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Investigations by Protein Film Electrochemistry of Alternative Reactions of Nickel-Containing Carbon Monoxide Dehydrogenase

Vincent C.-C. Wang[†], Shams T. A. Islam[†], Mehmet Can[‡], Stephen W. Ragsdale[‡], and Fraser A. Armstrong^{†,*}

[†]Department of Chemistry, Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QR, U.K.

[‡]Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48109-0606, United States

Abstract

Protein film electrochemistry has been used to investigate reactions of highly active nickel-containing carbon monoxide dehydrogenases (CODHs). When attached to a pyrolytic graphite electrode, these enzymes behave as reversible electrocatalysts, displaying CO₂ reduction or CO oxidation at minimal overpotential. The O₂ sensitivity of CODH is suppressed by adding cyanide, a reversible inhibitor of CO oxidation, or by raising the electrode potential. Reduction of N₂O, isoelectronic with CO₂, is catalyzed by CODH, but the reaction is sluggish, despite a large overpotential, and results in inactivation. Production of H₂ and formate under highly reducing conditions is consistent with calculations predicting that a nickel-hydrido species might be formed, but the very low rates suggest that such a species is not on the main catalytic pathway.

Graphical abstract

*Corresponding Author. Phone: +44 1865 272647. fraser.armstrong@chem.ox.ac.uk.

ASSOCIATED CONTENT

Supporting Information

Chronoamperometric results for the reaction of N₂O with CODH II_{Ch} at –760 mV. Chronoamperometric trace of CODH I_{Ch} attached to a MWCT-modified PGE electrode recorded at –760 mV under 100% CO₂ (40 scc/m) for 3 days. NMR result for the electrolytic solution that was poised at –760 mV using a MWCT-modified PGE electrode, without enzyme, in the presence of CO₂. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.5b03098.

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INTRODUCTION

Protein film electrochemistry (PFE) refers to a suite of electrochemical techniques in which the activity of a redox protein, typically a very active enzyme, is examined by attaching it to an electrode such that electrons exchange rapidly across the protein–electrode interface.^{1,2} Among the important features of PFE is the ability to measure catalytic activity directly as current, over a wide potential range; indeed, PFE allows the activity of an enzyme to be studied under potential conditions that are difficult to impose using chemical redox agents. In this paper, these aspects have been exploited to extract interesting information on an important class of enzyme that reduces CO₂.

Clean, efficient conversion of CO₂ to carbon-based fuels is an important scientific quest. In terms of the chemical energy that is stored, the first two-electron reduction of CO₂ to CO or formate is the most significant step in a reaction sequence that leads ultimately to chemical fuels, such as methanol and methane.³ In nature, carbon monoxide dehydrogenases (CODHs) catalyze CO₂/CO interconversion as shown in eq 1 ($E^{0'} = -520$ mV at pH 7.0)⁴ and PFE studies^{5–7} on two enzymes, CODH I and CODH II from *Carboxydotherrmus hydrogenoformans* (*Ch*), have shown that they achieve this with minimal overpotential requirement⁸—in strong contrast to synthetic molecular catalysts so far investigated.^{9,10} Attached to semiconductors, these enzymes also show how CO₂ can be reduced using light, in systems aimed at understanding artificial photosynthesis.^{11–15}

In terms of the wider implications for developing renewable energy catalysts, the nickel-containing CODHs belong to an increasing number of enzymes that behave as reversible electrocatalysts.⁸ Carbon dioxide is a difficult molecule to activate; therefore, understanding

how these enzymes catalyze CO₂ reduction so efficiently is important for designing the best catalysts for CO₂ reduction.¹⁶

The two enzymes CODH I_{Ch} and CODH II_{Ch} have high sequence similarity (59%) but different proposed cellular functions.¹⁶ Both proteins are homodimers with similar molecular weights, and their reported enzymatic activities and spectral features are almost identical, indicating similar active-site structures for both enzymes. The X-ray crystal structures of CODH II_{Ch}^{17–20} show (Figure 1) that each subunit contains a unique [Ni–4Fe–4S] cluster, known as the C-cluster, which is directly involved in the catalytic reaction, along with [4Fe–4S] clusters (the B-cluster and a shared D-cluster) that mediate electron transfer between the C-cluster and the external redox partner.¹⁶ A proposed catalytic cycle of CODH is shown in Scheme 1. On the basis of EPR and Mössbauer spectroscopy, several redox levels of the C-cluster, namely, C_{ox}, C_{red1}, and C_{red2}, have been identified.²¹ The oxidized “resting” state C_{ox} is inactive but readily activated by one-electron reduction. Studies by PFE have already shown how substrate analogues, cyanide (HCN/CN[−]) and cyanate (NCO[−]), selectively target the different redox levels.^{6,7}

