Experimental demonstration of radicaloid character in a $Ru^V = O$ intermediate in catalytic water oxidation

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Water oxidation is the key half reaction in artificial photosynthesis. An absence of detailed mechanistic insight impedes design of new catalysts that are more reactive and more robust. A proposed paradigm leading to enhanced reactivity is the existence of oxyl radical intermediates capable of rapid water activation, but there is a dearth of experimental validation. Here, we show the radicaloid nature of an intermediate reactive toward formation of the O-O bond by assessing the spin density on the oxyl group by Electron Paramagnetic Resonance (EPR). In the study, an ^{17}O-labeled form of a highly oxidized, short-lived intermediate in the catalytic cycle of the water oxidation catalyst cis,cis-[(2,2-bipyridine)$_2$(H$_2$O)Ru^{III}ORu^{III}(OH)$_2$(bpy)$_2$]$^{4+}$ was investigated. It contains Ru centers in oxidation states [4,5], has at least one $Ru^V = O$ unit, and shows $|A_{xx}| = 60G$ ^{17}O hyperfine splittings (hfs) consistent with the high spin density of a radicaloid. Destabilization of π-bonding in the $d^3 Ru^V = O$ fragment is responsible for the high spin density on the oxygen and its high reactivity.

blue dimer | water splitting | catalysis | solar fuels

Water has long been envisioned as a source of protons and electrons for fuel-forming reactions (1, 2), with O$_2$ the nonpolluting product that is converted back to H$_2$O in respiration and combustion. The considerable stability of the water molecule, however, hinders realization of artificial photosynthesis in a man-made device. Energetic barriers for water oxidation into O$_2$ can be lowered by use of a suitable catalyst. The most efficient water oxidation catalyst known to date is Photosystem II, which oxidizes water ($2H_2O + 4h\nu \rightarrow O_2 + 4H^+ + 4e^-$) in the process of natural photosynthesis with the help of an Mn$_4$Ca cluster catalyst embedded in a protein environment (1). Understanding the mechanism of water oxidation is critical for development of alternative energy solutions based on the concept of artificial photosynthesis.

With the aim of uncovering the mechanism of water oxidation, the oxygen evolving complex (OEC) of Photosystem II has been intensively studied by X-Ray Diffraction (XRD) (3–5) and multiple spectroscopic (6–10) and computational approaches (1, 11–15). Intriguingly, Density Functional Theory (DFT) analysis of the reaction barrier for water oxidation in the OEC has shown that formation of the Mn-O• radicaloid intermediate is necessary to reproduce the experimentally observed activation energy (11, 16–19). Several experimental observations are suggestive of the formation of the Mn-O• radicaloid as a species capable of reacting with H$_2$O with formation of an O-O bond (6, 7), but to the best of our knowledge, no direct experimental demonstration has been reported. The experimental difficulty is in the short lifetime of the S$_3$ state in the OEC in the Kok cycle for which formation of the Mn-O• radicaloid fragment has been proposed (7, 12, 20), which given its high spin state, makes EPR measurements particularly challenging.

Several Ru-based complexes have been developed as defined molecular catalysts for water oxidation (21–27). The first designed catalyst, the blue dimer (BD) cis,cis(bpy)$_2$(H$_2$O)Ru^{III}ORu^{III}(OH$_2$)(bpy)$_2$]$^{4+}$ [bpy is 2,2-bipyridine], shares similarities with the OEC, because they both undergo oxidative activation by proton-coupled electron transfer to reach higher oxidation states where water oxidation occurs (21–24). It is also the most studied catalyst for which experimental data on the reaction mechanism for

water oxidation are available. The results over the past 30 years are summarized in Fig. 1. BD [3,3] and [3,4] are known to be stable forms of the catalyst (numbers in square brackets denote the oxidation state of Ru centers). In BD catalytic cycle, the BD [4,5] and [5,5] intermediates were proposed to be active to O-O bond formation to produce peroxidic intermediates (24, 28–31). DFT analysis of the BD [5,5] intermediate showed unusually high spin density on the oxygen atom of the $d^3 Ru^V = O$ fragment (16, 32, 33) and suggested that this property is important for reactivity to water. Here, we show the radicaloid character of the $Ru^V = O$ intermediate experimentally by analyzing spin density distribution in the BD [4,5] intermediate. Early reports based on stopped flow measurements pointed to a transient nature of BD [5,5] (34), and we have preliminary data that a species previously reported as BD [5,5] (21, 30) is, indeed, a different form of BD [4,5] (a detailed analysis will be presented elsewhere). EPR results obtained for ^{17}O-labeled BD [4,5] are consistent with the hypothesis of the involvement of a radicaloid intermediate in the activation of water by this Ru-based water oxidation catalyst. Radicaloid character at the oxygen atom in the $Ru^V = O$ oxidant, with acceptor orbital largely based on the oxyl group, may facilitate O-O bond formation by extending the electron acceptor wave function to the O atom.

