# A Functional Mimic of the New Class of Tungstoenzyme, Acetylene Hydratase

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The unique tungstoprotein, acetylene hydratase (AH) isolated from the mesophilic anaerobe *Pelobacter acetylenicus*, catalyzes a net hydration reaction (eq 1) rather than a redox one.[1]
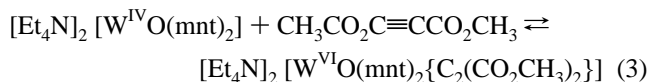
$$HC \equiv CH + H_2O \xrightleftharpoons{AH} CH_3CHO \qquad (1)$$
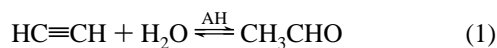
All other tungstoenzymes and all molybdoenzymes catalyze oxidoreductase-type reactions.[2] AH, a monomeric 73 kDa protein possessing tungsten with a pterin cofactor and Fe−S cluster(s),[1] thus closely resembles its prosthetic group content to the structurally characterized *Pyrococcus furiosus* aldehyde ferredoxin oxidoreductase (AOR).[3] Fe−S proteins are also known to catalyze nonredox hydration reaction[4] but the specific role of tungsten in the functioning of AH activity has been established.[1] In contrast to other tungstoenzymes, AH was isolated in air, although from an anaerobic bacterium, and was reported to be moderately stable under aerobic conditions. However, the enzyme does not show activity in air or in the presence of an oxygen-scavenging system but responds only after incubation with strong reductant such as titanium(III) citrate or sodium dithionite added to the assay mixture.[1] This requirement invited an alternate suggestion involving the initial reduction of acetylene to ethylene followed by hydration and subsequent oxidation.[2a,c] However, the anaerobe *P. acetylenicus* neither uses ethylene as the metabolite nor does AH catalyze the conversion of ethylene to acetaldehyde or ethanol, and furthermore, ethylene did not inhibit AH activity.[1] *Does the tungsten site of the AH of anaerobe P. acetylenicus get oxidized under aerobic isolation steps thus requiring strong reductant to regenerate its active form?* The likely participation of a reduced tungsten−pterin cofactor moiety for catalyzing the hydration of acetylene in AH is demonstrated by the model complex, $[Et_4N]_2[W^{IV}O(mnt)_2]$ **(1)**,[5] as shown[6] in eq 2.

$$HC \equiv CH + H_2O \xrightleftharpoons{(1)} CH_3CHO \qquad (2)$$

The corresponding oxidized complex, $[Et_4N]_2[W^{VI}O_2(mnt)_2]$,[5] did not catalyze eq 2, but after treatment with excess $Na_2S_2O_4$ responded equally well.[7] Similarly, a mixture of Na_2WO_4/Na_2-mnt (mnt = 1,2-dicyanoethylenedithiolate) (1:2 ratio) in aqueous medium in the presence of KH_2PO_4 (pH $\approx$ 6.5) remained inactive to catalysis of this transformation but became active upon addition of excess of $Na_2S_2O_4$.[8] We have already shown that $[W^{VI}O_2(mnt)_2]^{2-}$ formed by the interaction between Na_2WO_4 and Na_2mnt can be smoothly reduced in water to $[W^{IV}O(mnt)_2]^{2-}$ anion by dithionite.[5] Recovery of the active

species **1** in over 90% yield at different stages of eq 2[6] attests to its function as protoacetylene hydratase.

Unlike the reaction of $[Mo^{IV}O(dtc)_2]$ (dtc = dialkyldithio-carbamate) with C_2H_2,[9] we failed to observe any adduct formation between **1** with C_2H_2 by NMR or electronic spectroscopy in a C_2H_2-saturated solution of CH_2Cl_2 or CH_3COCH_3 (ambient conditions). However, the occurrence of eq 3[10] supports the oxidative addition reaction as encountered with elemental sulfur.[5a]

$$[Et_4N]_2 [W^{IV}O(mnt)_2] + CH_3CO_2C \equiv CCO_2CH_3 \rightleftharpoons$$
$$[Et_4N]_2 [W^{VI}O(mnt)_2\{C_2(CO_2CH_3)_2\}] \quad (3)$$

For reaction 3, K_{eq} {14($\pm$1) M^{-1} (CH_3CN, 25 °C)}, when compared with K_{eq} values for similar reactions of $[Mo^{IV}O(dtc)_2]$ with C_2H_2 (~20 M^{-1}) and with $CH_3CO_2C \equiv CCO_2CH_3$ (~20 000 M^{-1}),[11] justifies the above observation. The apparent lack of formation of a stable adduct of acetylene in the present system compared to the stability of $[MO(dtc)_2(C_2H_2)]$ (M = Mo, W)[9,11] is consistent with the essential dithiolene ligation from the pterin

