem} \ 2018, \ 2, \ 257 – 270\]
that of XFEL \cite{27} namely 5% reduction of the Mn–Mn distances of 3WU\cite{14} is necessary for production of the XFEL structure.\cite{27}

The Ca–Mn$_{a}$ distances were 3.75(3.77), 3.39(3.40), 3.35(3.34) and 3.50(3.51) (Å) respectively for SBSE(A) and SBSE(B). The corresponding Ca–Mn distances were 3.78(3.74), 3.40(3.39), 3.29(3.30) and 3.51(3.48) respectively for SBSE(A) and SBSE(B). The Ca–Mn distances were not so different between A- and B-monomers of the dimer structure of OEC of PSII in both samples, indicating a general tendency referred to as the rule \cite{15,16,17} for the XRD\cite{18} and XFEL\cite{19} structures (Eq. (5)).

\[
R(Ca–Mn_{a}) < R(Ca–Mn_{b}) < R(Ca–Mn_{a}) < R(Ca–Mn_{a}) \tag{5}
\]

The divalent Ca$^{2+}$ ion is therefore irrelevant to the X-ray damage.

### 3.2. Application of the Jahn–Teller Deformation Formula to the CaMn$_{a}$O$_{5}$ Cluster

The JT deformation formula [see Eqs. (2) and (3)] were applied to the low dose XRD structures by Tanaka et al.\cite{20} which elucidated subtle different structures between A- and B-monomers. The observed Mn$_{a}$–O$_{5}$ distances for SBSE(A) were 2.24(2.28) (Å) respectively, indicating that the Mn$_{a}$–O$_{a}$ distance was estimated to be 2.83(2.85) (Å) for O$_{3b}$=OH$^{-}$ and 2.82(2.83) (Å) for O$_{OH}$=O$^{2-}$ respectively, in accord with the parallel JT ($d_{z}$) elongation illustrated in Figure 1C. On the other hand, the Mn$_{a}$–O$_{a}$ distances were estimated to be 2.22(2.28) (Å) for O$_{3b}$=OH$^{-}$ and 2.26(2.38) (Å) for O$_{OH}$=O$^{2-}$ respectively, using the Mn$_{a}$–O$_{a}$ distances, namely 2.82 (2.85) (Å) for SBSE(A) and 2.83(2.85) (Å) for SBSE(B). The Equations (2) and (3) using the Mn$_{a}$–O$_{a}$ and Mn$_{a}$–O$_{a}$ distances were not conclusive for discrimination between O$_{3b}$=OH$^{-}$ and O$_{OH}$=O$^{2-}$ (except for SBSE(A) for which O$_{3b}$=OH$^{-}$) near the crossing region of the JT deformation (see Figure S2 in SI). Therefore, the second criterion, namely Mn$_{a}$–O$_{a}$ distance, was employed for the discrimination.\cite{21,22,23,24} As shown in Table 2, the observed Mn$_{a}$–O$_{a}$ distances by the LD XRD were 2.09 and 2.14 (Å) for SBSE(A) and SBSE(B) respectively. These values are rather consistent with the assumption of O$_{3b}$=OH$^{-}$ in Table S1, supporting the S$_{I}$ structure by XFEL\cite{25} and structure of A monomer of LD XRD.\cite{26}

The JT deformation formula were also applied to the low dose XRD structures of the B-bonomer.\cite{27} The observed Mn$_{a}$–O$_{a}$ distances for SBSE(B) were 2.17(2.12) (Å), providing that the estimated Mn$_{a}$–O$_{a}$ distances were 2.79(2.77) (Å) for O$_{3b}$=OH$^{-}$ and 2.80(2.78) (Å) for O$_{OH}$=O$^{2-}$ respectively. On the other hand, the Mn$_{a}$–O$_{a}$ distances for SBSE(B) were estimated to be 2.08(2.12) (Å) for O$_{OH}$=O$^{2-}$ and 1.98(2.06) (Å) for O$_{OH}$=O$^{2-}$ respectively, using the observed Mn$_{a}$–O$_{a}$ distance, namely 2.75 (2.77) (Å) for SBSE(B). Interestingly, the Mn$_{a}$–O$_{a}$ distance estimated using the Mn$_{a}$–O$_{a}$ distance (2.75 Å) of SBSE(B) was about 2.1 Å in agreement with the assumption of O$_{3b}$=OH$^{-}$ in Table S1. The observed Mn$_{a}$–O$_{a}$ distance for SBSE(B) was 2.07 Å, further supporting the assumption of O$_{3b}$=OH$^{-}$. The protonation of the O$_{3b}$ site is also consistent with the Mn$_{a}$–O$_{a}$ distance (2.12) for SBSE(B). Thus the JT distortion for the B-bonomer was consistent with the JT ($d_{z}$) deformation in Figure 1B. Interestingly, the longer Mn$_{a}$–O$_{a}$ distances of the B-bonomers are rather consistent with the longer Mn$_{a}$–O$_{a}$ distance (about 2.0 Å) by EXAFS.\cite{28} This indicates the d$_{z}$=JT-type B-structure (see Figure 1B) for the EXAFS results\cite{21,22} as shown in Table S6 (see the Supporting Information).

In order to confirm the above assignments, the Mn–O distances of the octahedral ligand fields for the Mn$_{a}$O$_{5}$ ion were depicted in Figure 3. The observed Mn$_{a}$–O$_{a}$ and Mn$_{a}$–O(W1) distances for SBSE(A) were 2.24(2.28) and 2.19(2.19) (Å) respectively, as shown in Figure 3 A(D), indicating the parallel JT elongation ($d_{z}$) illustrated in Figure 1C. The observed Mn$_{a}$–O$_{a}$ and Mn$_{a}$–O(W1) distances for SBSE(B) were 2.17(2.12) and 2.10(2.10) (Å) respectively, as shown in Figure 3 B(E), showing the shortening of 0.07(0.16) and 0.09(0.09) as compared with those of SBSE(A) and SBSE(B). On the other hand,

