Dalton Transactions

PAPER

View Article Online
View Journal | View Issue

Cite this: *Dalton Trans.*, 2015, **44**, 12618

Modeling of the hydrophobic microenvironment of water-soluble molybdoenzymes in an aqueous micellar solution†

Yuki Hasenaka, Taka-aki Okamura* and Kiyotaka Onitsuka

Received 20th March 2015,
Accepted 28th May 2015

DOI: 10.1039/c5dt01112d

www.rsc.org/dalton

A toluene-soluble molybdenum(VI) complex containing a bulky hydrophobic substituent, $(Et_4N)_2$-$[Mo^{VI}O_2\{1,2\text{-}S_2\text{-}3,6\text{-}(RCONH)_2C_6H_2\}_2]$ (R = (4-^{t}BuC$_6$H$_4$)$_3$C), was dissolved in the hydrophobic core of a micelle in an aqueous medium and catalyzed the biomimetic reduction of an amine *N*-oxide by an NADH analog. The kinetic isotope effect of solvent water clearly indicates that water molecules are essential for catalysis and are involved in the rate-determining step.

Introduction

Trimethylamine *N*-oxide reductase (TMAOR), a member of the DMSO reductase family, is a molybdoenzyme with two dithiolene ligands, called the pterin cofactor. This enzyme catalyzes oxygen-atom-transfer (OAT) reactions *via* the Mo(IV) and Mo(VI) oxidation states (Fig. 1).[1–4] The molybdenum(IV) center reductively eliminates an oxygen atom from amine *N*-oxides to form a Mo^{VI}=O bond, and is regenerated *via* protonation and reduction accompanied by the production of water. In the anaerobic respiratory chain, nicotinamide adenine dinucleotide (NADH) acts as an electron donor to reduce amine *N*-oxides, the terminal electron acceptors.[3,5] In the case of biotin sulfoxide reductase (BSOR), the direct hydride transfer from NADH phosphate to the active site has been proposed by kinetic analyses.[6–8]

The active site of TMAOR is located at the base of a large funnel-shaped depression, and the ligands are not exposed to the surface (Fig. 1).[1,9] The hydrophobic pocket formed by aromatic residues at the base of the depression is conserved in both TMAOR and BSOR.[1,7] The above results suggest the importance of hydrophobicity in the enzyme activity.

A number of molybdoenzyme models have been synthesized,[10–16] some of which have simulated the catalytic reduction of amine *N*-oxides by phosphines *via* coupled OAT reactions.[17–21] Monooxomolybdenum(IV) complexes with benzene-1,2-dithiolate (bdt) ligands can be used to eliminate the oxygen atom of trimethylamine *N*-oxide to yield a Mo^{VI}=O bond; the resulting dioxomolybdenum(VI) complexes can be reduced by benzoin to regenerate the original monooxomolybdenum(IV) complexes with the production of water.[22,23]

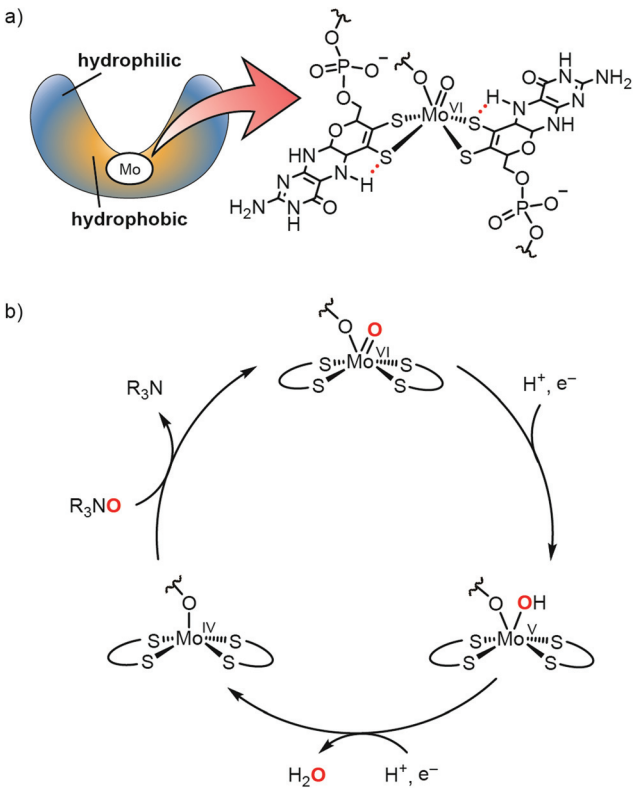


Fig. 1 (a) The structure of the active site and (b) catalytic cycle of trimethylamine *N*-oxide reductase.