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(6) Assay for catalytic activity: Through a solution of $[Et_4N]_2$-$[W^{IV}O(mnt)_2]$ **(1)** (0.25 mmol) dissolved in deaerated (Ar) CH_3CN/H_2O (10 mL:10 mL) was slowly bubbled C_2H_2 (*T* 30−35 °C), and the outflow of the gas was passed through a solution of 2,4-dinitrophenylhydrazine (DNP) in water HCl medium. During the passage of C_2H_2 for 1 h, the DNP solution became turbid, resulting in the precipitation of a yellow-orange solid. The passage of the C_2H_2 was continued for 4 h when appreciable quantity of the solid appeared. Electronic spectroscopy of the pink-colored reaction solution did not show any change in the absorption profile retaining the intensity of the absorption at 650 and 515 nm of the starting complex. The reaction mixture was diluted with large volume of water (100 mL) in the cold under argon, and the starting complex thus precipitated was recovered in over 90% yield. The filtrate, with a pale yellowish green color containing the reminder of the dissolved starting complex, was acidified with dilute HCl in the cold to precipitate traces of $[Et_4N]_2[W^{IV}(mnt)_3]$ formed under acidification of the starting complex (see ref 5a). To the colorless filtrate addition of DNP solution followed by warming to 50 °C and slow evaporation of the solution led to the precipitation of a yellow-orange solid which was centrifuged, washed with dilute HCl and with water, and air-dried. The yellow-orange precipitate obtained from the outflow of the gas mixture was similarly isolated. Both of the crops were identified as the same product by mp (147 °C) and by HPLC (Shimadzu SPD-6 AV, C-R3A chromatopac, 254 nm wavelength, eluted with MeOH at room temperature, flow rate 1 mL/min, retention time peak 3.072 min) which were identical to authentic hydrazone derivative of CH_3CHO. Controlled experiments (i) in the absence of **1** with passage of C_2H_2, (ii) in the presence of **1** with the passage of Ar, and (iii) in the absence of **1** with the addition of known amount of CH_3CHO in CH_3CN/H_2O with the passage of C_2H_2 (i) resulted in no formation of the hydrazone derivative, (ii) yielded recovery of **1** over 90% leaving trace amount of **1** in solution, and (iii) showed the presence of appreciable amount of CH_3CHO in CH_3-CN/H_2O solution even after 4 h of passage of C_2H_2 and similar lag period of ~30 min for the appearance of turbidity in the external monitoring DNP solution. In a typical turnover experiment, **1** (20 mg, 0.027 mmol) was dissolved in C_2H_2 saturated H_2O/CH_3CN (10 mL:5 mL) in a flask under C_2H_2 and sealed with a rubber septum. This was thermostated at 30 °C. After ca. 4 h, the flask was cooled, and by following the workup procedure as described above, the DNP hydrazone derivative of CH_3CHO was isolated in 43 mg yield which was about 9 turnovers (a lower limit due to appreciable solubility of the derivative in the large volume of the reaction medium) under the used assay conditions.

(7) A similar reaction carried out with $[Et_4N]_2[WO_2^{VI}(mnt)_2]$ did not respond to the formation of hydrazone derivative. Treatment of the reaction mixture with excess of $Na_2S_2O_4$ for ~30 min led to the formation of CH_3-CHO, which, however, remained in the reaction flask as the bisulfite (formed by the addition of dithionite) complex. Separation of the tungsten complex in its reduced form (~70% yield), acid decomposition in the cold to remove the rest of the dissolved complex as $[(Et_4N)_2][W^{IV}(mnt)_3]$ along with some elemental sulfur, and treatment of the colorless filtrate with DNP solution under evacuation to remove SO_2 slowly resulted in the precipitation of the hydrazone derivative of CH_3CHO which was characterized as described in ref 6.

(8) The reaction was carried out similarly as described above. After the addition of $Na_2S_2O_4$, the passage of C_2H_2 was carried out for 4 h and excess of $(Et_4N)Cl$ was added to precipitate the reduced complex (~60% yield). The rest of the procedure was identical to that described above to isolate the hydrazone derivative of CH_3CHO which was characterized as described in ref 6.

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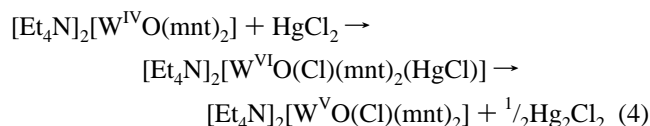* Address correspondence to this author at the following: tel +512-257265; Fax +512-590007; e-mail abya@iitk.ernet.in.
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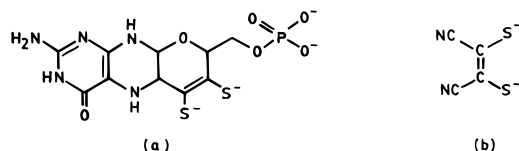
Figure 1. (a) Structure of the pterin cofactor containing a dithiolate functionality to chelate W, as observed in AOR.[3] (b) mnt^{2-} as model for a pterin dithiolate ligand.

cofactor (Figure 1) in the native protein.[1,2a,c] Here, the short-lived adduct should be the crucial requirement for the activation of acetylene for the subsequent hydration reaction. Equation 2 thus resembles the known Hg^{2+}-catalyzed hydration of acetylene.[12]

