

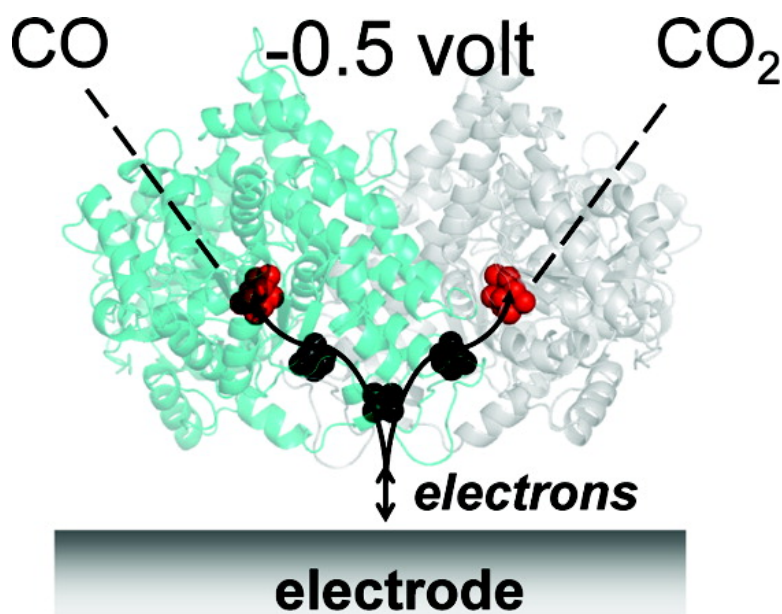
Communication

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Rapid and Efficient Electrocatalytic CO₂/CO Interconversions by *Carboxydothemus hydrogenoformans* CO Dehydrogenase I on an Electrode

Alison Parkin,[†] Javier Seravalli,[‡] Kylie A. Vincent,[†] Stephen W. Ragsdale,^{*,‡} and Fraser A. Armstrong^{*,†}

*Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QR, U.K., and
Department of Biochemistry, University of Nebraska, Lincoln, Nebraska 68588-0664*

Received May 21, 2007; E-mail: fraser.armstrong@chem.ox.ac.uk

Microbial interconversions between CO and CO₂ are catalyzed by carbon monoxide dehydrogenases (CODH).¹ 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 CO₂ reduction to acetyl CoA synthesis. The Ni-CODHs function in both directions, which is significant as both CO₂ and CO can be primary carbon sources and CO is an electron source. As evident from the standard reduction potential for reaction 1A ($E^{\circ} = -0.10$ V vs SHE at pH 0; corresponding to approximately -0.51 V at pH 7),² CO is more reducing than H₂, and this property is the basis for the industrial “water gas shift reaction” (1B) in which H₂ is produced from water. Both CO and CO₂ are unreactive without a catalyst, but enzyme-catalyzed reactions are fast, with turnover frequencies as high as 40000 s⁻¹ reported for CO oxidation by a Ni-CODH at pH 8, 70 °C.³



There is hope that (photo)electrochemical CO₂ reduction may provide a “green” alternative route to fuels and organic compounds,⁴ and CO₂/CO cycling in conjunction with reaction 1B could be used to split water.⁵ An ideal *electrocatalyst* for CO₂ reduction or CO oxidation would show high rates in either direction with minimal overpotential; in other words, CO₂/CO interconversion would behave as a reversible electrode reaction. Electrocatalysts producing CO or products such as formate or methanol have been reported,² but apart from some Cu alloys large overpotentials are observed.^{6,7} A recent paper described CO₂ reduction by a Ni-CODH, mediated by methyl viologen at a modest overpotential, but the reverse reaction was not reported.⁸

The strictly anaerobic thermophile *Carboxydothemus hydrogenoformans* (*Ch*) thrives at 70 °C and can use CO as a sole carbon and energy source.^{1,3} *Ch* produces at least five CODH complexes,⁹ 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⁺-pumping complex) and NADPH generation, respectively. The crystal structure of CODH II (Figure 1) reveals a [Ni4Fe-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.¹⁰ As part of larger complexes, Ni-CODH is a module for cycling and distributing electrons from CO/CO₂ interconversions. We now demonstrate that *Ch* Ni-CODH I¹¹ attached to an electrode displays facile, direct interfacial electron exchange and provides a benchmark for “reversible” CO₂/CO electrochemistry.