Results and Discussion

The BD catalyst intermediates BD [4,5] and BD [5,5], which are reactive to water, were characterized in previous studies (29, 35–37) and are characterized in more detail in this work. BD [4,5] can be prepared in 0.1 M HNO$_3$ by oxidation of the BD [3,4] with excess of cerium ammonium nitrate [Ce(IV)] and fast (less than 10–15 s) freezing (37). The BD [4,5] intermediate has been characterized by Ru K-edge X-Ray Absorption Near Edge Structure (XANES) (Fig. 2A). Extended X-Ray Absorption Fine Structure (EXAFS) analysis showed a short Ru = O bond (Fig. 2B, Table 1, and Table S1). The protonation state of the Ru^{IV} center in BD [4,5] in 0.1 M HNO$_3$ (whether it is Ru^{IV}-OH or Ru^{IV} = O) is not known with certainty (24). For example, BD in the [3,4] form, characterized by XRD, contains Ru^{III}-H$_2$O and Ru^{IV}-OH at pH = 1 (24). EXAFS fits for BD [4,5] were performed to model the protonation states Ru^V = O, Ru^{IV} = O (two short Ru = O distances per BD molecule) versus Ru^V = O, Ru^{IV}-OH (one short Ru = O distance in the BD molecule). A slightly better fit quality was obtained for the Ru^V = O, Ru^{IV}-OH formulation (Table 1, Fig. S1, and Table S1). To further assess the protonation states of BD [4,5], resonance Raman measurements were carried out on frozen BD [4,5] samples prepared by stopped-flow freeze quench of BD [3,4] mixed with 20 eq. of Ce(IV) (Fig. S2). The 816- to 818-cm^{-1} peak arises from the Ru = O stretch (35, 38, 39) and was observed in samples prepared at 100 ms-7s (Fig. S2), which is in agreement
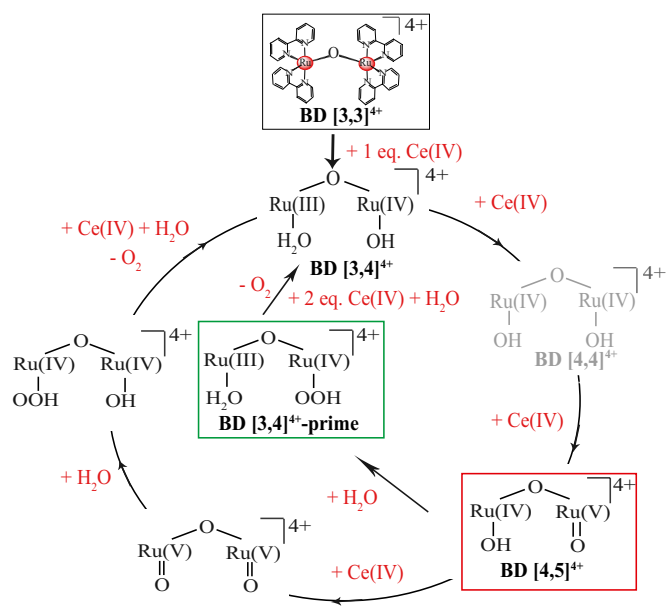
Fig. 1. Catalytic scheme of the water oxidation reactions with BD based on summary of available literature. BD [3,3] and BD [3,4] are stable forms of the catalyst. BD [4,4] has not been characterized and is considered to be unstable. BD [4,5] and BD [5,5] were described as species reactive to water with formation of peroxide species.

with earlier EPR characterization [figure 8 in the work by Moonshiram et al. (37)]. This peak undergoes a 35-cm^{-1} shift to lower frequency for BD [4,5] prepared in 0.1 M HNO$_3$/H$_2$^{18}O (Fig. S3). Based on earlier literature reports (38, 40), this shift is in good agreement with assignment of the 816-cm^{-1} band to a Ru^V = O vibration. The 816-cm^{-1} band grows in relative intensity from 100 ms to 4 s and then decreases after 7 s, giving rise to a band at 683–688 cm^{-1} previously assigned to a BD [3,4]-prime species (37). At the same time, the band for the Ru-O-Ru bridge mode shifts from 390 (at 4 s) to 410 cm^{-1} (at 15 s) (37). We were not able to resolve a separate band for the Ru^{IV} = O vibration, likely due to protonation of this group and formation of Ru^{IV}-OH. From these observations, BD [4,5] prepared as described above most likely contains only one Ru = O fragment.