In this paper, some further important properties of Ni–CODHs are explored by PFE. It is widely established that Ni–CODHs are extremely O₂-sensitive and undergo irreversible inactivation, but the nature of the reactions involved or target points for O₂ attack are still unclear.^{22–26} A greater understanding of the reaction mechanism may provide new insight for designing the crucial catalysts that can reduce CO₂ in the presence of air. Another substrate analogue of CO₂ is the isoelectronic molecule N₂O, and it is of interest to determine if and how this agent reacts. Although kinetically inert to most agents, N₂O is a powerful oxidant, the reduction potential for the N₂O/N₂ couple being 1.36 V vs SHE when corrected to pH 7.0. An earlier study of a carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) from *Moorella thermoacetica* showed that this larger complex of CODH catalyzes the reduction of N₂O to N₂, but it was unclear whether the reaction occurs at the C-cluster of CODH or at the metal cluster of ACS.²⁷ In this paper, we describe experiments on both CODH I_{Ch} (which has been better characterized, spectroscopically, and is superior at CO₂ reduction as it is less inhibited by CO) and CODH II_{Ch} (the crystallographically defined isozyme).

Recent quantum mechanics/molecular mechanics calculations have led to a proposal²⁸ that the electron-rich Ni site of the [Ni4Fe–4S] cluster in C_{red2} may exist as a hydrido species, i.e., as a Ni^{II}–H[−] entity (Scheme 1b) that would result from protonation of a site that is formally Ni(0) (Scheme 1). The fact that EPR studies^{21,29} reveal $g_{av} < 2$ values for both C_{red1} and C_{red2} states of the enzyme indicates that the Fe–S core remains in the same (reduced) oxidation level for both states. That the two redox equivalents required during catalysis are confined to the Ni subsite, specifically through the interconversion Ni^{II} ↔ Ni(0) (or Ni^{II}–H[−]), is further supported by XAS^{30,31} and Ni-ENDOR measurements.³² A low-valent Ni(0) subsite should indeed be strongly basic,³³ but its protonation then opens up the possibility of characteristic side reactions—production of formate (by insertion of CO₂ into the M–H bond)³⁴ and evolution of H₂ (a reduced terminal metal hydride, like that of a hydrogenase, should be prone to protonation, leading to H₂ release).³⁵ Exploiting the ability of the electrode to provide a variable high driving force for reduction, we have carried out

PFE experiments to quantify formate formation. We have also investigated the rate of evolution of H₂ during incubation with CO—in effect, the ability of CODH to catalyze the water–gas shift reaction.

MATERIALS AND METHODS

Isolation, purification, and activity measurements of CODH I and CODH II from *Carboxydotherrnus hydrogenoformans* were carried out as described previously.³⁶ The CO oxidation activities were 1300 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ for CODH I_{Ch} and 1000 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ for CODH II^{Ch} at 20 °C. For electrochemical measurements, all chemicals were of analytical or equivalent grade. Gases CO, CO₂ (CP grade), N₂O (99.99%), Ar (research grade), and O₂ (research grade) were obtained from BOC. Potassium cyanide was obtained from Fisher, and hydrogen peroxide (30% W/W) and polymyxin B sulfate were purchased from Sigma-Aldrich. Purified water (Milli-Q, Millipore 18.2 M Ω cm) was used throughout. Samples of O₂-saturated buffer (approximately 1.3 mM at 20 °C) were prepared by bubbling O₂ through a sealed vial for 20 min. The multiwalled carbon nanotubes (MWCTs, carbon >90% (trace metal basis)), 1-pyrenebutyric acid (Py), 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC), *N*-hydroxysulfosuccinimide (NHSS), and dimethylformamide (DMF), used for chemically modifying the PGE electrode, were purchased from Sigma-Aldrich. Buffers were prepared from 2-(*N*-morpholino) ethanesulfonic acid (MES) (Sigma-Aldrich) and adjusted to the desired pH value using NaOH (Sigma-Aldrich). Deuterium oxide D₂O (99.9%, Apollo Scientific) was used for NMR experiments. The gas channel in CODH II_{Ch} was simulated using the software “Hollow”.³⁷

