Benzoin Oxidation at Extreme Temperature by Bis(1,2-benzenedithiolato)dioxotungstate(VI) Complex: A Model Study for Hyperthermostable Tungsten Oxidoreductases

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The oxidation of benzoin by $(NEt_4)_2[M^{VI}O_2(S_2C_6H_4)_2]$ (M = W (**1**) and Mo (**2**)) ($S_2C_6H_4$ = 1,2-benzenedithiolate) was studied to clarify the role of tungsten ions in hyperthermostable enzymes. The reaction was analyzed by saturation kinetics, primary isotope effects of k_H/k_D, and the high temperture reaction at 100 °C. The rate constants were also compared with those of the selenolato analogs.

Recently, the biological role for tugsten has been established as well as for the chemically analogous element, molybdenum.[1] For example, tungsten oxidoreductases work in energy metabolism pathway of hyperthermophilic organisms which grow at extreme temperature (above 100 °C).[2] A crystallographic report by Rees et al. has revealed the *bis*(dithiolene) coordination to tungsten ion in aldehyde ferredoxin oxidoreductase.[3] The reported structure of tungsten cofactor closely resembles our previously reported model complexes, $(NEt_4)_2[W^{VI}O_2(S_2C_6H_4)_2]$ (**1**) ($S_2C_6H_4$ = 1,2-benezenedithiolato).[4] Both structures have bis(dithiolene) coordination on a hexa-coordinated tungsten atom.[5] To clarify the role of tungsten ions in thermostable enzymes, we have studied the oxidation of benzoin with the tungsten model complex, **1**. Benzoin is a suitable model substrate, if the aldehyde oxidation process of the tungstopterin enzyme is via an acetal state (RCH(OH)OH) similar to other aldehyde oxidases.[3] For some molybdopterin enzymes, a benzoin related substrate, (2*R*)-hydroxycarboxylate ($RCH(OH)COO^-$) was reported by H. Simon et al.[6]

The complexes, **1** and **2**, react with benzoin to give a reduced state model, $(NEt_4)_2[M^{IV}O(S_2C_6H_4)_2]$ (M = W (**3**) and Mo (**4**)) and benzil without side reaction (eq.1).[4,7] The clean formation of **3** or **4**, and benzil were detected by ^1H NMR spectra.

$$(M^{VI}O_2)^{2+} + PhCH(OH)COPh \longrightarrow (M^{IV}O)^{2+} + PhCOCOPh + H_2O \qquad (1)$$

Table 1. Deuterium isotope effect on the rate constants[14] (k_{obs} ($10^{-6}s^{-1}$)) of the oxidation of benzoin in DMF-d_7 at 30 °C

	1	2
benzoin	5.6 (± 0.6)	22 (± 4)
α-deuterated benzoin	2.2 (± 0.1)	5.4 (± 0.1)
k_H/k_D	2.5	4.0

((Reaction conditions: [**1**] and [**2**] = 1.0 mmol dm^{-3}, [benzoin] = 10 mmol dm^{-3}. Data were analyzed by pseudo first-order kinetics, d[benzil]/dt = k_{obs} [benzoin].)

In Figure 1, time conversion curves for **1** and **2** from ^1H NMR spectra are shown and were analyzed by the pseudo first-order kinetics. In the presence of 10- and 5-fold excess benzoin, the rate constant of **2** was nearly independent of benzoin concentration.

A primary isotope effect of k_H/k_D = 2.5 and 4.0 has been observed with the addition of a 10-fold excess of α-deuterobenzoin[8] for **1** and **2**, respectively. The rate-limiting step of the reaction thus involves abstraction of an α-hydrogen atom of the benzoin by an oxo ligand.

To investigate the reactivity at extreme temperature, we have examined the oxidation reaction of benzoin at 100 °C. Figure 2 shows the time-conversion curves and the obtained rate constants. At 30 °C the rate constant (k_W) for the W complex, **1**, was 2.4 times smaller than that (k_{Mo}) of the Mo analog, **2**. The yield of benzil was 7% for **1** and 13% for **2** after 25 h of the reaction. On the other hand, at 100 °C, the reactivity of the W complex, **1**, was increased significantly (k_{Mo}/k_W = 1.5). In addition, the benzil yield for **1** (90%) was higher than that for **2** (82%) after 25 h of the reaction. This reactivity improvement at high temperature probably implicate the reason why W ions are only incorporated into hyper- and meso-thermostable enzymes.[9,1] Actually in the hyper-thermophillic bacteria, only W-containing enzymes have been

