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Reduction of Bis(dithiolene)oxo(disulfido)tungsten(VI) Complex with Dihydrogen Related to the Chemical Function of the Fourth Tungsten-Containing Enzyme (WOR4) from *Pyrococcus furiosus*

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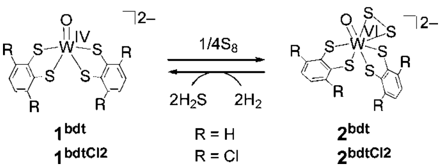
Discovery of the fourth tungsten oxidoreductase (WOR4) from hyperthermophilic archaeon *Pyrococcus furiosus* has expanded coordination and bioinorganic chemistry of heavy metal ions.[1,2] The WOR4 enzyme plays an important role in the elemental sulfur (S^0) metabolism, reducing S^0 to hydrogensulfide in the presence of dihydrogen (eq 1).[1,2] By this function, the WOR4 enzyme is distinguished from other tungsten enzymes.[2,3]

$$S^0 + H_2 \rightarrow H_2S \qquad (1)$$

The presence of a tungsten reaction center coordinated by two pyranopterin dithiolenes in the WOR4 enzyme has been suggested by a sequence comparison with the AOR family,[1,4] but both the sulfur activation mechanism and other coligands of the tungsten ion are still subjects of debate. Although the recent progress of biomimetic studies for the tungsten centers of AOR,[5] FDH,[4,6] and AH[4,7] families has significantly contributed to the elucidation of structure/reactivity relationships as well as the electronic structures of the tungsten centers,[2,3] a modeling study for the WOR4 reaction center has yet to be accomplished in detail. Recently, we reported the reductive activation of elemental sulfur by $[W^{IV}O(bdt)_2]^{2-}$ ($\mathbf{1}^{\mathbf{bdt}}$)[4] to give its disulfide adduct $[W^{VI}O(\eta^2\text{-}S_2)(bdt)_2]^{2-}$ ($\mathbf{2}^{\mathbf{bdt}}$),[8] providing a good model compound of the WOR4 reaction center. We herein report that the disulfide adduct $\mathbf{2}^{\mathbf{bdt}}$ reacts with dihydrogen (H_2) smoothly to give $\mathbf{1}^{\mathbf{bdt}}$ and 2 equiv of H_2S, the first functional model of the WOR4 enzyme (Scheme 1).

Scheme 1



Bis(dithiolene)$W^{IV}O$ complexes $\mathbf{1}^{\mathbf{L}}$ (L = bdt and $bdtCl_2$)[4] were readily converted to the corresponding disulfide adducts, $(Et_4N)_2$-$[W^{VI}O(\eta^2\text{-}S_2)(dithiolene)_2]$ ($\mathbf{2}^{\mathbf{bdt}}$ and $\mathbf{2}^{\mathbf{bdtCl2}}$), by the reaction with elemental sulfur (S_8) in CH_3CN at room temperature. The reaction of $\mathbf{1}^{\mathbf{L}}$ was monitored by the appearance of a strong absorption band at ~400 nm, and a titration experiment for the sulfurization indicated the 1:2 stoichiometry for $\mathbf{1}^{\mathbf{L}}$ vs S^0 (Figure S1). The observed rate constant k_{obs} for the formation of $\mathbf{2}^{\mathbf{bdt}}$ was 9.2×10^3 M^{-1} s^{-1} in CH_3CN at 25 °C, which was more than 10^3 times larger than that for $\mathbf{2}^{\mathbf{bdtCl2}}$ (5.6 M^{-1} s^{-1}) under the same conditions. X-ray analysis of $\mathbf{2}^{\mathbf{bdtCl2}}$ revealed a distorted pentagonal-bipyramidal tungsten center with two $bdtCl_2$, oxo, and side-on disulfide ligands as found in $\mathbf{2}^{\mathbf{bdt}}$ (Figure S2).[8] Although $[W^{IV}O(mnt)_2]^{2-}$ ($\mathbf{1}^{\mathbf{mnt}}$)[4] was reported to give a similar disulfide adduct $\mathbf{2}^{\mathbf{mnt}}$ with elemental sulfur, the sulfurization required more severe conditions (refluxing in CH_3CN for 24 h).[5c] Thus, the sulfur activation ability of the $W^{IV}O$ complexes increased in the order $\mathbf{1}^{\mathbf{mnt}} \ll \mathbf{1}^{\mathbf{bdtCl2}} < \mathbf{1}^{\mathbf{bdt}}$ as the electron donation by the dithiolene ligand increases.[9] Then, the reaction of $\mathbf{2}^{\mathbf{L}}$ with H_2 was investigated to construct a model system of the WOR4 catalytic cycle (Scheme 1).

