

Published in final edited form as:

*J Am Chem Soc.* 2007 August 29; 129(34): 10328–10329. doi:10.1021/ja073643o.

## Rapid and Efficient Electrocatalytic CO<sub>2</sub>/CO Interconversions by *Carboxydothemus hydrogenoformans* CO Dehydrogenase I on an Electrode

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Microbial interconversions between CO and CO<sub>2</sub> are catalyzed by carbon monoxide dehydrogenases (CODH).<sup>1</sup> These enzymes fall into three classes: Mo-CODH in which the active site is a CuMo-pterin, Ni-CODH in which the active site is a [Ni4Fe-5S] cluster, and Ni-CODH/ACS, in which a Ni-CODH is part of a larger complex coupling CO<sub>2</sub> reduction to acetyl CoA synthesis. The Ni-CODHs function in both directions, which is significant as both CO<sub>2</sub> and CO can be primary carbon sources and CO is an electron source. As evident from the standard reduction potential for reaction 1A ( $E^0 = -0.10$  V vs SHE at pH 0; corresponding to approximately  $-0.51$  V at pH 7),<sup>2</sup> CO is more reducing than H<sub>2</sub>, and this property is the basis for the industrial “water gas shift reaction” (1B) in which H<sub>2</sub> is produced from water. Both CO and CO<sub>2</sub> are unreactive without a catalyst, but enzyme-catalyzed reactions are fast, with turnover frequencies as high as 40000 s<sup>-1</sup> reported for CO oxidation by a Ni-CODH at pH 8, 70 °C.<sup>3</sup>



There is hope that (photo)electrochemical CO<sub>2</sub> reduction may provide a “green” alternative route to fuels and organic compounds,<sup>4</sup> and CO<sub>2</sub>/CO cycling in conjunction with reaction 1B could be used to split water.<sup>5</sup> An ideal *electrocatalyst* for CO<sub>2</sub> reduction or CO oxidation would show high rates in either direction with minimal overpotential; in other words, CO<sub>2</sub>/CO interconversion would behave as a reversible electrode reaction. Electrocatalysts producing CO or products such as formate or methanol have been reported,<sup>2</sup> but apart from some Cu alloys large overpotentials are observed.<sup>6,7</sup> A recent paper described CO<sub>2</sub> reduction by a Ni-CODH, mediated by methyl viologen at a modest overpotential, but the reverse reaction was not reported.<sup>8</sup>

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**Supporting Information Available:** Determination of apparent activation enthalpies, the formal reduction potential,  $K_M$  values, number of electrons involved in reactivation and complete ref 9. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The strictly anaerobic thermophile *Carboxydotherrmus hydrogenoformans* (*Ch*) thrives at 70 °C and can use CO as a sole carbon and energy source.<sup>1,3</sup> *Ch* produces at least five CODH complexes,<sup>9</sup> including CODH I (137.0 kDa) and CODH II (136.6 kDa), which are closely related homodimers involved in energy conservation (as part of a H<sup>+</sup>-pumping complex) and NADPH generation, respectively. The crystal structure of CODH II (Figure 1) reveals a [Ni<sub>4</sub>Fe-5S] and [4Fe-4S] cluster in each of the two subunits, which are bridged by a further, solvent-exposed [4Fe-4S] cluster that offers a route for electrons in and out of the enzyme.<sup>10</sup> As part of larger complexes, Ni-CODH is a module for cycling and distributing electrons from CO/CO<sub>2</sub> interconversions. We now demonstrate that *Ch* Ni-CODH I<sup>11</sup> attached to an electrode displays facile, direct interfacial electron exchange and provides a benchmark for “reversible” CO<sub>2</sub>/CO electrochemistry.

The voltammograms in Figure 2 reveal the intense electrocatalytic activity of *Ch* Ni-CODH I adsorbed on a pyrolytic graphite “edge” (PGE) electrode rotating at high speed in an anaerobic sealed cell.<sup>12</sup> Panels a and b reveal the separate reduction and oxidation activities under 100% CO<sub>2</sub> or 100% CO, while panels c and d show combined reduction and oxidation activities for a 1:1 CO<sub>2</sub>/CO gas mixture at two different pH values.

No nonturnover signals appeared without substrate, so the electroactive coverage must be very low,<sup>16</sup> nor was activity observed upon adding H<sub>2</sub> to CO/CO<sub>2</sub>-free experiments, confirming Ni-CODH I has no hydrogenase activity. For neither CO oxidation or CO<sub>2</sub> reduction does the catalytic current reach a limit, probably reflecting the high activity of enzyme molecules nonuniformly adsorbed on the PGE surface.<sup>13</sup> However, as discussed below, CO oxidation has an irregular potential dependence. Heating from 0 to 50 °C causes large increases in activity in both directions (higher temperatures decreased electrocatalytic activity, probably due to enzyme desorption, but the voltammetry was stable over several hours at 25 °C). The temperature dependence of rotation-rate independent currents yielded empirical activation enthalpies of  $\Delta H^\ddagger = 48 \text{ kJ mol}^{-1}$  for CO<sub>2</sub> reduction and 30 kJ mol<sup>-1</sup> for CO oxidation (Supporting Information). For a 1:1 gas mixture (panels c and d), the zero-current potential (average in each scan direction) approximates to the formal potential for the CO<sub>2</sub>/CO redox couple. We obtained  $E^0 = -500 \text{ mV}$  at pH 6.7, 25 °C (Supporting Information). Increasing the pH raises the CO oxidation activity relative to CO<sub>2</sub> reduction but the current always cuts sharply through the zero current axis at the CO<sub>2</sub>/CO reduction potential, so there is no enzyme-inherent overpotential in either direction. An inspiring conclusion is that CO<sub>2</sub>/CO redox cycling can be rendered “reversible”, similar to the 2H<sup>+</sup>/H<sub>2</sub> couple at Pt or hydrogenase-modified electrodes.<sup>14</sup>