Electrochemical experiments (cyclic voltammetry, CV, and chronoamperometry, CA) were carried out as described recently^{6,7} for CODH I_{Ch} and CODH II_{Ch} with an Autolab PGSTAT20 electrochemical analyzer. The cell was housed in an anaerobic glovebox (Vacuum Atmospheres, O₂ < 2 ppm). Pyrolytic graphite “edge” (PGE) electrodes (diameter 2 mm) were constructed as described previously.³⁸ Enzyme films of sufficient stability for most electrochemical experiments were obtained by spotting 2 μL of a 1:3 mixture (by concentration) of CODH and polymyxin onto the electrode surface. A high buffer concentration (0.2 M MES) was used to stabilize the pH in the presence of CO₂. All potentials were corrected to correspond to the standard hydrogen electrode (SHE) using the correction $E_{\text{SHE}} = E_{\text{SCE}} + 241 \text{ mV}$ at 25 °C.

To obtain a higher coverage of enzyme for detection of formate production, the PGE electrode was modified with MWCTs, in a similar manner to that described previously.³⁹ A suspension (15 μL) of MWCTs in DMF (1 mg/mL) was pipetted onto the PGE surface and left to dry overnight. The MWCT–PGE electrode surface was then treated with a 20 μL aliquot of a solution of Py in DMF (10 mM) and allowed to stand for 1 h. The modified electrode was washed with water to remove free Py and then taken into the glovebox for the next stage of modification. A mixture comprising 5 μL of EDC (0.4 M) and 5 μL of NHSS (0.1 M) was spotted onto the modified PGE surface; then, after 20 min, a solution of CODH I_{Ch} (10 μL) was pipetted on and left for 40 min with the electrode covered by an Eppendorf tube to preserve humidity. The resulting electrode was then placed in a 3 mL solution of 0.2 M MES (55.5% H₂O and 44.5% D₂O at pH 7) in the electrochemical cell and poised at

–760 mV with a continuous flow of CO₂ (40 scc/min) through the cell headspace. The electrode was rotated at 400 rpm. Samples were taken for NMR analysis (Bruker 500 MHz) after designated time periods.

Samples for measuring H₂ evolution by CODH I_{Ch} and CODH II_{Ch} under a CO atmosphere were prepared in a glovebox (<2 ppm of O₂). First, the Pyrex pressure vessel was sealed tightly with a rubber septum and purged with 96% CO/4% CH₄ for 20 min. Then, CODH I_{Ch} or CODH II_{Ch} in 0.2 M MES (pH 7.0) was injected into the vessel. A sample (20 μL) of the atmosphere in the headspace was withdrawn and analyzed for H₂ by gas chromatography (Agilent Technologies 7890A GC equipped with Restek ShinCarbon ST column and thermal conductivity detector) with N₂ as the carrier. For each measurement, the amount of H₂ formed was calculated against the internal standard CH₄.

RESULTS

Reactions with O₂

Potential Dependence—Chronoamperometry was used to investigate the potential dependence of the reaction of CODH II_{Ch} with O₂ under 100% CO at various potentials, as shown in Figure 2. The electrode was rotated at 2500 rpm, which ensures rapid mass transport of reagents to and from the electrode. At +140 mV (Figure 2a), the current recorded at the beginning of the experiment is due to the catalytic action of enzyme, which decreases with time (approximately 40% loss after 2 min) due mainly to formation of the oxidized inactive state C_{ox}.⁶ Under these high-potential conditions, the steady-state oxidation level of the C-cluster must be predominantly the most oxidized active one (C_{red1} or one termed C_{ox}' as inferred in previous investigations, uncharacterized but at the same oxidation level as C_{ox}). The potential was then stepped to –760 mV for 2 min to reactivate all C_{ox} that had formed and then stepped to +140 mV which revealed recovery of about 70% of the original activity. An aliquot of O₂-saturated solution was immediately added, resulting in rapid inactivation. After maintaining a flow of CO for about 20 min to flush away remaining O₂, the potential was again stepped to –760 mV for 2 min before stepping back to +140 mV to observe how much recovery occurred. On the basis of the current measured immediately before O₂ injection, about 50% of the activity was restored.