The EPR spectrum of BD [4,5] is shown in Fig. 2C. This signal has been observed earlier (21, 37) and was assigned to a signal from the S = 1/2 spin state with g_{xx} = 2.03, g_{yy} = 1.98, g_{zz} = 1.87 g-tensor; g_{xx} = 2.03 and g_{zz} = 1.87 are clearly resolved in the spectrum, whereas g_{yy} = 1.98 was derived from spectral simulations (Fig. 2C and Fig. S4). Addition of Ru A_{yy} = 40 ± 5G and A_{zz} = 25 ± 5G improves the agreement of simulated and experimental spectra (Fig. S5 A and B). The simulated EPR spectrum in Fig. 2C reflects the isotopic composition for Ru^{99,101} (I = 5/2) (details of EPR simulation in *SI Materials and Methods*). The EPR results are in agreement with earlier experiments for the BD complex prepared in the highly oxidized conditions at pH = 0.5, where apparent Ru^{99,101} hyperfine splitting (hfs) on the order of 40 G were reported (30, 41). A full Ru^{99,101} hfs-tensor was reported for the oxidized Ru^V complex derived from *cis*-[Ru^{II} (bpy)$_2$(H$_2$O)$_2$]$^{2+}$ (42). Notable Ru^{99,101} hfs in BD [4,5] is in agreement with DFT analysis (Table 2), showing considerable spin density on at least one Ru center in BD [4,5].

Samples highly enriched with BD [4,5] can be prepared by the automated freeze quench technique as discussed above (Fig. 2C) (37) or constant potential electrolysis redox titrations with carbon fiber flow electrode (21). However, neither of these approaches can be used for preparation of ^{17}O-enriched BD [4,5] because of the prohibitive cost of H$_2$^{17}O water. ^{17}O enrichment of BD [3,3] was achieved by dissolution of BD [3,3] powder in 60% enriched H$_2$^{17}O and incubation for 24 h. Under such conditions, H$_2$O molecules

coordinated to Ru centers in BD [3,3] are readily exchangeable (38); 1 eq Ce(IV) in H$_2$^{16}O/H$_2$N^{16}O$_3$ solution was added to 1 mM BD [3,3] in 60% enriched H$_2$^{17}O to acidify the solution to pH 1 and form BD [3,4]−^{17}O. Soon after, an excess of Ce(IV) (20 eq.) in H$_2$^{16}O/H$_2$N^{16}O$_3$ was added to form BD [4,5]−^{17}O, and solution was frozen within 30 s. Final H$_2$^{17}O enrichment was 50% because of dilutions. ^{17}O enrichment of the BD molecule is estimated to be between 50% and 60% because of the slower rate of H$_2$O exchange in BD [3,4]. Thus, we achieved the BD [4,5] intermediate with terminal Ru-O groups labeled with ^{17}O, whereas bridging oxygen is not exchangeable under conditions of the experiment (21).

The experiment with H$_2$^{17}O was repeated two times, and the same EPR spectra were obtained for reaction mixtures. Samples prepared by manual freezing in H$_2$O (control) and H$_2$^{17}O contain up to 50% BD [3,4]-prime species, which are a product of BD [4,5] reaction with water (Fig. 2D). X-Ray Absorption Spectroscopy (XAS) and EPR analysis of this product was reported previously (37). Melting of samples results in complete conversion of BD [4,5] into BD [3,4]-prime intermediate (Fig. 2D). Pure BD [4,5] EPR spectra were extracted by subtracting the contribution of BD [3,4]-prime product from the initial mixture. For BD [4,5] in regular water, spectra obtained by freeze quench and spectra obtained by subtraction were found to be similar (Fig. 2C).