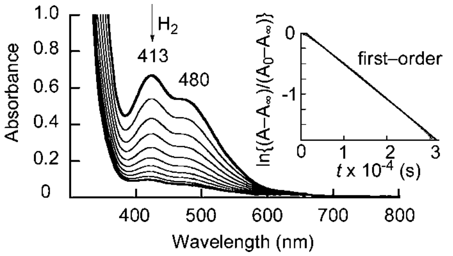


Figure 1. Spectral change for the reaction of $\mathbf{2}^{\mathbf{bdt}}$ (2.3×10^{-4} M) with H_2 (1 atm) in CH_3CN at room temperature. (Inset) First-order plot based on the decay of absorption at 413 nm.

Figure 1 shows a spectral change for the reaction of $\mathbf{2}^{\mathbf{bdt}}$ with H_2 (1 atm) in CH_3CN at room temperature, where characteristic absorption bands at 413 nm ($\epsilon = 2990$ M^{-1} cm^{-1}) and 480 nm ($\epsilon = 2490$ M^{-1} cm^{-1}) due to $\mathbf{2}^{\mathbf{bdt}}$ gradually decrease obeying first-order kinetics (Figure 1, inset, $k_{obs} = 1.0 \times 10^{-2}$ s^{-1}). The final spectrum was identical with that of $\mathbf{1}^{\mathrm{bdt}}$. The regeneration of $\mathbf{1}^{\mathrm{bdt}}$ from $\mathbf{2}^{\mathbf{bdt}}$ upon treatment with H_2 (1 atm) was also confirmed by ^{1}H NMR (Figure S3).[11] In a preparative scale reaction, $\mathbf{1}^{\mathbf{bdt}}$ was isolated in a 90% yield by a reaction of $\mathbf{2}^{\mathbf{bdt}}$ with H_2, and a formation of H_2S was confirmed by gas chromatography and $Pb(CH_3COO)_2$ test paper. Furthermore, addition of 2 equiv of $Cu(CH_3CO_2)_2 \cdot H_2O$ to a final reaction mixture of $\mathbf{2}^{\mathbf{bdt}}$ under H_2 (1 atm) gave CuS in a 176% yield based on $\mathbf{2}^{\mathbf{bdt}}$. These results clearly indicate that the reduction of bis(dithiolene)$W^{VI}O(\eta^2\text{-}S_2)$ complex $\mathbf{2}^{\mathbf{bdt}}$ with H_2 gives starting $W^{IV}O$ complex $\mathbf{1}^{\mathbf{bdt}}$ and 2 equiv of H_2S. Notably, the reduction of elemental sulfur (S^0) to H_2S by H_2 (1 atm) proceeded catalytically in the presence of a small amount of $\mathbf{2}^{\mathbf{bdt}}$ (1 mol %), where a turnover number of 24 for the catalyst was achieved after 24 h.

On the other hand, the reduction of $\mathbf{2}^{\mathbf{L}}$ with H_2 did not proceed at all when the weaker electron-donating dithiolene ligands $bdtCl_2$ and

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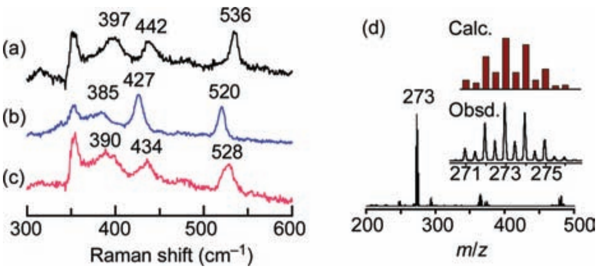

Figure 2. Resonance Raman spectra of (a) $\mathbf{2^{bdt}}$, (b) $\mathbf{2^{bdt}{-}^{34}S_2}$, and (c) their 1:1 mixture in CD_3CN with excitation at 488 nm. (d) Negative ESI-MS of 1:1 mixture of $\mathbf{2^{bdt}}$ and $\mathbf{2^{bdt}{-}^{34}S_2}$ in CH_3CN: isotope pattern simulated as 1:2:1 mixture of $\mathbf{2^{bdt}}$, $\mathbf{-^{32}S^{34}S}$, and $\mathbf{-^{34}S_2}$ is indicated as Calc.

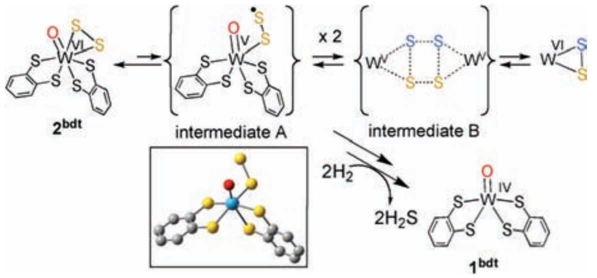

Figure 3. Proposed mechanism for the hydrodesulfurization and sulfur scrambling with $\mathbf{2^{bdt}}$. (Inset) DFT optimized structure of triplet intermediate A: tungsten (blue), sulfur (yellow), oxygen (red).