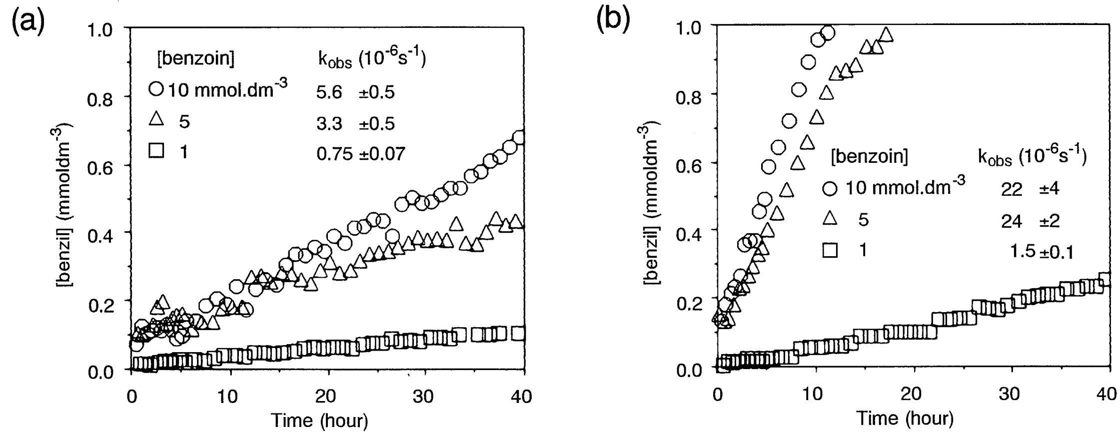

Figure 1. ^1H NMR time conversion curves of the reaction of $(NEt_4)_2[M^{VI}O_2(S_2C_6H_4)_2]$ (M = (a) W (**1**) and (b) Mo (**2**)) with benzoin in DMF-d_7 at 30 °C ([M] = 1.0 mmol dm^{-3}). Data were analyzed by pseudo first order kinetics, d[benzil] / dt = k_{obs}[benzoin].

Table 2. Comparison of rate constants[14] (k_{obs} ($10^{-6}s^{-1}$)) of the benzoin oxidation with selenolato and thiolato complexes

complex	k_{obs}
$(NEt_4)_2[W^{VI}O_2(S_2C_6H_4)_2]$ (**1**)	5.6 (± 0.6)
$(NEt_4)_2[W^{VI}O_2(SeSC_6H_4)_2]$ (**5**)	4.6 (± 0.5)
$(NEt_4)_2[W^{VI}O_2(Se_2C_6H_4)_2]$ (**6**).	4.7 (± 0.7)

(Reaction conditions: [W] = 1.0 mmol dm^{-3}, [benzoin] = 10 mmol dm^{-3}, in DMF-d_7 at 30 °C. Data were analyzed by pseudo first-order kinetics, d[benzil]/dt = k_{obs} [benzoin].)

isolated and any Mo-enzyme has not been found.[1]

Selenolate is a probable ligand in W and Mo oxidoreductases.[10] In the case of a Mo enzyme, formate dehydrogenase, the EPR spectra has proved the coordination of a selenocystein residue to a Mo ion.[11] To clarify the role of Se ligand in W enzymes, we have examined the reactivity of tungsten-selenolate complexes,[12] $(PPh_4)_2[W^{VI}O_2(SeSC_6H_4)_2]$ ($SeS_2C_6H_4$ = 1-selenolato-2-thiolatobenzene) (**5**) and $(PPh_4)_2[W^{VI}O_2(Se_2C_6H_4)_2]$ ($Se_2C_6H_4$ = 1,2-benzenediselenolato) (**6**). For the reaction system which contained a 10-fold excess of benzoin, the reaction behavior was compared with the thiolate complex, **1**, (k_{obs} = 5.6 (± 0.6) x 10^{-6} s^{-1}). As a result, the selenolate complexes, **5** and **6**, showed almost same rate constant, k_{obs} = ~4.7 x 10^{-6} s^{-1} (Table 2).[7,12] Interestingly, this does not correspond to the tendency of the oxo-releasing potential of **1**, **5** and **6**.[13]

In conclusion, based on our model study, W ion is relatively suitable for using at higher temperature. Because the relative reactivity (compared with the Mo analog for k_{Mo}/k_W and the yield of benzil) was improved with an increase in temperature. Presence of selenolato ligand does not affect the oxo-transfer reactivity.

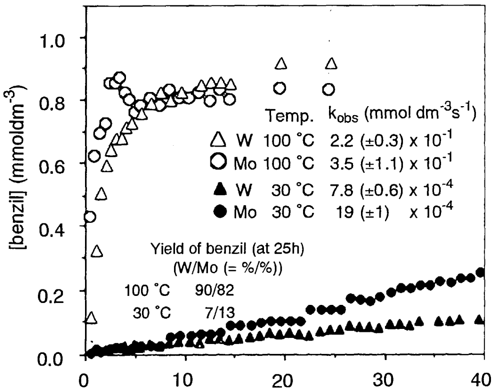

Figure 2. ^{1}H NMR time conversion curves of the reaction of $(NEt_4)_2[M^{VI}O_2(S_2C_6H_4)_2]$ (M = W (**1**) and Mo (**2**)) with benzoin in DMF-d_7 at 100 and 30 °C ([M] = 1.0 mmol dm^{-3}). Data were analyzed by second order kinetics, -d[benzoin] / dt = k_{obs}[benzoin][complex].