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<th>Table 2. The Mn$<em>{a}$–O$</em>{a}$ and Mn$<em>{a}$–O$</em>{a}$ distances (Å) of the CaMn$<em>{a}$O$</em>{5}$ cluster in the S$<em>{I}$ state of OEC of PSII based on the estimation procedure using the Mn$</em>{a}$–Mn$<em>{a}$ distance (Å) obtained by the mixing of the S$</em>{I}$ (C$<em>{I}$) and S$</em>{I}$ (C$_{I}$) structures (Eq. (7)).</th>
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</tbody>
</table>

[a] The geometrical parameters are given by the mixing of the S$_{I}$ (C$_{I}$) and S$_{I}$ (C$_{I}$) structures. [b] The mixing ratio $\alpha$(C$_{I}$) for the C$_{I}$ structure. [c] The Mn$_{a}$–O$_{a}$ distance for the (1–$\alpha$) S$_{I}$ (C$_{I}$) + $\alpha$ S$_{I}$ (C$_{I}$) structure. [d] The Mn$_{a}$–O$_{a}$ distance for the mixed (1–$\alpha$) S$_{I}$ (C$_{I}$) + $\alpha$ S$_{I}$ (C$_{I}$) structure. [e] Structural symmetry breaking (SSB) parameter. [f] The right-opened structure (R).
the Mn\textsubscript{st}–O\textsubscript{w} and Mn\textsubscript{st}–O(W2) distances for 5B5EA(5B66A) were 1.87(1.84) and 2.04(2.13) Å, respectively, as shown in Figure 3(A)(D). The corresponding values for 5B5EB(5B66B) are 2.07(2.10) and 2.17(2.15) Å as shown in Figure 3(B)(E), indicating the elongations of 0.20(0.26) and 0.13(0.02) Å, respectively, as shown in Figure 3(C)(F) in accord with the JT (d\textsubscript{J}) deformation. Therefore, 5B5EB(5B66B) are regarded as a JT (d\textsubscript{J}) deformed structure in Figure 1B, also suggesting that the EXAFS structure\textsuperscript{[8,10]} with the different topology [see Eq. (4b)] may be explained with the B-structure by the LD XRD. The LD XRD structures with no significant X-ray damage\textsuperscript{[30]} were not consistent with the R-structure with the JT (d\textsubscript{J}) distortion in Figure 1A, where the Mn\textsubscript{st}–Mn\textsubscript{st} and Mn\textsubscript{st}–O\textsubscript{w}(=O\textsuperscript{−}) distances are estimated to be about 2.7 and 1.8 Å, respectively.

3.3. Importance of the Mn\textsubscript{st}–O\textsubscript{w} Distances in the CaMn\textsubscript{ox} Cluster

The discrimination between O\textsuperscript{2−} and OH\textsuperscript{−} at the O\textsubscript{w} site is hardly possible based on the JT deformation formula in the region of the R(Mn\textsubscript{st}–O\textsubscript{w}) = 2.2 Å (see Figure S2). In this situation, the Mn\textsubscript{st}–O\textsubscript{w} bond lengths become an important JT deformation index for discrimination between O\textsuperscript{2−} and OH\textsuperscript{−} at the O\textsubscript{w} site of the CaMn\textsubscript{ox} cluster. The Mn\textsuperscript{IV}–O\textsubscript{w} bond lengths are usually about 1.8-1.9 Å for O\textsuperscript{2−}=O\textsuperscript{2−} because of no JT effect of Mn\textsuperscript{IV} ion, as shown previously (see Tables 5 and S5).\textsuperscript{[35–39]} The Mn\textsuperscript{IV}–O\textsubscript{w}–H bond length after protonation of the O\textsubscript{w} site is 2.0–2.1 Å because of no JT effect. On the other hand, the Mn\textsuperscript{IV}–O\textsubscript{w}–H bond length may be elongated to 2.3-2.4 Å if the JT elongation axis is parallel to the HO\textsubscript{w}–Mn\textsubscript{st}–O(Glu 354) bond in the CaMn\textsubscript{ox} cluster. In fact, Mn\textsubscript{st}–O\textsubscript{w} bond length by 3WU2 structure\textsuperscript{[48]} was 2.4 Å because of the reduction of Mn\textsuperscript{IV}\textsubscript{st} into Mn\textsuperscript{II}\textsubscript{st}.\textsuperscript{[130]} Thus, the JT deformation formula revealed by the computational results\textsuperscript{[35–39]} and available experiments for Mn complexes provide guiding principles for understanding of variations of Mn–O bond lengths of the CaMn\textsubscript{ox} cluster of OEC of PSII.

Nevertheless, several theoretical papers\textsuperscript{[41,42,44,46]} suggested the possibility of the radiation damage of the XFEL structure\textsuperscript{[27]} Previously\textsuperscript{[39]} we have estimated the fraction of the S\textsubscript{st} component in the observed XFEL structure\textsuperscript{[27]} on the basis of the following equation under the assumption of 2.0 and 2.4 Å for the Mn\textsubscript{st}–O\textsubscript{w} distances of the C\textsubscript{st} structures in the S\textsubscript{st} and S\textsubscript{ox} states, respectively [Eq. (6)]:

$$R(Mn^{IV}_{st}-O_{w}) = (1-\alpha)R(Mn^{IV}_{st}-OH_{(w)}) + \alpha R(Mn^{IV}_{st}-OH_{(w)}) = 2.0 \text{ for } S_1(C_8) + \alpha R(Mn^{IV}_{st}-OH_{(w)}) = 2.4 \text{ for } S_0(C_8)$$

(6)

where the C\textsubscript{st} structures are used for both S\textsubscript{st} and S\textsubscript{ox} states. Here the Equation (6) was applied for the low dose XRD structures.\textsuperscript{[148]} The weight (\alpha) of the S\textsubscript{st} component was estimated to be 17.5 and 35 (%) respectively for 5B5EA and 5B66A structures. The estimated contribution of the S\textsubscript{st}(C\textsubscript{st}) component for 5B5EA is smaller than the estimated value (25 %) for the no pre-flash experiment, whereas it seems non negligible for 5B66A structure\textsuperscript{[46]} under the assumption of no experimental uncertainty. However, the contamination of the S\textsubscript{st}(C\textsubscript{st}) structure in 5B66A resulted in a very small elongation (2.85–2.82 = 0.03 Å) of the Mn\textsubscript{st}–Mn\textsubscript{st} distance because of the same C\textsubscript{st} topology. Therefore, the observed structure of the A monomer by the LD XRD experiment\textsuperscript{[148]} is fully compatible with the
damage-free XFEL structure, particularly 4UB6 structure and reassigned EXAFS structure[39] (see Table S6).