The reported inhibitions by strong π-ligands like NO and CO for AH activity[1] attest to π-interactions between the tungsten center and the substrate. Explanation for the reduced activity of AH in the presence of HgCl$_2$ requires scrutiny with the model complex. Interestingly, HgCl$_2$ rapidly exhibits an oxidative addition reaction with **1**. The postulated heptacoordinated species so formed is unstable, subsequently undergoing W(VI)$-$Hg(II) bond cleavage[13] (Figure 2) as shown in eq 4.

$$[Et_4N]_2[W^{IV}O(mnt)_2] + HgCl_2 \rightarrow$$
$$[Et_4N]_2[W^{VI}O(Cl)(mnt)_2(HgCl)] \rightarrow$$
$$[Et_4N]_2[W^VO(Cl)(mnt)_2] + {}^1/_2Hg_2Cl_2 \quad (4)$$

The response of **1** toward oxidative addition reactions with elemental sulfur,[5a] CH$_3$CO$_2$C≡CCO$_2$CH$_3$, and HgCl$_2$ lends credence to the involvement of **1** in activating acetylene via electron donor$-$acceptor (EDA) complexation.[12] This model of study suggests that AH, when isolated under aerobic

(10) The complex was isolated as a red microcrystalline solid by reacting **1** with excess CH$_3$CO$_2$C≡CCO$_2$CH$_3$ under Ar in CH$_3$CN and precipitating by diethyl ether (60% yield). FAB$^-$: 622 (P$^-$), 752 (P$^-$ + Et$_4$N$^+$). IR (KBr): 915 s (ν(W=O)), 1700 vs (ν(C=O)), 1790 m (ν(C≡C)), 2220 vs (ν(CN)) cm^{-1}. λ_{max} (CH$_3$CN): 435 (ϵ 5350), 388 (5290), 326 (11 000) nm. ^{1}H NMR (Me$_2$SO-d_6): δ 3.86 (s, 3, ester CH$_3$), 3.83 (s, 3, ester CH$_3$), 3.24 (q, 16, cation CH$_2$), 1.21 (t, 24, cation CH$_3$). CV (DMF): $E_{pc} = -1.75$ vs Ag/AgCl (GCE, 100 mV/s, 10^{-3} M solution with 0.1 M Et$_4$NClO$_4$ as supporting electrolyte). Anal. Calcd for C$_{30}$H$_{46}$N$_6$O$_5$S$_4$W: C, 40.81; H, 5.25; N, 9.52; S, 14.53. Found: C, 40.46; H, 5.38; N, 9.70; S, 14.60.
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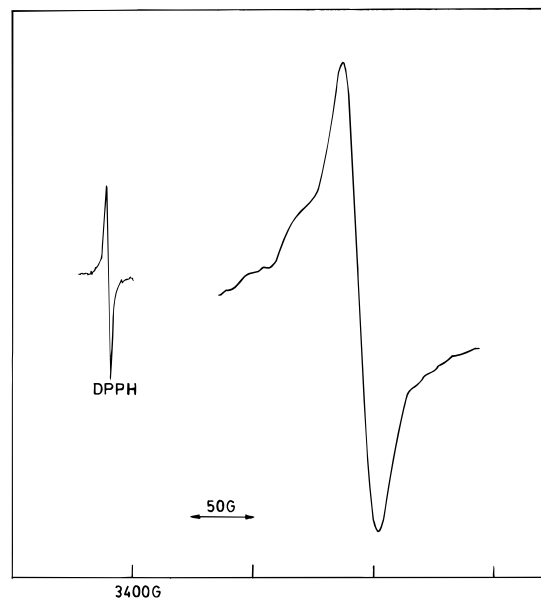
Figure 2. EPR spectrum of [W^{V}O(Cl)(mnt)$_2$]$^{2-}$ in CH$_2$Cl$_2$ at 25 °C.[13]

conditions, is reversibly oxidized and becomes inactive. Reduction of the oxidized tungsten center has to occur before catalytic activity can be restored. Once the tungsten center is reduced, no further changes in the redox state of tungsten occur during catalytic activity. For the stability of the reduced tungstoproteins, bis(dithiolene) coordination through pterin cofactors is proposed to be an obligate requirement[2a] as present in **1**. The role of the pterin part of AH may involve provision of structural stability through hydrogen bonding with the apoprotein (as observed in AOR[3]) and/or participation in the electron flow path between the tungsten and Fe$-$S centers during the necessary stages of activation or deactivation. Confirmation of these ideas await further characterization of AH.

(13) Compound **1** in CH$_2$Cl$_2$ under Ar reacted with 1 equiv of HgCl$_2$ leading to color change from pink to EPR inactive light redish-brown, which, after a lag time of $\sim$30 s, started to precipitate white Hg$_2$Cl$_2$ (tested by NH$_4$OH), and the solution displayed EPR active W(V) signal, $\langle g \rangle = 1.893$ (X band) characteristic of [Et$_4$N]$_2$[W^{V}OCl(mnt)$_2$] (see ref 5a).