Melting the ^{17}O samples resulted in the EPR spectrum of BD [3,4]-^{17}O-prime intermediate (Fig. 3B). Subtraction of the EPR of BD [3,4]-^{17}O-prime species from the EPR spectrum of the initial mixture allows extraction of the BD [4,5]-^{17}O spectrum (Fig. 3A). This spectrum is significantly broadened compared with the control, and components of ^{17}O hfs (I = 5/2) are clearly visible on the low field side of the spectrum (Fig. 3A). Splitting between these components is about 60 G. Upon adding ^{17}O hfs |A_{xx}| = 60G [taking into account lower estimate (50%) of ^{17}O enrichment] and using all
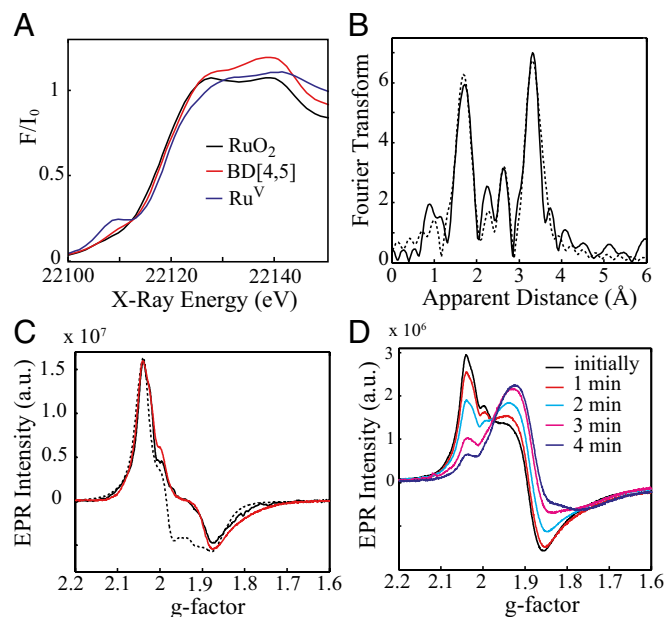

Fig. 2. (A) Normalized Ru K-edge XANES of the BD [4,5] compared with reference compound for Ru^V tetra-n-propylammonium bis-2-hydroxy-2ethylbutyrato(oxo) ruthenate(V) and RuO$_2$. (B) Fourier transforms of k^3-weighted Ru EXAFS of the BD [4,5] (black) and EXAFS fit (dashed) with moieties of protonated BD [4,5] [Ru(IV)-OH and Ru(V) = O]. (C) EPR spectra of the BD [4,5] obtained with freeze quench (0.5 mM in 0.1 mol HNO$_3$, 1-s freezing time; black) and quick manual mixing (1.2 mM in 0.1 mol HNO3) (Fig. 1) and subtraction of product spectrum (red). Simulation of the BD [4,5] EPR is shown by black dashed line. (D) EPR spectra of the reaction mixture obtained by quick manual mixing of BD [3,4] (1.2 mM in 0.1 mol HNO$_3$) starting catalyst and 20 eq Ce(IV). BD [4,5] converts into BD [3,4] prime intermediate on sample melting.

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Table 1. Structural parameters from EXAFS measurements of the BD [4,5] intermediate

Model species, fit qualities	EXAFS* shell: N** × distance (Å)
BD [4,5] in the Ru^V = O, Ru^{IV} = O protonation state R factor = 0.0003 Reduced χ^2 = 399	Ru-N: 4 × 2.09 Ru-O: 1 × 1.87 Ru-O: 1 × 1.70 Ru-C: 8 × 3.02 Ru-O-Ru angle: 166 ± 2° Ru-Ru: 1 × 3.71
BD [4,5] in the Ru^V = O, Ru^{IV}-OH protonation state R factor = 0.0003 Reduced χ^2 = 355	Ru-N: 4.5 × 2.09 Ru-O: 1 × 1.87 Ru-O: 0.5 × 1.71 Ru-C: 8 × 3.01 Ru-O-Ru angle: 166 ± 2° Ru-Ru: 1 × 3.71

*For comparison, XRD bond distances are 1.70 Å in Ru^V reference compound (49) and 1.81–1.83 Å in complexes with amino ligands (53, 54) with Ru^{IV}=O bond.

**N is the number of vectors given per Ru center.

other parameters as determined by the fit of the EPR spectrum of unlabeled BD [4,5], the experimentally observed splitting A_{xx} could be simulated well (Fig. 3A). There is a rather large uncertainty in the determination of A_{yy} and A_{zz} components of ^{17}O hfs tensor, because they are not clearly resolved. Upper limit for A_{zz} was estimated to be 25 G from analysis of the line broadening of the g_{zz} component. Additional simulations for effect of A_{yy} and A_{zz} values on spectral shape are shown in Fig. S5 C and D. Overall, the EPR data are consistent with the presence of one oxygen center with high spin density. Presence of one oxygen center with high spin density is in agreement with EXAFS and resonance Raman analysis suggesting the Ru^V = O, Ru^{IV}-OH protonation state for the BD [4,5] intermediate. ^{17}O-pulsed Electron Nuclear Double Resonance (ENDOR) measurements were not possible because of fast relaxation of BD [4,5] species. No echo signals were detected at 10 K for any BD compounds, including BD [3,4]-starting material and [3,4]-prime intermediate.