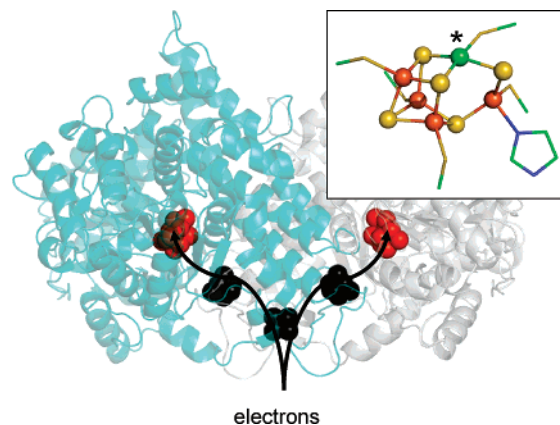


Figure 1. Structure of *Ch* CODH II showing the two subunits (blue and gray) and arrangement of Fe–S clusters (black) that relay electrons to and from the [Ni4Fe-5S] active sites (red). Inset shows the [Ni4Fe-5S] cluster where the proposed binding site for CO on the Ni atom is shown as *.¹⁰

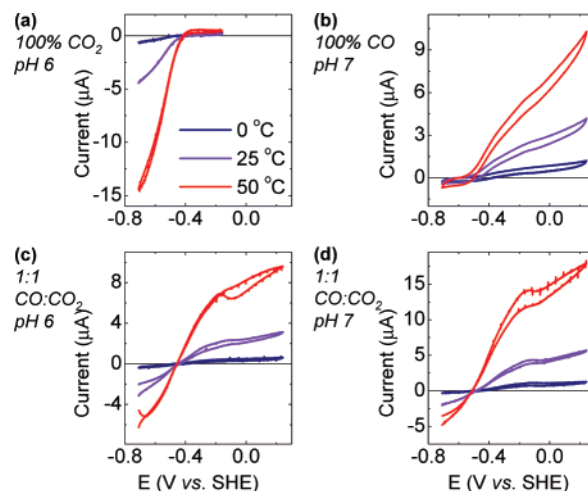


Figure 2. Protein film voltammograms showing CO₂ reduction and CO oxidation activities of *Ch* CODH I under atmospheres of 100% CO₂, 100% CO, or 1:1 CO₂/CO gas mixtures. Scan rate in panels a, c, and d was 10 mV/s; in panel b the scan rate was 30 mV/s. The electrode rotation was 4000 rpm. The pH is as 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.

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.¹² Panels a and b reveal the separate reduction and oxidation activities under 100% CO₂ or 100% CO, while panels c and d show combined reduction and oxidation activities for a 1:1 CO₂/CO gas mixture at two different pH values.

[†] University of Oxford.
[‡] University of Nebraska.

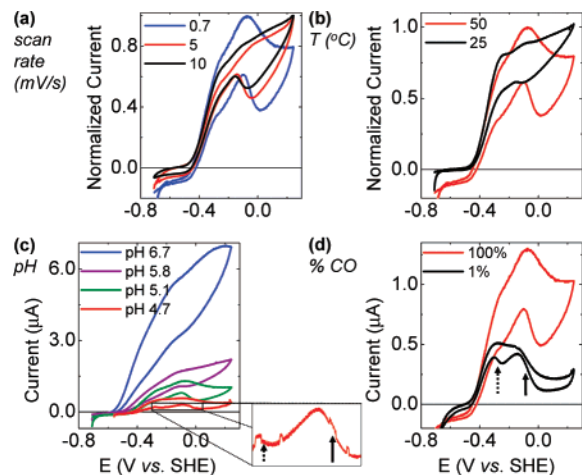


Figure 3. Observations of *Ch* Ni-CODH I activity at high potential, changing: (a) scan rate, (b) temperature, (c) pH, and (d) CO. Unless stated otherwise, conditions are 0.7 mV/s, 100% CO, rotation rate of 4000 rpm, pH 5.0, 50 °C. In panels a and b the current is normalized with respect to the maximum 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 CO₂ accumulates to be detected as a reduction current. It is clear that complete inactivation is not achieved even at 1% CO.

No nonturnover signals appeared without substrate, so the electroactive coverage must be very low,¹⁶ nor was activity observed upon adding H₂ to CO/CO₂-free experiments, confirming Ni-CODH I has no hydrogenase activity. For neither CO oxidation or CO₂ reduction does the catalytic current reach a limit, probably reflecting the high activity of enzyme molecules nonuniformly adsorbed on the PGE surface.¹³ 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 CO₂ reduction and 30 kJ mol⁻¹ 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 CO₂/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 CO₂ reduction but the current always cuts sharply through the zero current axis at the CO₂/CO reduction potential, so there is no enzyme-inherent overpotential in either direction. An inspiring conclusion is that CO₂/CO redox cycling can be rendered “reversible”, similar to the 2H⁺/H₂ couple at Pt or hydrogenase-modified electrodes.¹⁴

Our CO and CO₂ levels far exceed those of natural environments, so we used a procedure described by Léger et al.¹⁵ to compare K_M values for CO oxidation and CO₂ 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 (CO₂). 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 CO₂ means that in Figure 2, CO oxidation activity is saturated whereas CO₂ reduction is not, and at pH values below 6, k_{cat} for CO₂ reduction must actually be higher than for CO oxidation.¹⁶

Reversible inactivation at a potential more positive than -320 mV, pH 7.5, has been reported for another Ni-containing CODH,¹⁷ 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.¹¹) 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 H₂ levels.^{14,18}

In conclusion, *Ch* Ni-CODH linked to a PGE electrode displays intense electrocatalytic activity for CO oxidation and CO₂ 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 CO₂ electrochemistry. One important aspect will be to link the catalytic reversibility more closely to C-cluster interconversions that have been detected by EPR.¹

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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>.

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