The estimated fractions (α) of the S3(Ca) component by Equation (6) were 22 and 5 (%) respectively, for SB5EA and SB66A structures. The estimated contribution of the S3 component for SB5EA is smaller than 25%, whereas it seems negligible for SB66B structure for which the observed Mn3(b1)-O5(b1) distance is 2.02 Å. Therefore, the observed structure of the B monomer by the low dose (LD) XRD experiment[30] is fully compatible with the JT (dJ) deformed structure in Figure 1B instead of the JT (dJ) structure in Figure 1A. Thus, the nature of the chemical bonds of the CaMnO3 cluster of OEC is labile[39] indicating the structural deformations (see Figure 4).

![Figure 4](image)

**Figure 4.** The Mn–O distances and hydrogen bonding interactions in the dimer of OEC of PSI by low-dose XRD experiments. (A) A-monomers by SB5EA(SB66A) and (B) B-monomers of SB5EA(SB66B).

**3.4. Estimation of Radiation Damage by the Mn3(b1)–Mn4(a1) Distances in the CaMnO3 Cluster**

The pre-flash procedure for generation of the pure S1 state was not performed for the LD XRD experiments[30] indicating a possibility of the contamination of the S3 component. The JT deformation formula indicate that the Mn3(b1)–Mn4(a1) distances can be used for estimation of the possible fraction of the S3 component, under the assumption of reduction of the high-valent MnV3(b1) ion into MnIV3(b1) as follows [Eq. (7)]:

\[
R(\text{Mn}_{3(b1)}-\text{Mn}_{4(a1)}) = (1-\alpha)R(\text{Mn}_{3(b1)}-\text{Mn}_{4(a1)} = 2.80) \text{ for S1(Ca)} \\
+ \alpha R(\text{Mn}_{3(b1)}-\text{Mn}_{4(a1)} = 3.10) \text{ for S3(Ca)}
\]

(7)

where the C1 structure for the S1(3343) state was employed for estimation. The mixing coefficient (α) are summarized in Table 2. The α-values are 6.7 and 16.7 (%) respectively, for SB5EA and SB66A for which the Mn3(b1)–Mn4(a1) distances are 2.82 and 2.85 (Å) respectively. Therefore, the S3 components for the A-monomers are smaller than 25%, showing the normal behavior. The Mn3(b1)–O5(b1) distances estimated by the α-values are 2.22(2.24) and 2.28(2.28) (Å) respectively, where the observed distances are given in parentheses. The estimated (observed) Mn3(b1)–O5(b1) distances are 2.03(2.09) and 2.07(2.14) (Å), respectively. The JT deformation formulae [see Eqs. (2) and (3)] work well for examination of the S3 contamination for the LD XRD structures.[10] The A-monomer by the LD XRD[30] is essentially regarded as the S3(Ca) structure even if the partial S3(Ca) contamination is taken into account.

We have assumed the reduction of the high-valent MnIV3(b1) into the MnIII3(b1) ion in the S1 to S2 transition in Equation (7). However, the reduction of MnIV3(b1) into MnIII3(b1) by chemical origins[40] is also conceivable, yielding the S2 state with the (2443) valence configuration.[31] The optimized Mn3(b1)–Mn4(a1), Mn3(b1)–Mn3(b1), Mn3(b1)–Mn3(b1), Mn3(b1)–Mn4(a1) and Mn4(a1)–O5(b1) distances were 2.97, 2.81, 2.75, 3.32 and 2.44 (Å) respectively for the S3(Ca) state with the (2443) configuration.[40] Therefore the Mn3(b1)–Mn4(a1) distances are also estimated as follows [Eq. (8)]:

\[
R(\text{Mn}_{3(b1)}-\text{Mn}_{4(a1)}) = (1-\alpha)R(\text{Mn}_{3(b1)}-\text{Mn}_{4(a1)} = 2.80) \text{ for S1(Ca)} \\
+ \alpha R(\text{Mn}_{3(b1)}-\text{Mn}_{4(a1)} = 2.97) \text{ for S3(Ca)}
\]

(8)

The α-values calculated by using R(\text{Mn}_{3(b1)}-\text{Mn}_{4(a1)}) distance of S3(Ca) are 11.8 and 29.4 (%), respectively. Therefore, the estimated MnIV3(b1)–O5(b1) distances for SB5EA and SB66A are 2.21(2.24) and 2.26(2.28) (Å) respectively, where the observed distances are given in parentheses. The main component of the A-monomer[30] based on the Mn3(b1)–Mn4(a1) and Mn4(a1)–O5(b1) distances is regarded as the S3(Ca) structure in Figure 1C.

Several theoretical papers[44, 46] claimed that the “damage-free” XFEL structure[27] may be regarded as the S3 structure. The α-values by Equation (7) assuming the mixing of the S3(Ca)(3343) configuration are 20, 10, 27 and 37 (%) respectively for 4UB6A(2.86 Å), 4UB6a(2.83 Å), 4UB8A(2.88 Å) and 4UB8A(2.91 Å) for which the observed Mn3(b1)–Mn4(a1) distances are given in parentheses. On the other hand, the corresponding α-values by Equation (8) assuming the mixing of the S3(Ca)(2443) configuration are 35 (92), 18 (62), 47(77) and 65 (67) (%) respectively, where the corresponding values estimated by the EXAFS line simulations using 2.83, 2.86, 2.83 and 2.88 (Å) for R(\text{Mn}_{3(b1)}-\text{Mn}_{4(a1)}) respectively, are given in parentheses.[40] High(HO)- and low(LO)-oxidation paradigms[42, 47] have been proposed for the valence state of the CaMnO3 cluster in OEC of PSI (see Ref. [47] and section SV in the Supporting Information). According to the HO and LO paradigms,[2, 47] MnII and MnIII ions are involved in the S3 state, respectively. The Equation (7), consistent with the HO paradigm, suggests that the fraction of the S3-component for 4UB6 is normal for the no pre-flash experiment,[27] whereas the estimated value for 4UB8 suggests the non-negligible uncertainty.[44, 46] 4UB6 is also acceptable for the Equation (8) for the LO paradigm.[45] Therefore, our conclusion is different from the assumption of the better quality of 4UB8 than that of 4UB6 employed for the electron...
density maps analysis by Wang et al.,[46] which predicted 100, 88, 77 and 78 (%) S1 to S9 reductions for 4UB6A, 4UB6B, 4UB8A and 4UB8B respectively. The LO paradigm reported by Petrie et al.[37] provided different explanations of the XRD structure[36] as described in the Supporting Information, Section SV.