Earlier theoretical studies of BD [5,5] showed the radicaloid character of the Ru^V = O fragment by computing high spin densities on the oxygen (32). We performed DFT analysis of BD [4,5] with three combinations of functional/basis sets (Table 2) to analyze how well DFT can reproduce geometry (Table S2) and magnetic resonance parameters of this molecule. The Ub3lyp/dgdzvp DFT approach predicted an asymmetric structure for the BD molecule with different Ru-O bond distances in the Ru-O-Ru bridge (Table S2), which does not agree with EXAFS results (Table 1). The local density approximation (LDA) with polarized triple-ζ basis set (LDA/TZP) geometry optimization resulted in the structure, which is in a good agreement with EXAFS data (Table S2). Differences in the bond lengths for Ru-O(bridge) and Ru^V = O were within 0.05 Å between the experiment and calculation. To further verify how well LDA/TZP can reproduce BD geometry, we calculated the structure of BD [3,4], a molecule with structure that is known from XRD analysis (22, 43). Results are shown in Table S2. DFT simulations done with LDA/TZP closely reproduced geometry of the BD [3,4].

All DFT calculations confirmed the high spin density on the oxygen in the Ru^V = O fragment of BD [4,5] (Table 2). Although the sign of experimental $|A_{xx}|$ = 60G is likely negative, the signs of the A_{yy} and A_{zz} components are not known, preventing us from evaluating the isotropic and anisotropic terms of ^{17}O A-tensor for determination of 2s and 2p spin densities. To overcome this limitation, we computed ^{17}O hfs A-tensor using DFT and compared its values with experiment. Ub3lyp/dgdzvp calculations predicted the ^{17}O hfs A-tensor with two large components: 82.5 G and 66.2 G, which does not agree with experiment. Relativistic LDA/TZP and General-Gradient Approximation XC-functional parametrized by Becke-Ernzerhoff (GGAOPBE)/TZP calculations were carried out with Zeroth-Order Regular Approximation (ZORA). Earlier, these potential/basis sets were used by us to compute electronic structure and Ru L-edge XANES spectra for a model complex [Ru(NH$_3$)$_6$]$^{3+}$, and BD [3,3], and it was shown that these two basis sets well reproduce experimental XANES (44, 45). LDA/TZP was used here for geometry optimization because of prohibitive computational cost of GGAOPBE/TZP for the BD. Magnetic resonance parameters were evaluated by both LDA and the more sophisticated

Table 2. Spin densities and ^{17}O hfs from DFT analysis

Species	Method, charge, spin	Spin density* on oxygen and Ru	^{17}O hfs from DFT, Gauss a_{iso}	A-tensor
BD [4,5] Ru—O(2)—Ru ‖ | O(1) OH(3)	GW09 unb3lyp /dgdzvp 4+, S = 1/2	O (1) 1.07 O (2) −0.13 O (3) −0.16 Ru 1.09 Ru −0.86	O (1) −45.65	A$_{11}$ = 11.75, A$_{22}$ = −66.18, A$_{33}$ = −82.5
	ADF LDA/TZP 4+, S = 1/2	O (1) 0.68 O (2) −0.11 O (3) 0.01 Ru 0.55 Ru −0.16	O (1) −7.95	A$_{11}$ = −53.17, A$_{22}$ = −0.45, A$_{33}$ = 29.76 A-tensor in principle axes of g-tensor A$_{xx}$ = 28.97, A$_{xy}$ = 6.54, A$_{xz}$ = 3.16 A$_{yx}$ = 6.54, A$_{yy}$ = −52.30, A$_{yz}$ = −4.02 A$_{zx}$ = 3.16, A$_{zy}$ = −4.02, A$_{zz}$ = −0.53 g-tensor: g$_{xx}$ = 2.08, g$_{yy}$ = 2.02, g$_{zz}$ = 1.89
	ADF GGAOPBE /TZP 4+, S = 1/2	O (1) 0.82 O (2) −0.21 O (3) 0.03 Ru 0.57 Ru −0.24	O (1) −21.67	A$_{11}$ = −63.86, A$_{22}$ = −22.24, A$_{33}$ = 21.08 A-tensor in principle axes of g-tensor A$_{xx}$ = 12.27, A$_{xy}$ = 2.8, A$_{xz}$ = −17.39 A$_{yx}$ = 2.89, A$_{yy}$ = −63.72, A$_{yz}$ = 0.53 A$_{zx}$ = −17.39, A$_{zy}$ = 0.53, A$_{zz}$ = −13.57 g-tensor: g$_{xx}$ = 2.11, g$_{yy}$ = 2.02, g$_{zz}$ = 1.90
OH radical	GW09 unb3lyp /dgdzvp 0, S = 1/2	O 1.0	−39.17	A$_{11}$ = 12.10 A$_{22}$ = 11.16 A$_{33}$ = −140.77