Figure 2b shows a similar experiment but with O₂ injected when the electrode potential is held at –260 mV. The initial activity decrease (slower than observed at +140 mV) is now due mainly to general deterioration of the enzyme film (film loss). After stepping down to –760 mV and then back to –260 mV and injecting O₂-saturated solution, the cell was again purged by the flow of CO for 1000 s before stepping to –760 mV to reactivate any recoverable enzyme. In contrast to the experiment carried out at +140 mV, there was only a very small recovery (approximately 1%).

A further experiment (Figure 2c) was carried out to determine whether the inactive C_{ox} state is less prone to damage by O₂. First, the electrode potential was set at –260 mV with 100% CO in the headspace to estimate the normal rate of film loss. The potential was then stepped to +140 mV, and the gas supply was changed from 100% CO to 5% CO in Ar to accelerate the electrochemical formation of C_{ox}. Once the catalytic current had dropped to ca. 0 μA,

registering completion of the formation of the inactive C_{ox} state, O_2 -saturated buffer (0.5 mL) was injected. The electrode potential was maintained at +140 mV for a further 1000 s, while O_2 was flushed from the cell; then, it was stepped to -560 mV for reductive reactivation during which time the gas composition was changed back to 100% CO . After 10 min, the electrode potential was stepped to -260 mV, upon which more than 70% of the CO oxidation activity was recovered. This result showed that the enzyme is significantly protected against degradation by O_2 when it is in the oxidized resting C_{ox} state.

Protection by Cyanide—Further PFE experiments were carried out to examine the nature of the irreversible inactivation by O_2 , which was now established to be much increased under more reducing conditions. As shown in Scheme 1, cyanide binds reversibly to the Ni atom of the C-cluster and targets the C_{red1} state but not C_{red2} . Previous studies showed that catalytic activity in the presence of cyanide is strongly suppressed at electrode potentials more positive than ca. -0.65 V but recovers rapidly when the potential is stepped below -0.76 V.^{6,7} We therefore carried out a potential-step experiment with CODH II_{Ch} , as shown in Figure 3, to see if cyanide binding protects the enzyme against irreversible attack by O_2 as expected if the C-cluster is the target site.

A rotating disc electrode with a film of CODH II_{Ch} was set up as before: the electrode potential was held at -260 mV, and then, an aliquot of concentrated KCN solution was injected to give a final concentration of 10 mM in the cell. Once the electrocatalytic current had dropped to approximately zero, O_2 -saturated buffer (0.5 mL into 3.0 mL of cell solution) was injected and the potential was maintained for at least 1000 s during which time O_2 was flushed out by CO purging. The cell solution was then replaced with fresh buffer (thus rapidly removing any cyanide remaining in solution), and the potential was switched to -760 mV for 60 s to allow cyanide to dissociate from the enzyme before a second exchange with fresh buffer was carried out. A step back to -260 mV revealed that approximately 50% of the catalytic CO oxidation current was restored, as opposed to less than 1% current recovery without cyanide protection, under the same conditions (as shown in Figure 2b). The result shows that cyanide, which binds strongly to the active C_{red1} state, protects against irreversible inactivation by O_2 .

Reactions with N_2O

Cyclic voltammetry of CODH I_{Ch} conducted under 100% N_2O shows that a negative current commences below -0.55 V (Figure 4a), which is much smaller than observed when the experiment is conducted under 100% CO_2 . To eliminate the possibility of the small reduction current being due to traces of CO_2 in the solution, cyclic voltammetry was also performed with a stationary electrode (Figure 4b). Were the electrocatalytic current to be due to CO_2 reduction, a positive (oxidation) peak would be expected at approximately -0.4 V due to reoxidation of the CO product remaining in the vicinity of the electrode surface. A large oxidation peak is observed when the experiment is conducted under 100% CO_2 but no trace of a peak was observed when 100% N_2O was used, showing that the small reduction current is due to N_2O reduction and not residual CO_2 in solution. Experiments with CODH I_{Ch} and CODH II_{Ch} under a N_2O atmosphere gave similar results.