The spin densities (Q) on the Mn4, Mn3 and O4i atoms are another chemical index[37,38] for elucidation of the reduction of the Mn4 and Mn3 ions, for which the Q values are about 4.0 and 3.0 respectively. Table 3 summarizes the calculated Q values by the QM (UB3LYP) method.[46] The spin densities on the Mn4 are in the range: 4.23 - 4.37, indicating the internal reduction by the spin polarization (SP) of the Mn4-O4i bond under the assumption of W2 = H2O and O4i=O2−. Therefore, the renormalized spin densities (Qa) are obtained by using the negative spin densities on the O4i atom (see Table 3). The Q values for the Mn4 site are about 4.0, indicating the Mn4 valence state. The SP effect for the Mn4-O4i bond is small for the case: W2 = O4i=OH−, indicating Q≈4.0 on Mn4. The spin densities (Q) on the Mn3 ion are also 3.7 - 3.8 in agreement with the Mn4 valence state. The Q-values on the Mn3 site are 2.9 – 3.2 in compatible with the Mn4 valence state. On the other hand, spin densities (Q) on the Mn3 site are 3.21, 3.11, 3.49 and 3.64, respectively, for 4UB6A, 4UB6B, 4UB8A and 4UB8B. The spin densities of the Mn3 site for 4UB8 were significantly larger than 3.0, suggesting that not negligible reduction of the Mn3 into Mn3, namely, the mixing of S3(C4)[3343] under the assumption of no experimental uncertainty. Present and previous[25] computational results indicate that the XFEL structure (4UB6) by Suga et al.[27] corresponds to the structure against the claim by other groups[44,46] and it is compatible with the structure of A monomer by LD XRD.[36] Young et al. also used the XFEL structure[27] for analysis of the new SFX results (SKAF)[29] for dark stable state.

4. Discussion

4.1. Examination of the Right-Opened structure in the S4 State

The structures of A-monomers with R(Mn3b-Mn4a) > 2.8 Å by the LD XRD[36] are compatible with the XFEL structures[36] and the re-assigned EXAFS structure (see Table S6).[37-39] On the other hand, the Mn3b-Mn4a distances of 5B5EB and 5B66B[66] are shorter than 2.80 Å, suggesting that the geometrical structures of B-monomers may be explained by mixing of the right-opened (R) S4 structure (see Figure 1A) with the slightly right (C3)- and left (C4)-elongated quasi-central S4 structures (see Figure S1) as follows (X = R or L) [Eq. (9)],

\[ R(Mn3b-Mn4a) = (1-\alpha)R(Mn3b-Mn4a) + \alpha R(Mn3b-Mn4a) = 2.70 \text{ for S4 (R)} \]

\[ + \alpha R(Mn3b-Mn4a) = R(Mn4) \text{ for S4 (C4)} \]

where R4 is 2.80 and 3.10 Å for S4(C4) and S4(C4) respectively. Table S2 summarizes the mixing coefficients (\(\alpha\)) and estimated Mn4-O4i and Mn3b-O4i distances. From Table S2 (see SI I), the mixing is acceptable for 5B5EB. However, the calculated Mn4-O4i distance is 1.93 Å for both mixing cases, and in contradiction to the observed value of 2.17 Å. The calculated Mn3b-O4i distances are 1.95 and 1.88 Å, respectively, for the S4(C4) and S4(C4) mixings, but the observed value is 2.07 Å. Therefore the observed Mn4-O4i and Mn3b-O4i distances for 5B5EB are hardly explained by the mixing in Equation (9) based on the Mn–Mn distances.

The \(\alpha\)-values calculated by using R(Mn3b-Mn4a) = 2.75 Å for 5B5EB are 25 and 12.5 (%), respectively, for the S4(C4) and S4(C4) mixings into the S4(R) structure, indicating that the S4(R)-structure is compatible for 5B5EB. However, the calculated Mn4-O4i distance is 1.93 Å for both mixing cases, and in contradiction to the observed value of 2.17 Å. The calculated Mn3b-O4i distances are 1.95 and 1.88 Å, respectively, for the S4(C4) and S4(C4) mixings, but the observed value is 2.07 Å. Therefore the observed Mn4-O4i and Mn3b-O4i distances for 5B5EB are hardly explained by the mixing in Equation (9) based on the Mn–Mn distances.

The \(\alpha\)-values for 5B66B with R(Mn3b-Mn4a) = 2.77 Å[66] are calculated to be 35 and 17.5 (%), respectively, for the S4(C4) and S4(C4) mixings into the S4(R) structure, indicating that the former value for the S4(C4) mixing is over the normal value (25%). On the other hand, the latter value for the S4(C4) mixing is normal, indicating that the S4(R)-structure in Figure 1A seems feasible for 5B66B. The calculated Mn4-O4i and Mn3b-O4i distances for the latter mixing are 1.99(2.12) and 1.91(2.02) Å respectively, where the corresponding observed values are given in parentheses (see Section SI in the Supporting Information). The high-resolution LD XRD structure with experimental uncertainty smaller than 0.1 Å is desirable for further discussion of the S4(R) structure in Figure 1A.