*Spin densities were computed using scalar Hamiltonian ZORA approximation, allowing one to perform spin-polarized DFT calculations, whereas EPR properties were extracted from spin-orbit ZORA calculations.
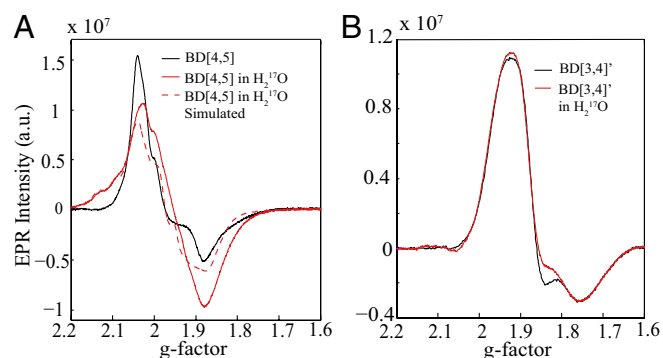
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Fig. 3. (*A*) EPR spectra of BD [4,5] (0.5 mM in 0.1 mol HNO_3) and BD [4,5]-^{17}O (1.2 mM in 0.1 mol HNO_3) extracted by subtracting EPR spectrum of BD [3,4]-prime and BD [3,4]-^{17}O-prime (1 mM in 0.1 mol HNO_3) products from initially obtained reaction mixtures. Simulation of the BD [4,5]-^{17}O spectrum is shown by the red dashed line. (*B*) Comparison of the EPR spectra of BD [3,4]-prime and BD [3,4]-^{17}O-prime species (1 mM in 0.1 mol HNO_3).

GGAOPBE approach with no significant difference in results (Table 2). Calculations with both potentials result in somewhat poor agreement for computed and experimental g-tensors, with up to 0.08 deviation for g_{xx}, 0.04 deviation for g_{yy}, and 0.03 deviation for g_{zz}. However, both methods predicted ^{17}O A-tensor with one large component: −64 G and −53 G (Table 2), which is in good agreement with experiment. The orientation of the g-tensor axis relative to the axis of the BD [4,5] molecule is not known, and will likely remain unknown, as it is not possible to crystallize BD [4,5] due to its short lifetime. Evaluation of the computed ^{17}O A-tensor in the principle axis of the g-tensor showed that DFT predicts the largest ^{17}O-hfs along the g_{yy} direction of the g-tensor, which does not agree with experiment. The error likely arises from poor results of the g-tensor calculations. To summarize results of DFT analysis, we can note that (*i*) all calculations show high spin density on the oxygen in the Ru^V = O fragment; (*ii*) LAD/TZP and GGAOPBE/TZP reproduce well the largest principle component of the ^{17}O A-tensor; and (*iii*) the predictive power of DFT for computation of the g-tensor remains qualitative (46) rather than quantitative, at least for complicated molecules such as the BD with two Ru atoms.

Formation of a π-bond in the M = O fragment was previously discussed in molecular orbital terms (47, 48). We propose that destabilization of π-bonding in the Ru^V = O fragment is responsible for the high spin density on the oxygen. To illustrate this proposed hypothesis, we analyzed MOs with a high contribution of oxygen (from the Ru^V = O fragment) 2p and Ru 4d orbitals, which are responsible for Ru^V = O bonding. MOs analysis of the BD is quite involved because of low symmetry and electronic delocalization. Thus, a simplistic model structure, $[RuO(H_2O)_5]^{3+}$, and an intermediate model, $[RuO(OH)(bpy)_2]^{2+}$, were analyzed, both S = 1/2, and parallel to the analysis of BD (Fig. 4, Table 3, and Table S3). In the simplistic model $[RuO(H_2O)_5]^{3+}$, an antibonding orbital (in this case, it is the HOMO) becomes populated with a single electron, and this electron is distributed between oxygen and Ru. Similar orbitals were observed for the intermediate model and BD (Table 3). In all cases, occupied molecular orbitals with significant contribution of the oxygen p-orbitals from the Ru^V = O fragment were identified, which were antibonding for the d^3 Ru^V = O fragment (Table 3). These singly occupied (α) orbitals had their symmetric (β) orbital counter partner among unoccupied orbitals (Table S3), allowing (at least in qualitative terms) an explanation for the high spin density on oxygen. Thus, we are able to understand the origin of the high spin density on the oxygen in the d^3 Ru^V = O fragment. Analysis of the role of the radicaloid oxygen in the activation of H_2O molecule is outside of the scope of this manuscript and will be presented elsewhere.