Department of Macromolecular Science, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan.
E-mail: tokamura@chem.sci.osaka-u.ac.jp; Fax: +81 6 6850 5474; Tel: +81 6 6850 5451

†Electronic supplementary information (ESI) available: The whole spectra of Fig. 3, kinetic plot of the catalytic reduction of DDAO by BNAH in water-containing toluene-d_8. See DOI: 10.1039/c5dt01112d

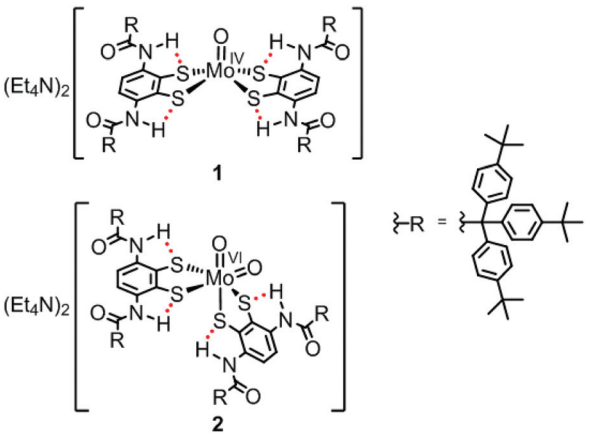


Chart 1 Designation of molybdenum complexes.

We have recently reported toluene-soluble monooxomolybdenum(IV) and dioxomolybdenum(VI) complexes, $(Et_4N)_2[Mo^{IV}O\{1,2\text{-}S_2\text{-}3,6\text{-}(RCONH)_2C_6H_2\}_2]$ (**1**), (R = $(4\text{-}^tBuC_6H_4)_3C$) and $(Et_4N)_2[Mo^{VI}O_2\{1,2\text{-}S_2\text{-}3,6\text{-}(RCONH)_2C_6H_2\}_2]$ (**2**) (Chart 1). The introduction of bulky hydrophobic substituents covered the polar molybdenum center completely with a hydrophobic barrier, and the approach of Me_3NO to the active center was found to be more efficient in hydrophobic media.[24] If the complexes are solubilized in an aqueous medium, the biomimetic catalysis using protons of water is expected to be achieved.

We present here the biomimetic catalytic reduction of an amine *N*-oxide by an NADH analog in an aqueous micellar solution. There are a few reports on models promoting the OAT reaction in aqueous media; however, the reports are limited to the use of a mixture of polar solvents and water or wet organic solvents.[25,26] Our trial in this paper is to simulate heterogeneous environments around the active sites of enzymes. The model complex in the hydrophobic core of a micelle mimics the active site in the hydrophobic pocket. The outside of a micelle resembles the surroundings of water-soluble enzymes although it does not represent a pure aqueous solution.

Experimental

All procedures were performed under an argon atmosphere by using the Schlenk technique. All organic solvents were dried and distilled under argon before use. Reagents were obtained commercially and used without further purification. $(Et_4N)_2[Mo^{VI}O_2(1,2\text{-}S_2\text{-}3,6\text{-}\{(4\text{-}^tBuC_6H_4)_3CCONH\}_2C_6H_2)_2]$ (**2**)[24] was prepared by reported methods.

Stoichiometric reaction between 1 and DDAO and the subsequent reduction by BNAH in toluene/THF

The procedure was similar to that described in the previous paper[24] for Me_3NO in toluene, except for the use of DDAO (30 mM, 20 μL) in THF. After the complete formation of **2** was confirmed, a solution of BNAH (30 mM, 20 μL) in THF was added. After 20 min, H_2O (10 μL, 180 mM) was added, and the time course of the reaction was monitored by using the absorption maximum of BNAH.

Catalysis in toluene-d_8

To an NMR tube cooled at −78 °C were successively added a solution of DDAO (0.80 mg, 3.5 μmol) in a mixture of toluene-d_8 (0.3 mL)/H_2O (0.2 μL, 11 μmol), a solution of BNAH (0.74 mg, 3.5 μmol) in toluene-d_8 (0.3 mL), and a solution of **2** (0.51 mg, 0.21 μmol) in toluene-d_8 (0.1 mL). To start the reaction, the contents were quickly warmed up to 27 °C with shaking. The reaction was monitored at 27 °C by using ^{1}H NMR spectroscopy.