The mixing for S4(R) with S4(C4) is also conceivable as follows [Eq. (10)],

Table 3. The spin densities on the Mn4b, Mn3b, Mn3a, Mn4a and O4i ions of the XFEL structures[27] of the CaMn4O8 cluster of OEC of PSII by UB3LYP method.[46]

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</table>

[a] The renormalized spin density Q4i = Q(Mn4a) + Q(O4i) to remove the internal reduction of Mn ion by the spin polarization of the Mn4-O4i bond is given in parentheses.
The estimated \(\alpha\)-values are 18.5 and 25.9 (%) respectively, for SB5EB and SB66B, indicating that the \(d_{xy}\)-JT type R-structure seems acceptable. However, the elongated Mn_{\text{ca}}–O_{\text{ca}} distances for SB5EB and SB66B are estimated to be 1.90 (2.17) and 1.95 (2.12) (Å) respectively, where the corresponding observed values are given in parentheses. Therefore the observed Mn_{\text{ca}}–O_{\text{ca}} and Mn_{\text{ca}}–O_{\text{ca}} distances for SB5EB are hardly explained by the mixing in Equation (10).

The valence configuration of the Mn_{3b} site should be smaller than the formal Mn\(^{6+}\) (Q = 3.0) of the pure S\(_1\) configuration if the S\(_2\)s(C\(_2\)) or S\(_3\)s(C\(_3\)) with the (3343) configuration were mixed in the LD XRD structure without the pre-flash (see Table 3). On the other hand, the valence configuration of the Mn_{4a} site should be smaller than the formal Mn\(^{6+}\) (Q = 4.0) with mixing of the S\(_2\)s(C\(_2\)) with the (2443) configuration. Therefore, precise determination of the valence state of each Mn ion in the LD XRD structures by the X-ray spectroscopy is desirable for discrimination between the mixing schemes (9) and (10) on the experimental ground.\(^{[25]}\) The HO and LO paradigms\(^{[24,25]}\) relating to valence states of Mn ions are discussed in the Supporting Information, Section SV.

4.2. X-ray Induced Atomic Displacements by XFEL

Several experimental and theoretical studies\(^{[49–52]}\) on X-ray-induced atomic displacements within the XFEL pulse durations have been performed in relation to X-ray damages of serial femtosecond crystallography (SFX) (see SIII). Nagaya et al.\(^{[49,50]}\) investigated the electronic and nuclear dynamics of I-containing organic molecules such as 5-iodouracil (5-IU) induced by intense hard X-ray pulses at the XFEL facility (SACLA), elucidating that the changes of C–C, C–N and C–C distances of 5-IU were less than several % at the 10 fs pulse duration, and in contrast, the I–C length of 5-IU did not change in 30 fs. Amin et al.\(^{[51,52]}\) performed ab initio molecular dynamics simulation of OEC, where the nuclei move classically in a full quantum potential created by electron density under the effect of strong laser pulse in the Ehrenfest dynamics regime (see details SIII). The computational results\(^{[51,52]}\) showed that the Mn–Mn and Mn–Ca distances were less affected by radiation damage due to their heavy masses, while the O_{\text{ca}} atom moved significantly. The Mn_{\text{ca}}–Mn_{\text{ca}} (Ca–O_{\text{ca}}) distances for the S\(_1\) structure (see Figure 1A) were calculated to be 2.89(2.47), 2.90(2.47) and 3.08(2.53) (Å) respectively, after 0, 10 and 50 fs duration of the XFEL pulse.\(^{[51,52]}\) Therefore, the elongations by X-ray damage were 0.01(0.00) and 0.19(0.06) (Å) for 10 and 50 fs irradiations, respectively. The calculated Mn_{\text{ca}}–O_{\text{ca}} (Mn_{\text{ca}}–O_{\text{ca}}) distances\(^{[51,52]}\) for the S\(_1\) structure were 1.88(1.87), 1.92(1.89) and 2.34(2.01) (Å) respectively, after 0, 10 and 50 fs irradiation of XFEL. The elongations by X-ray damage were 0.04(0.02) and 0.46(0.14) (Å) for 10 and 50 fs irradiations, respectively, indicating the 2.13(1.07) and 24.4 (7.49) % elongations. The Mn\(^{4+}\)–O_{\text{ca}} bond was sensitive to the radiation damage as compared with the Mn\(^{6+}\)–O_{\text{ca}} bond.

According to the above computational results,\(^{[51,52]}\) the Mn\(^{4+}\)–O_{\text{ca}} bond lengths of the XFEL structures\(^{[27]}\) were estimated using the Coulomb explosion distance (\(\Delta_{\text{EXFEL}}\)) as follows (Eq. (11)):

\[
R(\text{Mn}_{\text{ca}}–\text{O}_{\text{ca}})_{\text{correct}} = R(\text{Mn}_{\text{ca}}–\text{O}_{\text{ca}})_{\text{XFEL}} – \Delta_{\text{EXFEL}}
\]

where \(\Delta_{\text{EXFEL}}\) were given by the above 2.13 (10 fs) and 24.4 (50 fs) % elongations of the Mn\(^{4+}\)–O_{\text{ca}} distance of XFEL structures.\(^{[27]}\) The 5 and 10 (%) elongations were also examined for weak and medium explosions, respectively. Table 4 summarizes the calculated Mn_{\text{ca}}–O_{\text{ca}} distances, for which the Mn_{\text{ca}}–Mn_{\text{ca}} distances are estimated using Equation (3). The explosion distances (\(\Delta_{\text{EXFEL}}\)) were about 0.05, 0.1, 0.2 and 0.6 (Å) respectively, for 2.13 (10 fs), 5 (a fs), 10 (b fs) and 24.4 (50 fs) % elongations, where 0 < a < b < 50. The slightly elongated Mn_{\text{ca}}–O_{\text{ca}} distances (about 2.3 Å) of the XFEL structures at 10 fs pulse duration (SACLA)\(^{[27]}\) are shortened by about 0.05 – 0.1 Å, and the corrected Mn_{\text{ca}}–O_{\text{ca}} distances by Equation (11) are therefore compatible with those of the A-monomers of the LD XRD structure.\(^{[30]}\) On the other hand, the corrected Mn_{\text{ca}}–O_{\text{ca}} distances for the medium (10%) and long (50 fs) pulse durations are formally compatible with the Mn_{\text{ca}}–O_{\text{ca}} distances of the B-monomers of the LD XRD\(^{[30]}\) and R-structures in Figure 1A, respectively. The computational results suggest that the atomic displacement of the Mn\(^{4+}\)–O_{\text{ca}} bond of the CaMn_{3}O_{6} cluster by XFEL\(^{[27]}\) is small (<0.1 Å) because of the short pulse width (10 fs) at SACLA.\(^{[33]}\) The situation is the same for the Mn\(^{4+}\)–O_{\text{ca}} bond (see Table S5). The high-resolution XFEL (~40 fs) structure at LCLS\(^{[26]}\) is really desirable for comparison (see SIII).