Although the spectrum of BD [4,5]-^{17}O is significantly affected by large ^{17}O splitting, spectra of BD [3,4]-prime and BD [3,4]-^{17}O-prime intermediates are similar, and no line broadening can be noted. The molecular structure of the BD [3,4]-prime intermediate remains unknown. Previous assignment of the 683-cm^{-1} band detected in the resonance Raman experiment, which showed a 46-cm^{-1} shift on ^{16}O/^{18}O substitution, to the O-O vibration shifted to an unusual frequency because of coupling with the vibration in the Ru-O-Ru bridge was premature (37). An isotope scrambling experiment in 50% H_2^{16}O and 50% H_2^{18}O did not reveal the 1:2:1 intensity pattern expected for the O-O fragment (Fig. S6). This Raman pattern still does not rule out the possibility that the detected vibration is a Ru-O vibration in the Ru-OOH peroxide fragment. Properties of the BD [3,4]-prime intermediate will be analyzed in the future by DFT computation of magnetic resonance parameters and Raman frequencies and by X-ray scattering. Low spin density on oxygen is consistent with a peroxide intermediate with end-on Ru-OOH coordination (Fig. 1). The O-O vibration in such a fragment might have low intensity in the resonance Raman spectrum.

In summary, we have experimentally shown high spin density on the oxygen in the d^3 Ru^V = O fragment of the BD [4,5] intermediate, which is reactive in catalytic water oxidation. We are not aware of other experimental studies of the spin density in M = O units. The Ru^V = O unit is the first to show large radicaloid character and associated large ^{17}O hfs. Ru^V= O fragment is a radical based on localized ligand orbitals. We cannot exclude that more radicals of this type will be discovered in the future by analysis of highly reactive M = O units with different metal ions with electronic configuration similar to Ru^V = O. DFT calculations reproduce the high spin density on the oxygen in Ru^V = O fragment. The presence of a singly occupied antibonding MO with a high contribution of oxygen p-orbital is responsible for the high spin density on oxygen and its activated radicaloid character. These conclusions suggest that the design of catalysts with partially stabilized oxyl radicals may be an important element in the development of water oxidation catalysts.
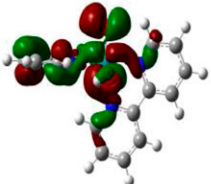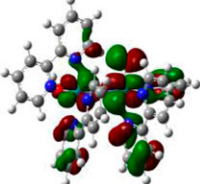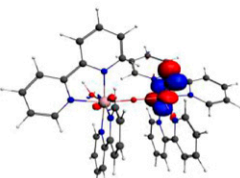
Materials and Methods
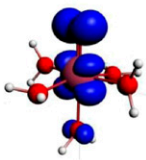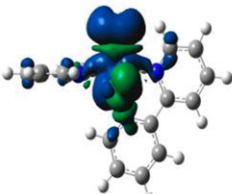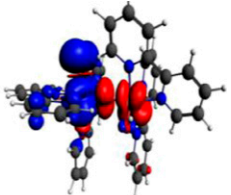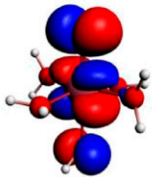
Fig. 4. Schematic MO diagram for $[RuO(H_2O)_5]^{3+}$ model from AFD, LDA/TZP, ZORA, and scalar calculations; more details are in Table S3. Energy of MOs is given in Hartrees (scale on the left); Ru and O atomic orbitals are shown on an arbitrary energy scale. Orbitals essential for Ru^V = O interaction are shown as black levels, and all other levels are gray. Unoccupied orbitals are colored in cyan and orange. An unpaired electron on the HOMO orbital (antibonding for Ru^V = O) is distributed between oxygen and Ru, contributing to considerable spin density on the oxygen in the Ru^V = O fragment.

Table 3. Visualization of spin density and MOs from DFT

Minimal model: $RuO(H_2O)_5]^{3+}$*	Intermediate model: $[RuO(OH)(bpy)_2]^{2+}$	BD [4,5]-OH	BD [4,5]-OH*
	Spin density on the oxygen in $Ru^V = O$ fragment		
0.51	0.70	1.06	0.68
	Visualization of the spin density		

Selected MOs contributing to high spin density on the oxygen.[†] These MOs have oxygen p character and are antibonding for $Ru^V = O$ fragment.