Preparation of a micellar solution of 2

To a solution of **2** (1.55 mg, 0.63 μmol) in THF (0.3 mL) was added a 0.1 M THF solution of Triton™ X-100 (0.9 mL, 90 μmol). The resulting reddish-brown solution was concentrated under reduced pressure, and water (1.8 mL) was added to afford a 0.35 mM aqueous micellar solution of **2**.

Catalysis in a micellar solution

A reaction system containing **2**, DDAO, and BNAH was monitored in the 280–1000 nm region. The measurements were carried out in a 1 mm UV cell at 27 °C. After the thermal equilibrium, a 90 mM aqueous solution of DDAO (10 μL, 0.9 μmol) and a 30 mM THF solution of BNAH (30 μL, 0.9 μmol) were added to a 0.35 mM aqueous micellar solution of **2** (0.26 mL, 0.09 μmol), and the cell contents were quickly mixed by shaking. The absorption at 355 nm (BNAH) was monitored at 27 °C.

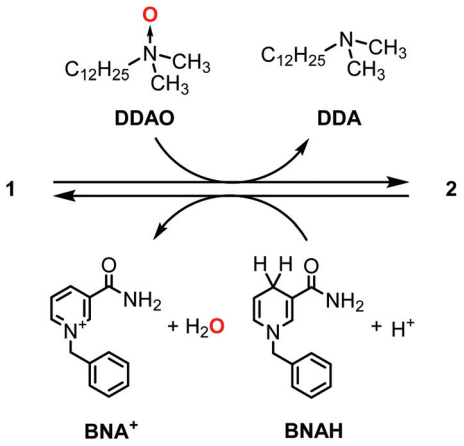
Physical measurements

^{1}H NMR spectra were recorded using a JEOL ECA-500 spectrometer in toluene-d_8 at 27 °C. UV-visible absorption spectra were recorded using a SHIMADZU UV-3100PC spectrometer.

Results

Stoichiometric and catalytic reactions in nonpolar solvent

Because of the limitations of the solubility of the reagent in organic solvents, we designed a model system of a catalytic reaction using dodecyldimethylamine *N*-oxide (DDAO) and an NADH analog, 1-benzyl-1,4-dihydronicotinamide (BNAH). At first, a stoichiometric reaction in organic solvents was examined using UV-vis spectroscopy (Scheme 1, Fig. 2). When 2 equivalents of DDAO were added to a toluene solution of complex **1** (0.1 mM), a rapid OAT reaction occurred in a manner similar to the reaction of Me_3NO (Fig. 2a, from orange to green line).[24] Subsequently, 2 equivalents of BNAH were added, but no reaction occurred (Fig. 2a, red line). The characteristic absorption of BNAH at 355 nm remained unchanged for 20 min. The absorption obviously decreased upon the addition of excess of water, showing the consumption of



Scheme 1 The OAT reaction of DDAO and regeneration of **1** from **2** by BNAH.

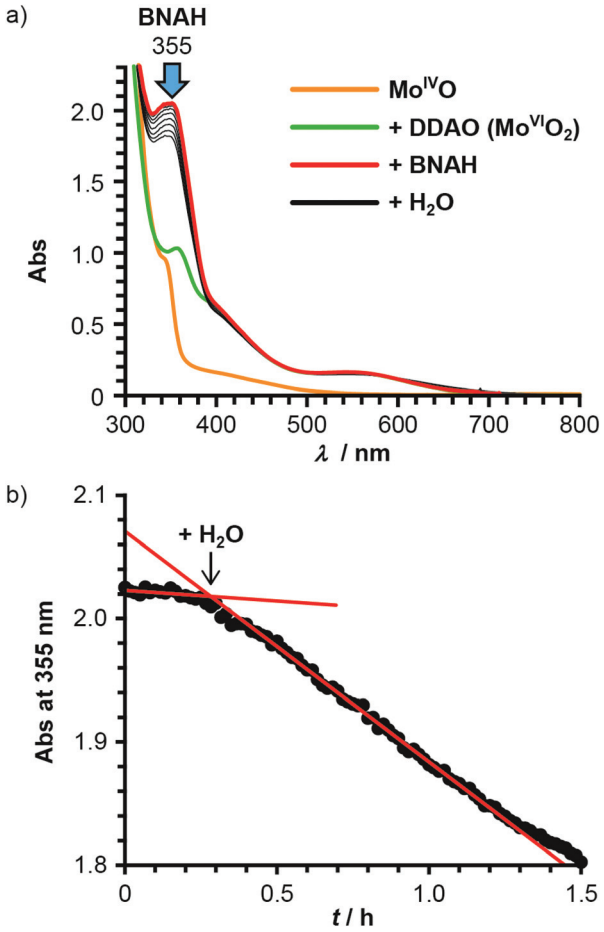


Fig. 2 (a) UV-vis spectral change and (b) time-course of the reaction of **1** with DDAO and BNAH in toluene/THF. Red lines were added for clarity.

