

## CO<sub>2</sub> fixation by [W<sup>IV</sup>O(S<sub>2</sub>C<sub>2</sub>(CN)<sub>2</sub>)<sub>2</sub>]<sup>2-</sup>: functional model for the tungsten-formate dehydrogenase of *Clostridium thermoaceticum*

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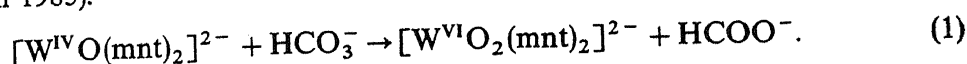
**Abstract.** (NEt<sub>4</sub>)<sub>2</sub>[W<sup>IV</sup>O(S<sub>2</sub>C<sub>2</sub>(CN)<sub>2</sub>)<sub>2</sub>] (1), isolated by reaction of Na<sub>2</sub>WO<sub>4</sub>, Na<sub>2</sub>S<sub>2</sub>C<sub>2</sub>(CN)<sub>2</sub> (Na<sub>2</sub>mnt) in acidified (pH 5.5) aqueous medium in the presence of excess of sodium dithionite and NEt<sub>4</sub>Br, reduces CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> (pH 7.5) to yield HCOO<sup>-</sup> and (NEt<sub>4</sub>)<sub>2</sub>[W<sup>VI</sup>O<sub>2</sub>(S<sub>2</sub>C<sub>2</sub>(CN)<sub>2</sub>)<sub>2</sub>] (2) mimicking tungsten-formate dehydrogenase (W-FDH) activity. (1) reacts with Na<sub>2</sub>MoO<sub>4</sub> in acidic medium to produce [Mo<sup>IV</sup>O(S<sub>2</sub>C<sub>2</sub>(CN)<sub>2</sub>)<sub>2</sub>]<sup>2-</sup> implicating the displacement of tungsten by molybdenum from the cofactor complex in W-FDH.

**Keywords.** CO<sub>2</sub> 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 CO<sub>2</sub> to HCOO<sup>-</sup> 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<sup>IV</sup> = O} moiety with a dithiolene chelated ligand in its reduced form. The ability of synthesized [Mo<sup>VI</sup>O<sub>2</sub>(mnt)<sub>2</sub>]<sup>2-</sup> 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 (NEt<sub>4</sub>)<sub>2</sub>[W<sup>IV</sup>O(S<sub>2</sub>C<sub>2</sub>(CN)<sub>2</sub>)<sub>2</sub>] (1), which is the first synthesized tungsten compound functionally similar to W-FDH in relation to the reduction of biological substrate CO<sub>2</sub>, and the displacement of W from (1) by Mo using Na<sub>2</sub>MoO<sub>4</sub>, a reaction used for the reconstitution assay of W-FDH (Deaton *et al* 1984).

Na<sub>2</sub>WO<sub>4</sub> (2 mmol) and Na<sub>2</sub>mnt (4 mmol) were dissolved in 100 ml of water under dinitrogen atmosphere and the pH was adjusted to 5.5 by adding CH<sub>3</sub>COOH. 10 g of sodium dithionite were added into it and (1) was precipitated as a pink coloured solid by adding Et<sub>4</sub>NBr (4 mmol). Recrystallization from CH<sub>3</sub>CN-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<sup>-1</sup>; <sup>13</sup>C-NMR 140.41 (C=C), 118.9(CN) ppm; λ<sub>max</sub> (MeCN) 649 (ε = 145), 521 (219) and 370 (2190) nm; CV in MeCN (GCE), 100 mV/s containing 0.1 M Et<sub>4</sub>NClO<sub>4</sub> reversible one-electron oxidation at +0.465 V vs NHE, in MeCN with 3% water irreversible 2-electron oxidation with *E*<sub>pa</sub> 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;  $\lambda_{max}$ (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.

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