

CO₂ fixation by [W^{IV}O(S₂C₂(CN)₂)₂]²⁻: functional model for the tungsten-formate dehydrogenase of *Clostridium thermoaceticum*

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MS received 3 February 1992

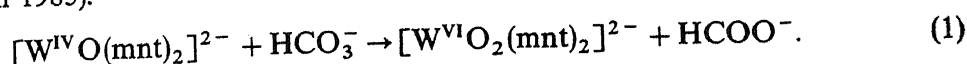
Abstract. (NEt₄)₂[W^{IV}O(S₂C₂(CN)₂)₂] (1), isolated by reaction of Na₂WO₄, Na₂S₂C₂(CN)₂ (Na₂mnt) in acidified (pH 5.5) aqueous medium in the presence of excess of sodium dithionite and NEt₄Br, reduces CO₂/HCO₃⁻ (pH 7.5) to yield HCOO⁻ and (NEt₄)₂[W^{VI}O₂(S₂C₂(CN)₂)₂] (2) mimicking tungsten-formate dehydrogenase (W-FDH) activity. (1) reacts with Na₂MoO₄ in acidic medium to produce [Mo^{IV}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 {W^{IV} = O} moiety with a dithiolene chelated ligand in its reduced form. The ability of synthesized [Mo^{VI}O₂(mnt)₂]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 (NEt₄)₂[W^{IV}O(S₂C₂(CN)₂)₂] (1), 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 (1) by Mo using Na₂MoO₄, 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 (1) was precipitated as a pink coloured solid by adding Et₄NBr (4 mmol). Recrystallization from CH₃CN-diethyl ether led to analytically pure (1) in 60% yield (diamagnetic; negative ion FAB mass spectrum, molecular anion with isotropic pattern centred at *m/z* = 482; IR (KBr) 935 *vs*, *v*(W = O), 2192 *vs*, *v*(CN) cm⁻¹; ¹³C-NMR 140.41 (C=C), 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 Et₄NClO₄ reversible one-electron oxidation at +0.465 V vs NHE, in MeCN with 3% water irreversible 2-electron oxidation with *E*_{pa} at +0.48 V vs NHE at 25°C). (1) is very sensitive to oxygen which decomposes it. However, under anaerobic

conditions it reduces $K_3[Fe(CN)_6]$ 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, $\nu(W=O)$ (for *cis*{ WO_2 } moiety), 2200.5 vs, $\nu(CN) cm^{-1}$; ^{13}C NMR 141 (C=C), 118.42 (CN) ppm; λ_{max} (MeCN) 440 sh, 380 ($\epsilon = 11600$) 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_3CN-H_2O suggests that the oxidation of W(IV) is thermodynamically possible by CO_2 as the $CO_2/HCOO^-$ couple has $E^\circ = -0.42$ V. Under strict anaerobic conditions, (1) in CH_3CN-H_2O (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 $50^\circ C$ 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 $NaHCO_3/Na_2CO_3$. This is subjected to chromotropic 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).



Interestingly, (1) in CH_3CN-H_2O (1:1), when treated with excess of Na_2MoO_4 at \sim pH 5 (CH_3COOH), showed quick quantitative conversion as shown in reaction (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_2MoO_4 in acidic medium (Deaton *et al* 1984). Detailed kinetics of reactions (1) and (2) are in progress and will be reported later.

Acknowledgements

The authors thank the Council of Scientific and Industrial Research, and the Department of Science and Technology, New Delhi, for financial support and the Regional Sophisticated Instrumentation Centre, Lucknow, for negative ion FAB mass and ^{13}C NMR spectral measurements.

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