BNAH (Fig. 2a, black lines, Fig. 2b). These results indicate that water is essential for the reduction of complex **2** in the catalytic cycle.

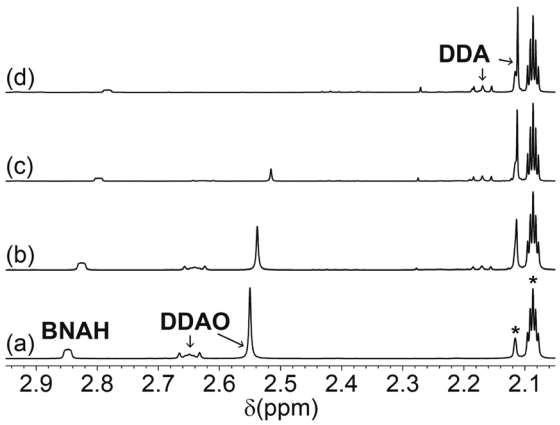


Fig. 3 ^{1}H NMR spectral change in water-containing toluene-d_8 during the catalytic reduction of DDAO by BNAH with 2 mol% of **2** at 27 °C after (a) 0.5 h, (b) 96 h, (c) 373 h, and (d) 887 h. The asterisk (*) denotes toluene.

The catalytic reduction of DDAO by BNAH in water-containing toluene-d_8 was then monitored using ^{1}H NMR spectroscopy (Fig. 3, Fig. S1†). The consumption of DDAO and BNAH followed by the production of DDA was observed. The pseudo-first-order reaction rate constant k_{obs} (1.9×10^{-6} s^{-1}) in the presence of 2 mol% of complex **2** was approximately 20 times higher than that observed in the absence of the catalyst (7.9×10^{-8} s^{-1}), and the turnover number (TON) of the catalysis achieved with the complete consumption of DDAO was 46 although the reaction apparently occurred without a catalyst (Fig. S2†).

Catalysis in an aqueous micellar solution

In order to dissolve the hydrophobic molecules in an aqueous medium, we used micelles. In the process of searching for a surfactant to solubilize the complex, Triton™ X-100, a liquid non-ionic surfactant,[27,28] could successfully form a transparent micellar solution of complex **2**. Because complex **2** was insoluble in water, but readily soluble in toluene and Triton™ X-100, the complex must be located in the hydrophobic core of the micelles (Fig. 4). The catalytic reduction of DDAO by BNAH in an aqueous micellar solution was monitored using UV-vis spectroscopy (Fig. 5). The pseudo-first-order rate constant of the catalytic reaction ($k_{obs} = 36 \times 10^{-6}$ s^{-1}) was about 10 times higher than that of the reaction without the complex (3.8×10^{-6} s^{-1}). When D_2O was used instead of H_2O, the reaction slowed down (KIE = k_H/k_D = 6.5), which indicates that solvent water molecules (or protons) are involved in the rate-determining step and are crucial for catalysis. A weak and broad absorption band was observed at 770 nm during the catalytic reaction (Fig. 5), which suggests the presence of a monooxomolybdenum(v) species[23,29,30] generated by one-electron reduction as a side reaction.

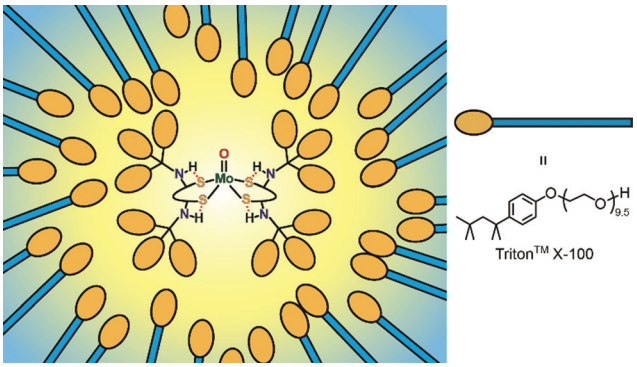


Fig. 4 Schematic drawing of the complex in an aqueous micellar solution (orange oval: hydrophobic group, blue stick: hydrophilic group).