mnt were employed,[12] as revealed by the intact UV−vis and ^{1}H NMR spectra of acetonitrile solutions containing $\mathbf{2^L}$ with H_2 (1 atm) even at an elevated temperature of 80 °C. To gain mechanistic insights into the reduction of $\mathbf{2^{bdt}}$, resonance Raman (rR) and electrospray ionization mass spectroscopic (ESI-MS) techniques were employed. As indicated in Figure 2a, $\mathbf{2^{bdt}}$ exhibited three isotope sensitive rR bands in CD_3CN, where the band at 536 cm^{-1} was assigned to the ν(S−S) stretching vibration and the other two at 442 and 397 cm^{-1} were assigned to ν(W−S) ones based on the observed isotope shifts with $\mathbf{2^{bdt}{-}^{34}S_2}$ (Figure 2b).[13] When the CD_3CN solution of $\mathbf{2^{bdt}}$ was mixed with that of labeled $\mathbf{2^{bdt}{-}^{34}S_2}$ in a 1:1 ratio, a rR spectrum that corresponds to a mixture of $\mathbf{2^{bdt}{-}^{32}S_2}$, $\mathbf{-^{32}S^{34}S}$, and $\mathbf{-^{34}S_2}$ in a 1:2:1 ratio was obtained (Figure 2c). The ESI-MS of an acetonitrile solution containing $\mathbf{2^{bdt}{-}^{32}S_2}$ and $\mathbf{2^{bdt}{-}^{34}S_2}$ (1:1) also gave a peak cluster around m/z = 273 assignable to a mixture comprised of $\mathbf{2^{bdt}{-}^{32}S_2}$, $\mathbf{-^{32}S^{34}S}$, and $\mathbf{-^{34}S_2}$ in a 1:2:1 ratio (Figure 2d). In the case of $\mathbf{2^{bdtCl2}}$ supported by the weaker electron-donating dithiolene, however, such an isotope scrambling between the $^{32}S_2$ and $^{34}S_2$ derivatives was not observed at all in both rR and ESI-MS experiments.[14] Thus, it can be concluded that only a bis(dithiolene)$W^{VI}O(S_2)$ complex which can be reduced by H_2 exhibits a dynamic behavior accompanying the scrambling of sulfur atoms of the WS_2 moiety.

On the basis of these results, a mechanism shown in Figure 3 is proposed for the reduction of $\mathbf{2^{bdt}}$ with H_2. The sulfur scrambling may occur through a dimeric W(V) intermediate **B**,[15] which may be generated from $\mathbf{2^{bdt}}$ via a six-coordinate triplet W(V) species with an end-on S_2^- group (intermediate **A**).[16] The intermediate **A** is the most plausible active species that reacts with H_2, since the reaction obeys first-order kinetics with respect to the tungsten complex and the reaction proceeds only from the disulfide adduct $\mathbf{2^{bdt}}$ showing the sulfur scrambling (no reduction took place from neither $\mathbf{2^{bdtCl2}}$ nor $\mathbf{2^{mnt}}$ which exhibit no sulfur scrambling). The DFT calculation at the (U)B3LYP level of theory indicated that the intermediate **A** is 9 kcal/mol higher in energy than $\mathbf{2^{bdt}}$ itself.[18] The intermediate **A** binds H_2 at its open seventh coordination site to form an adduct such as $W^V(H_2)(\eta^1\text{-}S_2)$ from which H_2S is evolved.[19] A stronger electron donation by bdt to the W center may enhance the rearrangement of the disulfide group from side-on to end-on by *trans* influence. Such a rearrangement may not occur in the case of $\mathbf{2^{bdtCl2}}$, having a stronger Lewis acidic W center due to the weaker electron-donating ability of $bdtCl_2$.[20]

In summary, an efficient catalytic model system of the WOR4 enzyme, reduction of S_8 to H_2S *via* $W^{VI}O(S_2)$ complex with the aromatic dithiolene ligand, has been successfully constructed for the first time. Further studies are now being undertaken to elucidate the mechanistic details of the disulfide reduction process and ligand effects using olefinic dithiolene ligands like pyranopterin dithiolene in the enzyme.

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Supporting Information Available: Experimental details and spectroscopic and the crystallographic data of $\mathbf{1^L}$ and $\mathbf{2^L}$, and computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) Abbreviation: AOR (aldehyde oxidoreductase), FDH (formate dehydrogenase), AH (acetylene hydratase), bdt (1,2-benzenedithiolate), $bdtCl_2$ (3,6-dichloro-1,2-benzenedithiolate), mnt (1,2-dicyanoethylene-dithiolate).
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(11) In addition to $\mathbf{1^{bdt}}$, a small amount (∼10%) of $[WO_2(bdt)_2]^{2-}$ was formed. Substitution of the S_2^{2-} group with contaminated H_2O in the NMR sample might take place.
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(13) These frequencies are in the ranges of ν(S−S) and ν(W−S) stretching values reported in the literature. Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 5th ed.; John Wiley & Sons: New York, 1997.
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(20) The weaker Lewis acidity of the W center in $\mathbf{2^{bdt}}$ is reflected by the longer W−S (disulfide) bond lengths (2.496(5) Å) and the largely negative redox potential (−1.25 V vs SCE) for the W(VI)/(V) redox couple as compared with those of $\mathbf{2^{bdtCl2}}$ (2.4538(10) Å; −0.95 V, respectively).

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