4.3. Comparisons Between Low-Dose XRD and New SFX Structures

The \(d_{xy}\)-JT type structures (see Figure 1B) of B-monomers by LD XRD\(^{[30]}\) were in agreement with the EXAFS structure with the (2, 1, 0) Mn–Mn distances\(^{225}\) under the assumption of protonation of the O_{\text{ca}} site (O_{\text{ca}}=OH\(^{+}\)). Detailed discussions on the EXAFS results\(^{[24,25]}\) relating to LD XRD\(^{[30]}\) were given in Section SIV in the Supporting Information.\(^{[39]}\) Here the JT deformation Equations (1)–(3) applied for the analysis of very recent SFX structures with and without preflash in the dark stable state reported by Suga et al.\(^{[24]}\) and Young et al.\(^{[26]}\). Table 5 summarizes the observed and calculated Mn_{\text{ca}}–Mn_{\text{ca}} and Mn_{\text{ca}}–O_{\text{ca}} distances and SSB parameters for these SFX structures. From Table 5, the SFX results for A-monomer of 5GTH without preflash\(^{[26]}\) elucidated that the Mn_{\text{ca}}–Mn_{\text{ca}} and Mn_{\text{ca}}–O_{\text{ca}} distances were 2.98(2.91), 2.33(2.34), 2.03(2.02) and 0.20(0.19) (Å) respectively, where the corresponding values for B-monomer were given in parentheses. The Mn_{\text{ca}}–Mn_{\text{ca}} distances estimated by using the observed Mn_{\text{ca}}–O_{\text{ca}} distance for 5GTH(A) were 2.88(2.88) and 2.84(2.84) (Å) for O_{\text{ca}}=OH\(^{+}\) and O_{\text{ca}} = O\(^{2–}\), respectively. On the other hand, the Mn_{\text{ca}}–O_{\text{ca}} distances estimated by using the observed Mn_{\text{ca}}–Mn_{\text{ca}} distance for
Table 4. The calculated Mn$_2$-Mn$_1$ distances (Å) of the XFEL$^{[25]}$ and SFX$^{[24,26]}$ structures of the CaMn$_4$O$_8$ cluster of OEC of PSII based on the Mn$_{\text{Ca}}$–O$_{\text{Ca}}$ distances (Å) shortened by the corrections of the XFEL expansions.$^{[24]}

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(a) The Mn$_{\text{Ca}}$–O$_{\text{Ca}}$ distances of the XFEL structures$^{[25]}$ were estimated by using the Coulomb explosion distance ($\Delta$Mn$_{\text{Ca}}$) in Equation (11). (b) The Mn$_{\text{Ca}}$–Mn$_{\text{Ca}}$ distances were estimated by using Equations (2) and (3) under the assumption of a1) O$_{\text{Ca}}$=OH$^-$ and b2) O$_{\text{Ca}}$=O$^2$.$^*$. (c) The Mn$_{\text{Ca}}$–O$_{\text{Ca}}$ distances for SGTHA(B)$^{[24]}$ and SKAFA(B)$^{[24]}$ by SFX were the same as that of 4UB8B, providing the same estimation results.

Table 5. The Mn$_1$–Mn$_2$ distances (Å) of the CaMn$_4$O$_8$ cluster in the S$_1$ state of OEC of PSII by SFX$^{[24,26]}$ with and without preflash and estimation procedure [Eqs. (2) and (3)].

<table>
<thead>
<tr>
<th>Structures</th>
<th>Mn$_1$–Mn$_2$ XRD</th>
<th>Mn$_1$–Mn$_2$ (Estimation)</th>
<th>Mn$<em>{\text{Ca}}$–O$</em>{\text{Ca}}$ XRD</th>
<th>Mn$<em>{\text{Ca}}$–O$</em>{\text{Ca}}$ (Est.)</th>
<th>O$_{\text{Ca}}$</th>
<th>SSBB</th>
<th>Topology$^{[4]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5GTHA</td>
<td>2.98</td>
<td>(2.88)$^{[4]}$</td>
<td>2.33</td>
<td>2.03</td>
<td>O$_{\text{Ca}}$=OH$^-$</td>
<td>0.20</td>
<td>C</td>
</tr>
<tr>
<td>(no preflash) dark SFX</td>
<td>2.98</td>
<td>(2.84)$^{[4]}$</td>
<td>(2.54)$^{[24]}$</td>
<td>(2.0)$^{[4]}$</td>
<td>O$_{\text{Ca}}$=O$^2$</td>
<td>0.20</td>
<td>C</td>
</tr>
<tr>
<td>5GTHB</td>
<td>2.91</td>
<td>(2.88)$^{[4]}$</td>
<td>2.34</td>
<td>2.02</td>
<td>O$_{\text{Ca}}$=OH$^-$</td>
<td>0.19</td>
<td>C</td>
</tr>
<tr>
<td>(no preflash) dark SFX</td>
<td>2.91</td>
<td>(2.84)$^{[4]}$</td>
<td>(2.90)$^{[24]}$</td>
<td>(1.8)$^{[4]}$</td>
<td>O$_{\text{Ca}}$=O$^2$</td>
<td>0.37</td>
<td>C</td>
</tr>
<tr>
<td>SWSSA</td>
<td>2.77</td>
<td>(2.86)$^{[4]}$</td>
<td>2.29</td>
<td>2.02</td>
<td>O$_{\text{Ca}}$=OH$^-$</td>
<td>0.24</td>
<td>C</td>
</tr>
<tr>
<td>(preflash dark) SFX</td>
<td>2.77</td>
<td>(2.83)$^{[4]}$</td>
<td>(2.12)$^{[24]}$</td>
<td>(2.0)$^{[4]}$</td>
<td>O$_{\text{Ca}}$=OH$^-$</td>
<td>0.41</td>
<td>R</td>
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<tr>
<td>2.77</td>
<td>(2.06)$^{[24]}$</td>
<td>(1.8)$^{[4]}$</td>
<td>O$_{\text{Ca}}$=O$^2$</td>
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<td>SWSSB</td>
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<td>(2.86)$^{[4]}$</td>
<td>2.29</td>
<td>2.03</td>
<td>O$_{\text{Ca}}$=OH$^-$</td>
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<td>C</td>
</tr>
<tr>
<td>(preflash dark) SFX</td>
<td>2.75</td>
<td>(2.83)$^{[4]}$</td>
<td>(2.08)$^{[24]}$</td>
<td>(2.0)$^{[4]}$</td>
<td>O$_{\text{Ca}}$=O$^2$</td>
<td>0.45</td>
<td>R</td>
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<tr>
<td>2.75</td>
<td>(1.98)$^{[24]}$</td>
<td>(1.8)$^{[4]}$</td>
<td>O$_{\text{Ca}}$=O$^2$</td>
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<tr>
<td>SKAFA(B)</td>
<td>2.87</td>
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<td>2.33</td>
<td>2.20</td>
<td>O$_{\text{Ca}}$=OH$^-$</td>
<td>0.20</td>
<td>C</td>
</tr>
<tr>
<td>(no preflash) dark SFX</td>
<td>2.87</td>
<td>(2.84)</td>
<td>(2.32)$^{[4]}$</td>
<td>(2.0)</td>
<td>O$_{\text{Ca}}$=O$^2$</td>
<td>0.20</td>
<td>C</td>
</tr>
</tbody>
</table>