50-α HOMO	110-α HOMO-2	215-α HOMO-4	171-α HOMO

Calculations were performed with Gaussian09, unb3lyp/dgdzvp.
*Armsterdam Density Functional (ADF), LDA/TZP, ZORA, scalar ZORA model.
[†]Molecules were oriented to provide optimal visualization of the MOs localized to $Ru^V = O$ fragment.

oxidation with excess Ce(IV). The reaction mixtures were fast-freeze quenched into a precooled pentane bath (−120 °C) using an SFM 20 Stopped-Flow System (Bio-Logic) equipped with an umbilical connector with a built-in ejection nozzle. For isotopic exchange, BD [3,3] was dissolved in 98% O^{18} water and 60% ^{17}O water (Icon) and incubated for 24 h. The $Ru^V = O$ reference, tetra-n-propylammonium bis-2-hydroxy-2ethylbutyrato(oxo) ruthenate (V), was prepared as described by Dengel et al. (49). Its EPR spectrum was recorded at 20 K and found to be identical to the spectrum of the original report (49).

EPR Measurements. Low-temperature X-band EPR spectra were recorded using a Bruker EMX X-band spectrometer. The sample temperature was maintained at 20 K, unless otherwise indicated, by use of an Air Products LTR liquid helium cryostat. Spectrometer conditions were as follows: microwave frequency, 9.65 GHz; field modulation amplitude, 10 G at 100 kHz; microwave power, 31.70 mW. Field calibration was checked vs. 2-2,-diphenyl-1-picrylhydrazyl (DPPH) standard. EPR simulations were done with SimFonia software.

XAS and EXAFS Measurements. XAS spectra were collected at the Advanced Photon Source (APS) at Argonne National Laboratory (ANL) on bending magnet beamline 20 at electron energy of 23 keV and average current of 100 mA. The radiation was monochromatized by a Si(110) crystal monochromator. The intensity of the X-rays was monitored by three ion chambers (I_0, I_1, and I_2) filled with 70% nitrogen and 30% argon and placed before (I_0) and after the sample (I_1 and I_2). Plastic (Lexan) EXAFS sample holders (inner dimensions of $12 \times 3 \times 3$ mm) filled with freeze-quenched samples of BD [4,5] were inserted into a precooled (20 K) cryostat. The samples were kept at 20 K in an He atmosphere at ambient pressure. Data were recorded as fluorescence excitation spectra using a 13-element energy-resolving detector. The solid Ru^V sample was diluted with Boron Nitride (BN) powder in a 1:5 ratio, pressed between Mylar tape, and measured in the cryostat in transmission mode. To reduce the risk of radiation damage by X-ray radiation, 80% flux was used in the defocused mode (beam size = 1×10 mm), and no damage was observed scan after scan to any samples. The samples were also protected from the X-ray beam during spectrometer movements by a shutter synchronized with the scan program. Additionally, low-flux measurements (only 10% of beamline flux) were done on reactive intermediate BD [4,5] to show the same EXAFS results. No more than five scans were taken at each sample position at any condition.

The Ru XAS energy was calibrated by the first maxium in the second derivative of the Ru metal XANES spectrum (22,117 eV). EXAFS scan with 10-eV steps in the preedge region (21,967–22,102 eV), 1-eV steps (22,102–22,117 eV) through the edge, and 0.05-$Å^{-1}$ steps from k = 2.0–16 $Å^{-1}$ were used. As reference compound for BD [4,5], Ru(IV) and Ru(V), Ru(IV) oxide and tetra-n-propylammonium bis-2-hydroxy-2-ethylbutyrato(oxo) ruthenate(V) (49) were used (Fig. 2A). Additional Exafs analyses are provided in *SI Materials and Methods*.

DFT Calculations. DFT calculations performed with Gaussian09 used B3LYP exchange correlation (XC) functional and dgdzvp basis set, with all electrons of the Ru atom explicitly included in calculations (no frozen cores) (50). For DFT performed with the Amsterdam Density Functional (ADF) 2010 package (51) exploiting Slater-type orbitals expansion as opposed to Gaussian-type ones from Gaussian09 suite, the LDA XC functional parameterized by Vosko et al. (52) and general gradient approximation XC functional parameterized by Becke-Ernzehoff (GGAOPBE) were used with polarized triple-ζ basis set (LDA/TZP and GGAOPBE/TZP). These basis sets include three Slater functions for each orbital; for Ru atom, it includes two 5p and three more 4f additional polarized functions. No frozen core approximation was used. Relativistic effects were accounted for by simulations within ZORA Hamiltonian. ADF calculations were performed for systems in gaseous phase. All DFT calculation were done for the whole molecules treating all ligands explicitly.

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