Chronoamperometry was used to study the reaction of CODH I_{Ch} with N_2O in more detail. Figure 5 shows experiments in which the effect of N_2O was measured at increasingly negative potentials. Analogous results obtained for CODH II_{Ch} are shown in the Supporting Information, Figure S1. At a potential of -209 mV, the enzyme was first subjected to two CO/Ar cycles in order to establish the background “film loss” rate. The gas supply was then switched from 100% Ar to 100% N_2O , upon which there was no change in current. After replacing the gas supply by Ar and then switching to 100% CO, an oxidation current was observed, which was substantially lower than that recorded before the reaction with N_2O . When CO was replaced directly by N_2O (at 5900 s), the current decreased to zero and then recovered, again partly, when the gas composition was restored to 100% CO. Experiments with similar cycling of gases at more negative potentials (-439 and -760 mV) revealed that a reduction current appears under 100% N_2O , which is much more marked at -760 mV, demonstrating that N_2O is a reducible substrate. (Note that a much longer time is required to remove CO_2 from the solution compared to CO.) In addition, from the extent of activity lost at the end of each series of gas cycles, it is clear that N_2O also causes increasing degrees of irreversible inactivation as a more negative electrode potential is applied.

Production of Formate and H_2

Figure 6 shows the 1H NMR spectrum of the solution resulting from the reduction of CO_2 by CODH I_{Ch} adsorbed on a MWCT-modified PGE electrode held at -760 mV over a period of 3 days. Owing to the length of time required, we used CODH I_{Ch} because of its greater activity compared to CODH II_{Ch} . The characteristic 1H peak for $HCOO^-$ is clearly evident at 8.4 ppm, and similar to that obtained for a pure solution of formate at the same pH. Indeed, adding a small aliquot of pure potassium formate solution to the NMR tube resulted in a single peak at 8.4 ppm, thus establishing unambiguously that the product is formate. The amount of formate produced by CODH I_{Ch} in 3 days was approximately 75 nmol (concentration 25 μM in the solution) compared to 9.4 μmol of the total two-electron product (CO + formate) estimated by integrating the current–time profile of the chronoamperometric trace (Supporting Information, Figure S2). The amount of formate detected must be an underestimate because only $HCOO^-$ is observed by 1H NMR. No formate was detected in a control experiment in which the MWCT-modified PGE electrode without the enzyme was poised at -760 mV under a continuous flow of CO_2 for 3 days (Supporting Information, Figure S3). Neither was any formate detected in experiments in which the MWCT-modified PGE electrode, with or without CODH I_{Ch} adsorbed, was held at open circuit potential under CO for 4 days.

The time courses for H_2 evolution by CODH I_{Ch} and CODH II_{Ch} in the presence of 96% CO/4% CH_4 at 20 °C are shown in Figure 7. Rates of H_2 evolution determined at -260 mV were 78.4 nmol min $^{-1}$ mg $^{-1}$ (0.16 s $^{-1}$) for CODH I_{Ch} and 7.5 nmol min $^{-1}$ mg $^{-1}$ (0.016 s $^{-1}$) for CODH II_{Ch} . To establish that H_2 formation stems from the activity of CODH and not through contamination by hydrogenase, an aliquot of KCN solution (to a total concentration of 0.2 mM) was added (H_2 evolution by hydrogenase is not inhibited by cyanide). Production of H_2 ceased upon introducing cyanide to the reaction cell, establishing that the H_2 evolving activity is due to CODH.

DISCUSSION

Our experiments show that the reversibility of reaction of O_2 with Ni-CODHs is potential dependent. Unlike cyanide and cyanate which are not redox active at an electrode, O_2 cannot be studied as an inhibitor at low potentials without it also being reduced at the electrode. Use of a rotating electrode offers a good partial solution because a steady supply of O_2 is sustained and products such as superoxide and peroxide are rapidly removed from the electrode surface.