[a] The Mn$_{\text{Ca}}$–Mn$_{\text{Ca}}$ distances were estimated by using the experimental Mn$_{\text{Ca}}$–O$_{\text{Ca}}$ distance by SFX structures$^{[24,26]}$ with and without preflash in Equations (2) and (3) under the assumption of a1) O$_{\text{Ca}}$=OH$^-$ and a2) O$_{\text{Ca}}$=O$^2$.$^*$. (b) The Mn$_{\text{Ca}}$–Mn$_{\text{Ca}}$ distances were estimated by using the experimental Mn$_{\text{Ca}}$–Mn$_{\text{Ca}}$ distance in Equations (2) and (3) under the assumption of a1) O$_{\text{Ca}}$=OH$^-$ and b2) O$_{\text{Ca}}$=O$^2$.$^*$. (c) The Mn$_{\text{Ca}}$–O$_{\text{Ca}}$ distances were estimated to be 2.0 and 1.8 for c1) O$_{\text{Ca}}$=OH$^-$ and c2) O$_{\text{Ca}}$=O$^2$., respectively. (d) Assignment of the O$_{\text{Ca}}$ site. (e) Structural symmetry breaking (SSBB) parameter. (f) Topology.

5GTHA(B) were 2.54(2.40) and 2.90(2.62) (Å) for O$_{\text{Ca}}$=OH$^-$ and O$^2$., respectively. The estimated Mn$_{\text{Ca}}$–O$_{\text{Ca}}$ distances supported O$_{\text{Ca}}$=OH$^-$ . The structural parameters for SGTHA(B) without preflash were fully consistent with those of previous SFX structure without preflash.$^{[27]}$ They were also compatible with SKAFA(B) structure without preflash by Young et al.$^{[29]}$ the structure of A monomer by LD XRD$^{[30]}$ and the reassigned EXAFS structure (see Tables S6 and S7).$^{[39]}

The SFX results for A(B)-monomer of 5WS5 with preflash indicated that the Mn$_{4(a)}$-Mn$_{4(b)}$, Mn$_{4(a)}$-O$_{50}$, and Mn$_{4(b)}$-O$_{50}$ distances for were 2.77(2.75), 2.29(2.29), 2.02(2.03) and 0.24(0.24) (Å), respectively. The Mn$_{4(a)}$-Mn$_{4(b)}$ distances estimated by using the observed Mn$_{4(a)}$-O$_{50}$ distance for 5WS5A(B) were 2.86(2.86) and 2.83(2.83) (Å) for O$_{50}$=OH$^-$ and O$^-$, respectively. The estimated Mn$_{4(a)}$-Mn$_{4(b)}$ distances were longer by about 0.1 Å than the corresponding observed values. On the other hand, the Mn$_{4(a)}$-O$_{50}$ distances estimated by using the observed Mn$_{4(a)}$-Mn$_{4(b)}$ distance for 5WS5A(B) with preflash were 2.12(2.08) and 2.06(1.98) (Å) for O$_{50}$=OH$^-$ and O$^-$, respectively. The estimated Mn$_{4(a)}$-O$_{50}$ distances were shorter by about 0.2 Å than the observed distances, indicating the similarity to the corresponding observed values of B-monomer of LD XRD. Moreover, the Mn$_{4(a)}$-O$_{50}$ distance estimated by 5% reduction of the observed values of 5WS5A(B) by SFX(SACLA) is 2.18(2.18) which is in compatible with those of the B-monomer of LD XRD at 1.85 Å resolution as shown in Table 4. Judging from the estimated Mn$_{4(a)}$-O$_{50}$ distances, and the observed Mn$_{4(a)}$-Mn$_{4(b)}$ and Mn$_{4(b)}$-O$_{50}$ distances, SW5A(B) structure with preflash was similar to B-monomer of LD XRD. Preflash effect was significant for successive investigation of the S$_{1}$-to-S$_{2}$ transition investigated by SFX. Further examinations of the SFX results after two flash illuminations were given in the Section SV in the Supporting Information (Table S8).

4.4. Importance of Hydrogen-Bonding Interactions in the Protein Field

Subtle structural differences between A- and B-monomers by low-dose XRD were examined for elucidation of important roles of the environmental effects around the CaMn$_{4}$O$_{8}$ cluster. Figure 4 illustrates the observed Mn-O distances, together with hydrogen bonding interactions for the CaMn$_{4}$O$_{8}$ cluster. The O$_{3(b)}$-N(His 337) distances for 5B5EA(5B66A) and 5B5EB(5B66B) were 2.46(2.48) and 2.75(2.74) (Å), respectively. The O$_{3(b)}$-N distances of the A-monomers are shorter by 0.29(0.26) Å than those of the B-monomers, indicating that the O$_{3(b)}$-H--N(His 337) hydrogen bonding interaction is very strong for the A-monomers. The Mn$_{40}$-O$_{3(b)}$ bond lengths for 5B5EA(5B66A) and 5B5EB(5B66B) were 2.27(2.18) and 1.96(1.95) (Å), respectively. The Mn$_{40}$-O$_{3(b)}$ distances of the A-monomers are longer by 0.31(0.23) Å than those of the B-monomers, indicating the elongation induced by the strong hydrogen bonding interaction.