Our CO and CO<sub>2</sub> levels far exceed those of natural environments, so we used a procedure described by Léger et al.<sup>15</sup> to compare  $K_M$  values for CO oxidation and CO<sub>2</sub> reduction. In this method (Supporting Information) the activity is recorded over a large, continuous concentration range down to extremely low levels. At 25 °C, pH = 6.0, the  $K_M$  (CO) value (13 experiments) is ca. 0.002 atm (ca. 2 μM) but only a lower limit (ca. 0.06 atm) could be determined for  $K_M$  (CO<sub>2</sub>). Although 25 °C is colder than normal growth conditions for *Ch*, the values reflect very well the enzyme’s ability to scavenge low-level CO. The very high  $K_M$  for CO<sub>2</sub> means that in Figure 2, CO oxidation activity is saturated whereas CO<sub>2</sub> reduction is not, and at pH values below 6,  $k_{\text{cat}}$  for CO<sub>2</sub> reduction must actually be higher than for CO oxidation.<sup>16</sup>

Reversible inactivation at a potential more positive than -320 mV, pH 7.5, has been reported for another Ni-containing CODH,<sup>17</sup> so the unusual shape of voltammograms in the CO oxidation region was investigated further. (Our electrochemical results were obtained over much shorter time scales than required to observe *irreversible* inactivation at high CO levels.) Figure 3 shows that reversible inactivation is resolved by lowering the scan rate

(a) or if higher temperatures (b), lower pH (c), and lower CO levels (d) are used. At pH 4.7, 1% CO, not one but *two* reversible activation/inactivation processes are distinguished, at ca.  $-250$  and ca.  $-50$  mV. That we may be addressing two different forms of the enzyme merits further investigation. We assigned the high-potential activation as a one-electron process from its Nernstian reactivation profile (Supporting Information). The behavior mirrors that of hydrogenases which also undergo reversible anaerobic inactivation, particularly at low H<sub>2</sub> levels.<sup>14,18</sup>

In conclusion, *Ch* Ni-CODH linked to a PGE electrode displays intense electrocatalytic activity for CO oxidation and CO<sub>2</sub> reduction. This not only provides a good way to study this enzyme under strict potential control but it also sets a standard for future studies of CO and CO<sub>2</sub> electrochemistry. One important aspect will be to link the catalytic reversibility more closely to C-cluster interconversions that have been detected by EPR.<sup>1</sup>

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

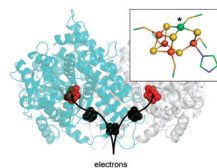
## Acknowledgments

This research is supported by the EPSRC and BBSRC (Grant 43/E16711, F.A.A.) and NIH (Grant GM39451, S.W.R.).

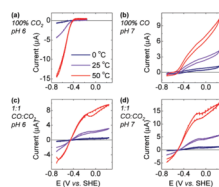
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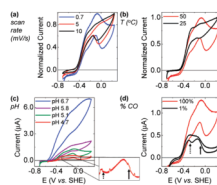
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**Figure 1.** Structure of *Ch* CODH II showing the two subunits (blue and gray) and arrangement of Fe–S clusters (black) that relay electrons to and from the [Ni4Fe-5S] active sites (red). Inset shows the [Ni4Fe-5S] cluster where the proposed binding site for CO on the Ni atom is shown as \*.<sup>10</sup>



**Figure 2.** Protein film voltammograms showing CO<sub>2</sub> reduction and CO oxidation activities of *Ch* CODH I under atmospheres of 100% CO<sub>2</sub>, 100% CO, or 1:1 CO<sub>2</sub>/CO gas mixtures. Scan rate in panels a, c, and d was 10 mV/s; in panel b the scan rate was 30 mV/s. The electrode rotation was 4000 rpm. The pH is as indicated and the temperature color scheme given in panel a is used in other panels. Buffer compositions are given in ref 12. Electroactive coverage varies between different experiments.



**Figure 3.** Observations of *Ch* Ni-CODH I activity at high potential, changing: (a) scan rate, (b) temperature, (c) pH, and (d) CO. Unless stated otherwise, conditions are 0.7 mV/s, 100% CO, rotation rate of 4000 rpm, pH 5.0, 50 °C. In panels a and b the current is normalized with respect to the maximum oxidation current. In panels c and d black vertical arrows indicate resolution of *two* activation processes. Where a large amount of CO is oxidized, sufficient CO<sub>2</sub> accumulates to be detected as a reduction current. It is clear that complete inactivation is not achieved even at 1% CO.