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References and Notes

1 A. Kletzin and M. W. W. Adams, *Fems Microbiol. Rev.* ,**18**, 5 (1996).
2 M. W. W. Adams and R. M. Kelly, *Biocatalysis at Extreme Temperatures*. (American Chemical Society, Washigton, DC, 1991).
3 M. K. Chan, S. Mukund, A. Kletzin, M. W. W. Adams, and D. C. Rees, *Science*, **267**, 1463 (1995).
4 N. Ueyama, H. Oku, and A. Nakamura, *J. Am. Chem. Soc.*, **114**, 7310 (1992).
5 H. Oku, N. Ueyama, and A. Nakamura, *Chem. Lett.*, **1996**, 31.
6 T. Trautwein, F. Krauss, F. Lottspeich, and H. Simon, *Eur. J. Biochem.*, **222**, 1025 (1994).
7 a) N. Ueyama, H. Oku, M. Kondo, T. Okamura, N. Yoshinaga, and A. Nakamura, *Inorg. Chem..*, **35**, 643 (1996). b) Reaction systems containing the $(M^{VI}O_2)^{2+}$ complex (M = W (**1** and **5**) and Mo (**2** and **6**)) and benzoin were monitored using a 400 MHz ^{1}H-NMR spectrometer (JEOL GSX-400) with measurements of the ^{1}H signals of benzil and benzoin. A solution of benzoin (DMF-d_7) in a 5 mm NMR gas tight tube containing a benzenedithiolato complex solution in DMF-d_7 was prepared under argon atmosphare at 27 °C.
8 α-C-deuterated benzoin was prepared by the reported method of A. G. Harrison and R. K. M. R. Kallury, *Org. Mass Spectrom.*, **15**, 249 (1980). Based on 400 MHz ^{1}H NMR spectra, 98% of α-C-hydrogen atom was found deuterated.
9 R. A. Schmitz, M. Richter, D. Linder, and R. K. Thauer, *Eur. J. Biochem..*, **207**, 559 (1992).
10 a) V. N. Gladyshev, S. V. Khangulov, M. J. Axley, and T. C. Stadtman, *Proc. Natl. Acad. Sci. USA* **91**, 7708 (1994). b) J. C. Deaton, E. I. Solomon, G. D. Watt, P. J. Wetherbee, and C. N. Durfor, *Biochem. Biophys. Res. Commun.*, **149**, 424 (1987).
11 The synthesis of **5** and **6** was done by the similar procedure to the synthesis of **1**.[4] Anal. Calcd for **5**, $C_{60}H_{48}O_2P_2WSe_2S_2$: C, 56.79 H, 3.81. Found: C, 56.59; H, 3.87. For **6**, $C_{60}H_{48}O_2P_2WSe_4$ C, 52.89; H, 3.55. Found: C, 52.78; H, 3.55.
12 Benzoin concentration dependency was studied with the reaction systems containing the $(W^{VI}O_2)^{2+}$ complexes (**1**, **5**, and **6**, [M] = 1 mmol.dm^{-3}) and benzoin (1, 5, 10, and 20 mmol dm^{-3}) in DMF. The systems were monitored using a HPLC (JASCO880-PU) and a C-18 ODS column. The obtained rate constants[14] (k_{obs}) were as follows:

[benzoin] (mmol.dm^{-3})	**1** ($10^{-6}s^{-1}$)	**5** ($10^{-6}s^{-1}$)	**6** ($10^{-6}s^{-1}$)
1	1.7 (± 0.3)	1.1 (± 0.2)	1.7 (± 0.5)
5	1.6 (± 0.3)	1.2 (± 0.2)	1.7 (± 0.4)
10	3.2 (± 0.2)	5.0 (± 0.9)	5.0 (± 0.9)
20	4.9 (± 0.7)	5.6 (± 0.6)	4.6 (± 0.6)

13 In DMF, **1**, **5** and **6** exhibited irreversible peaks at -1.26, -0.88, and -0.55 V vs SCE, respectively, presumably assignable to the W(VI)/W(V) redox process and the successive O-atom releasing reactions when scanned in the cathodic direction. L. J. DeHayes, H. C. Faulkner, J. W. H. Doub, and D. T. Sawyer, *Inorg. Chem.*, **14**, 2110 (1975).
14 All errors in parences are random errors estimated at the 99% confidential level (2.5σ).