The O$_{4(a)}$-O$_{11}$ distances for 5B5EA(5B66A) and 5B5EB(5B66B) were 2.66(2.71) and 2.44(2.45) (Å), respectively. The O$_{4(a)}$-O$_{11}$ distances of the B-monomers are shorter by 0.22(0.26) Å than those of the A-monomers, indicating very strong O$_{4(a)}$-H--O$_{11}$(W11) hydrogen bonding interaction. The Mn$_{4(a)}$-O$_{4(a)}$ bond lengths for 5B5EA(5B66A) and 5B5EB(5B66B) were 1.87(1.84) and 2.07(2.10) (Å), respectively. The Mn$_{4(a)}$-O$_{4(a)}$ distances of the B-monomers are longer by 0.20(0.26) Å than those of the A-monomers, indicating the elongation induced by the very strong hydrogen bonding interaction. The elongation of the Mn$_{4(a)}$-O$_{4(a)}$ distance is consistent with the JT distortion in Figure 1B. Thus, the LD XRD experiments opened the door for understanding important roles of confinement effects of protein such as hydrogen bonding networks for subtle geometry changes of the CaMn$_{4}$O$_{8}$ cluster in OEC of PSII, indicating the following structural fluctuations ($\Delta R_{\text{protein}}$ < 0.1 Å) depending on states of protein fields [Eq. (12)]:

$$R(Mn_{4(a)}-Mn_{3(b)})_{\text{protein}} = 2.80 \pm \Delta R_{\text{protein}} (\text{Å})$$

The large-scale QM/MM models involving hydrogen bonding networks are necessary for theoretical investigation of $\Delta R_{\text{protein}}$ at the atomic level for the CaMn$_{4}$O$_{8}$ cluster controlled by several environmental effects, such as pH, hydrogen bonding and packing structures of OEC of PSII.

4.5. Implication of Present Results in Artificial Photosynthesis

The CaMn$_{4}$O$_{8}$ cluster in OEC of PSII examined here is a roadmap for artificial photosynthesis. Present results indicate that the orbital degree of freedom and related Jahn–Teller (JT) deformation of the CaMn$_{4}$O$_{8}$ cluster were investigated by EXAFS and ground spin states observed by EPR. In this paper, the orbital degree of freedom and related Jahn–Teller (JT) deformation of the CaMn$_{4}$O$_{8}$ cluster were investigated by elucidate possible origins of the two different geometrical structures by low dose (LD) XRD. Hydrogen bonding networks around the CaMn$_{4}$O$_{8}$ cluster play important roles for subtle regulation of its geometrical structures. Present theoretical results in turn indicate that SCES such as magnetic transition-metal clusters developed in the field of molecular magnetism may be converted into active catalysts for water oxidation in artificial photosynthesis, as proposed previously. To this end, the CaMn$_{4}$O$_{8}$ cluster in OEC of PSII can be replaced with other SCESs constructed of abundant 3d transition metals such as Fe, Co, Cu etc. for development of artificial photo-catalysts for which precious metals have been used for catalytic sites. Protein field is also replaced with other robust confinement materials such as metal organic framework (MOF), polyoxo metallate (POM), nanotube (NT) as illustrated in Figure 5. Masaoka et al. recently made a great breakthrough for conversion of the Fe$_{6}$ magnetic cluster into an active catalyst for water conversion by electrochemical hole-doping techniques. Design of appropriate ligands for hole doping by solar energy are desirable for future developments of molecular catalysts for water oxidation.
Confinements of SCES by several reaction fields

![Diagram](image)

**Figure 5.** Our theoretical proposal of artificial photosynthesis where the protein field of OEC of PSII confines the CaMnO cluster which is a typical example of hole-doped strongly correlated electron systems (SCES). The CaMnO cluster can be replaced with other SCESs constructed of abundant 3d transition, such as, for example, Mn, Fe, and Co. The protein field is replaced with other confinement materials, such as metal–organic frameworks (MOF), polyoxometallates (POM) and nanotubes (NT).

5. Conclusions

The JT deformation formula[37–39] are found to be useful and applicable for understanding and qualitative prediction of JT deformations of the CaMnO cluster in OEC of PSII. In fact, the JT deformation formula[37–39] was successfully applied for elucidation of structural deformations of the CaMnO cluster in the A- and B-monomers of the dimer structure of PSII by the XRD experiments under low dose 0.03 Mgy (585E) and 0.12 Mgy (5866) conditions.[40] The Mm3O–Mn2O6, Mn2O4–O(a) and Mn3O–O(b) distances of the A-monomer are about 2.8, 2.2 and 2.0 (Å) respectively, showing the JT (dJ) elongation of the Mn2O4–O(b) distance responsible for the C8 structure in Figure 1C. The corresponding distances of the B-monomer are 2.75, 2.1 and 2.0 (Å) respectively, indicating the JT (dJ) deformation (see Figure 1B). The structure of the A-monomer by LD XRD[40] is consistent with the refined SFX structure without XFEL damage[8–10] (see Table 4) and the reassembled EXAFS[37–39] structures, whereas the structure of the B-monomer by LD XRD is compatible with the original EXAFS structure[40] (see Table 5) and the recent SFX structure with preflash[39] (see Table 5). The EXAFS structure[40] has been referred to as the reference structure to support the theoretical S8 models with the JT deformation formulae were successfully applied for theoretical analysis of the recent SFX results[38,39] after two flash (see Section SV in the Supporting Information). Finally, implications of the present results for artificial photosynthesis by the use of abundant transition metals[14–15] have been touched briefly.

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Conflict of Interest

The authors declare no conflict of interest.

**Keywords:** artificial photosynthesis · oxygen evolving complex · photosystem II · theoretical models · X-ray diffraction


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