It is clear from the substantial protection afforded by cyanide, as well as the experiments carried out after inactivating the enzyme to the C_{ox} state, that the C-cluster is the primary (but not necessarily sole) target. It is less clear which active oxidation level of the enzyme is most susceptible to O_2 . Electrochemistry can drive and measure reactions but cannot provide structural information, nor can steady-state levels of different intermediates be fixed with certainty; the influence of the electrode potential must be matched against the binding of substrates, products, and inhibitors. The observation that more negative potentials lead to irreversible inactivation by O_2 contrasts with the observation made with hydrogenases for which oxidizing conditions render O_2 most potent.^{40,41} At the highest potential used, +140 mV, the enzyme is already inactivating (converting rapidly to C_{ox}) without any O_2 being added. Addition of O_2 at this potential, most likely to favor a high steady-state level of C_{red1} (or the hypothetical state C_{ox}' suggested⁶ in a previous study), causes rapid inactivation but the enzyme can be reactivated upon reduction; therefore, C_{ox} (or an equivalent state) is being formed. The fact that cyanide, which targets C_{red1} , offers protection against irreversible inactivation suggests that C_{red1} is a specific target for O_2 ; if so, the avoidance of such irreversible inactivation at +140 mV is further evidence for a state C_{ox}' . In this reasoning, we have not considered the influence of the redox status of the electron-transferring B- and D-clusters, and thus, further speculation is unwarranted. Calculations using “Hollow” software showed that a gas channel connects the protein surface to the C-cluster but not the B-cluster, making it less likely that O_2 reacts with the B-cluster, although it could attack the surface-exposed D-cluster.

The cyclic voltammetry and chronoamperometry results show clearly that N_2O is catalytically reduced by CODH. Noting that N_2O is a much stronger oxidant than CO_2 , the sluggish nature of this reaction is clear from comparing the results obtained at -439 and -760 mV which show that an excessively high driving force (overpotential) is still required (over 2 V). The potential at which catalysis occurs is probably set by that required for electrons to enter the enzyme at the D-cluster and regenerate C_{red2} on each cycle; the reduction potential of the D-cluster is not known, but the C_{red1}/C_{red2} potential lies close to that of the CO_2/CO couple.¹⁶ After removing N_2O from the cell, the decrease in electrocatalytic currents for CO oxidation or CO_2 reduction shows that N_2O also causes irreversible inactivation, the extent of which increases as the electrode potential is made more negative.

The ability of CODH I_{Ch} to generate formate from CO_2 is difficult to verify by chemical means due to the low reduction potential ($E < -0.5$ V at pH 7.0) being difficult to sustain by chemical titrants. This problem is easily overcome by using an electrode to drive the

reaction. Analysis of the solution after electrolysis for 3 days showed that a small amount of formate was indeed formed but at a level at least 2 orders of magnitude lower than CO (based on faradaic efficiency). More significant production of formate would have provided reasonable evidence for involvement of a Ni(II)-hydrido species, because insertion of CO₂ into M–H bonds to produce formate is well established for molecular metal catalysts.^{42,43} A metal-bound hydrido ligand can also be protonated to yield H₂; indeed, our results agree with earlier studies⁴⁴ which demonstrated that CODH displays low H₂ evolution activity, but the activity (accordingly abolished by the presence of cyanide) is 4–5 orders of magnitude lower than that for CO oxidation (approximately ~1000 s⁻¹ at 20 °C for CODH I). Detection of significant H₂ formation would have pointed to a greater similarity between the water–gas shift (WGS) reaction⁴⁵ and oxidation of CO by CODH, as shown in Scheme 1. However, the very low side-reaction activities argue against C_{red2} being a Ni(II)-hydrido species, since such a species would be expected to be much more reactive for formate and H₂ production.