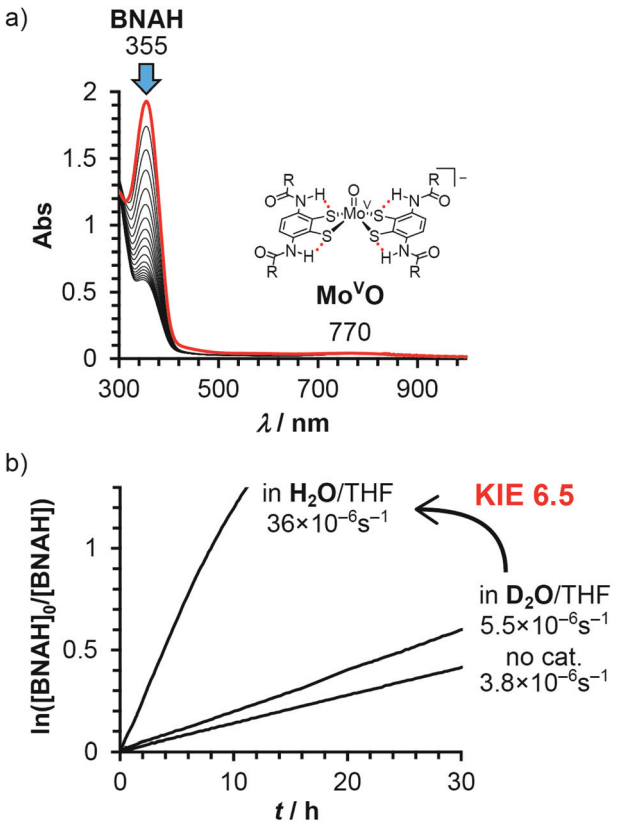
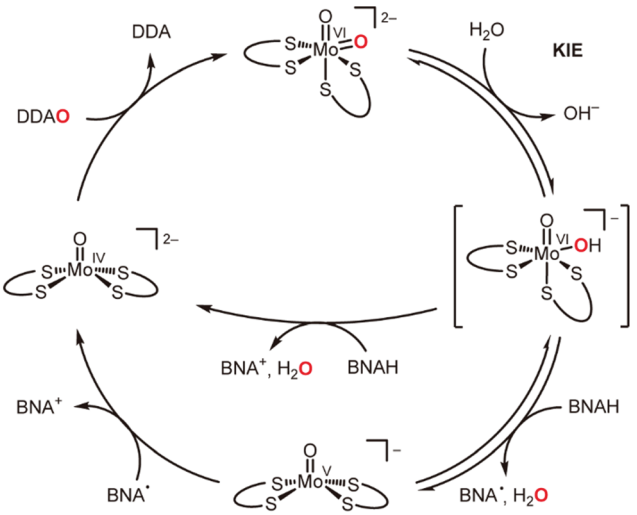


Fig. 5 (a) UV-vis spectral change and (b) kinetic plot of the catalytic reduction of DDAO by BNAH in an aqueous micellar solution.

Discussion

Dioxomolybdenum(VI) complex **2** catalyzes the oxygen-atom-transfer (OAT) reaction of an amine *N*-oxide in water using an NADH analog, BNAH, in both organic and aqueous media. The stoichiometric reaction in non-aqueous media clearly showed the essential role of water molecules in the reduction



Scheme 2 Proposed mechanism of the catalysis.

step. Moreover, the catalytic OAT reaction could be directly observed using ^{1}H NMR spectroscopy in water-containing toluene-d_8.

The non-ionic surfactant, Triton™ X-100, efficiently covered the complex containing bulky hydrophobic groups and created a heterolytic microenvironment in water as shown in Fig. 4. As a result of kinetic experiments, the reaction mechanism of the catalysis is considered as shown in Scheme 2. The large kinetic isotope effect (KIE) of the solvent indicated that the protonation of the dioxomolybdenum(VI) complex by water was involved in the rate-determining step. Furthermore, the observation of the molybdenum(V) species suggested the presence of a one-electron reduction process, which is similar to the proposed catalytic cycle of TMAOR, where the one-electron reduction associates with the protonation of the terminal oxo ligand (Fig. 1).[1]

Conclusions

We constructed a water-soluble molybdoenzyme model containing a hydrophobic microenvironment in an aqueous micellar solution. The model catalyzes the biomimetic reduction of an amine *N*-oxide by an NADH analog in aqueous media. The hydrophobic microenvironment likely promotes the uptake of substrates from the outer aqueous media into the polar reactive site. The present catalytic system, which reduces the substrate followed by the production of water, is one of the most well designed enzyme models.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number 26410072.

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