CO₂ fixation by [W⁴⁺O(S₂C₂(CN)₂)₂]²⁻: functional model for the tungsten-formate dehydrogenase of Clostridium thermoaceticum

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Abstract. (NE₄)₂[W⁴⁺O(S₂C₂(CN)₂)₂] (I), isolated by reaction of Na₂WO₄, Na₂S₂C₂(CN)₂ (Na₄mnt) in acidified (pH 5-5) aqueous medium in the presence of excess of sodium dithionite and NE₄Br, reduces CO₂/HCO₃⁻ (pH 7-5) to yield HCOO⁻ and (NE₄)₂[W⁴⁺O₂(S₂C₂(CN)₂)₂] (2) mimicking tungsten-formate dehydrogenase (W-FDH) activity. (I) reacts with Na₃MoO₄ in acidic medium to produce [Mo⁶⁺O(S₂C₂(CN)₂)₂]²⁻ implicating the displacement of tungsten by molybdenum from the cofactor complex in W-FDH.

Keywords. CO₂ fixation; functional model; tungsten-formate dehydrogenase; Clostridium thermoaceticum; nit-1 reconstitution assay.

The tungsten formate dehydrogenase endogeneous to Clostridium thermoaceticum catalyses NADPH-dependent reduction of CO₂ to HCOO⁻ as the first step in acetogenic glucose fermentation (Andersen and Ljungdahl 1974; Yamamoto et al 1983; Adams and Mortenson 1985). This functional tungsten enzyme has been shown to contain the cofactor common to molybdenum hydroxylases (Kramer et al 1987) by Neurospora crassa nit-1 reconstitution assay (Deaton et al 1984) and thus may contain a {W⁴⁺ = O} moiety with a dithiolene chelated ligand in its reduced form. The ability of synthesized [Mo⁶⁺O₂(mnt)₄]²⁻ to mimic sulphite oxidase activity (Sarkar and Das 1992) prompted us to search for similar complexes of tungsten. Herein we report the novel chemistry of (NE₄)₂[W⁴⁺O(S₂C₂(CN)₂)₂] (I), which is the first synthesized tungsten compound functionally similar to W-FDH in relation to the reduction of biological substrate CO₂, and the displacement of W from (I) by Mo using Na₃MoO₄, a reaction used for the reconstitution assay of W-FDH (Deaton et al 1984).

Na₂WO₄ (2 mmol) and Na₂mnt (4 mmol) were dissolved in 100 ml of water under dinitrogen atmosphere and the pH was adjusted to 5-5 by adding CH₃COOH. 10 g of sodium dithionite were added into it and (I) was precipitated as a pink coloured solid by adding Et₄NBr (4 mmol). Recrystallisation from CH₃CN-diethyl ether led to analytically pure (I) in 60% yield (diamagnetic; negative ion FAB mass spectrum, molecular anion with isotropic pattern centred at m/Z = 482; IR (KBr) 935 vs, ν(W = 0), 2192 vs, ν(CN) cm⁻¹; ¹³C-NMR 140-41 (C=O), 118-9(CN) ppm; λ_max (MeCN) 649 (ε = 145), 521 (219) and 370 (2190) nm; CV in MeCN (GCE), 100 mV/s containing 0.1 M Et₄NCIO₄, reversible one-electron oxidation at +0.465 V vs NHE, in MeCN with 3% water irreversible 2-electron oxidation with Eₚ at +0.48 V vs NHE at 25°C). (I) is very sensitive to oxygen which decomposes it. However, under anaerobic conditions.
conditions it reduces $K_3[\text{Fe(CN)}_6]$ or methylene blue quantitatively with the formation of (2), which can be followed by electronic spectroscopy. (2) was directly synthesized by following a procedure similar to that described for (1) but by adding sodium sulphite instead of sodium dithionite. (2): diamagnetic; negative ion FAB mass spectrum, molecular anion with isotropic pattern centred at $m/z = 498$; IR (KBr) 905.7 vs, 860 vs, $v(W=O)$ (for cis $\{\text{WO}_2\}_2$ moiety), 2200-5 vs, $v(\text{CN})\text{cm}^{-1}$; $^{13}\text{C NMR}$ 141 (C=C), 118.42 (CN) ppm; $\lambda_{\max}$ (MeCN) 440 sh, 380 ($\varepsilon = 11600$) nm; CV in MeCN, similar conditions as for (1) irreversible reduction at $-1.20$ V vs NHE). The $E_{pa}$ at $+0.48$ V vs NHE in CH$_3$CN–H$_2$O suggests that the oxidation of W(IV) is thermodynamically possible by CO$_2$ as the CO$_2$/HCOO$^-$ couple has $E^0 = -0.42$ V. Under strict anaerobic conditions, (1) in CH$_3$CN–H$_2$O (1:1) in the presence of excess of CO$_2$/HCO$_3^-$ (pH 7-5 adjusted by HCl), slowly changes to (2) within a day and on keeping the reaction mixture at a temperature of 50°C this change is completed after 6 h (followed by electronic spectroscopy). From the reaction mixture, (2) is removed by repeated acetone treatment with the precipitation of a white solid largely containing NaHCO$_3$/Na$_2$CO$_3$. This is subjected to chromatropic acid test for formate assay (Grant 1948) which proves the presence of 55% formate based on (1) thus demonstrating the reaction (1) which is the fundamental reaction of W-FDH in Clostridium thermoaceticum (Andersen and Ljungdahl 1974; Yamamoto et al 1983; Adams and Mortenson 1985).

$$[\text{W}^{IV}\text{O}(\text{mnt})_2]^{2-} + \text{HCO}_3^- \rightarrow [\text{W}^{VI}\text{O}_2(\text{mnt})_2]^{2-} + \text{HCOO}^-.$$  \(1\)

Interestingly, (1) in CH$_3$CN–H$_2$O (1:1), when treated with excess of Na$_2$MoO$_4$ at $\sim$ pH 5 (CH$_3$COOH), showed quick quantitative conversion as shown in reaction (2):

$$[\text{W}^{IV}\text{O}(\text{mnt})_2]^{2-} + \text{MoO}_4^{2-} \rightarrow [\text{Mo}^{IV}\text{O}(\text{mnt})_2]^{2-} + \text{WO}_4^{2-}.$$  \(2\)

However, in neutral or in slightly basic medium reaction (2) is complete after two weeks. This reaction in acidic medium is parallel to the reconstitution assay of W-FDH with the inactive assimilatory nitrate reductase of the nit-1 mutant of Neurospora crassa in the presence of Na$_2$MoO$_4$ in acidic medium (Deaton et al 1984). Detailed kinetics of reactions (1) and (2) are in progress and will be reported later.

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