Development of efficient catalysts (or photocatalysts) for CO₂ reduction plays an important role in converting CO₂ to carbon-based fuel in a clean and sustainable way.³ Knowledge about how Ni-CODHs perform CO₂ reduction so efficiently is valuable because the 3D structure of the active site of the enzymes is known in considerable detail—the outer coordination shell in particular.⁸ The experiments we have described add to this knowledge by helping to define the extent of alternative activities, including degradation by O₂ and the extent of parallel production of formate and H₂.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Vincent KA, Parkin A, Armstrong FA. Investigating and Exploiting the Electrocatalytic Properties of Hydrogenases. *Chem. Rev.* 2007; 107:4366–4413. [PubMed: 17845060]
2. Léger C, Bertrand P. Direct Electrochemistry of Redox Enzymes as a Tool for Mechanistic Studies. *Chem. Rev.* 2008; 108:2379–2438. [PubMed: 18620368]
3. Hansen HA, Varley JB, Peterson AA, Norskov JK. Understanding Trends in the Electrocatalytic Activity of Metals and Enzymes for CO₂ Reduction to CO. *J. Phys. Chem. Lett.* 2013; 4:388–392. [PubMed: 26281729]
4. Bender G, Pierce E, Hill JA, Darty JE, Ragsdale SW. Metal Centers in the Anaerobic Microbial Metabolism of CO and CO₂. *Metallomics.* 2011; 3:797–815. [PubMed: 21647480]
5. Parkin A, Seravalli J, Vincent KA, Ragsdale SW, Armstrong FA. Rapid and efficient electrocatalytic CO₂/CO interconversions by *Carboxydotherrmus hydrogenoformans* CO dehydrogenase I on an electrode. *J. Am. Chem. Soc.* 2007; 129:10328–10329. [PubMed: 17672466]

6. Wang VCC, Can M, Pierce E, Ragsdale SW, Armstrong FA. A Unified Electrocatalytic Description of the Action of Inhibitors of Nickel Carbon Monoxide Dehydrogenase. *J. Am. Chem. Soc.* 2013; 135:2198–2206. [PubMed: 23368960]
7. Wang VCC, Ragsdale SW, Armstrong FA. Investigations of two Bidirectional Carbon Monoxide Dehydrogenases from *Carboxydotherrmus hydrogenoformans* by Protein Film Electrochemistry. *ChemBioChem.* 2013; 14:1845–1851. [PubMed: 24002936]
8. Armstrong FA, Hirst J. Reversibility and efficiency in electrocatalytic energy conversion and lessons from enzymes. *Proc. Natl. Acad. Sci. U. S. A.* 2011; 108:14049–14055. [PubMed: 21844379]
9. Benson EE, Kubiak CP, Sathrum AJ, Smieja JM. Electrocatalytic and Homogeneous Approaches to Conversion of CO₂ to Liquid Fuels. *Chem. Soc. Rev.* 2009; 38:89. [PubMed: 19088968]
10. Savéant JM. Molecular Catalysis of Electrochemical Reactions. Mechanistic Aspects. *Chem. Rev.* 2008; 108:2348–2378. [PubMed: 18620367]
11. Woolerton TW, Sheard S, Reisner E, Pierce E, Ragsdale SW, Armstrong FA. Efficient and Clean Photoreduction of CO₂ to CO by Enzyme-Modified TiO₂ Nanoparticles Using Visible Light. *J. Am. Chem. Soc.* 2010; 132:2132–2133. [PubMed: 20121138]
12. Woolerton TW, Sheard S, Chaudhary YS, Armstrong FA. Enzymes and bio-inspired electrocatalysts in solar fuel devices. *Energy Environ. Sci.* 2012; 5:7470–7490.
13. Chaudhary YS, Woolerton TW, Allen CS, Warner JH, Pierce E, Ragsdale SW, Armstrong FA. Visible light-driven CO₂ reduction by enzyme coupled CdS nanocrystals. *Chem. Commun.* 2012; 48:58–60.
14. Bachmeier A, Hall S, Ragsdale SW, Armstrong FA. Selective Visible- Light- Driven CO₂ Reduction on a p- Type Dye- Sensitized NiO Photocathode. *J. Am. Chem. Soc.* 2014; 136:13518–13521. [PubMed: 25237714]
15. Bachmeier A, Wang VCC, Woolerton TW, Bell S, Fontecilla-Camps JC, Can M, Ragsdale SW, Chaudhary YS, Armstrong FA. How Light-Harvesting Semiconductors Can Alter the Bias of Reversible Electrocatalysts in Favor of H₂ Production and CO₂ Reduction. *J. Am. Chem. Soc.* 2013; 135:15026. [PubMed: 24070184]
16. Can M, Armstrong FA, Ragsdale SW. Structure, Function, and Mechanism of the Nickel Metalloenzymes, CO Dehydrogenase, and Acetyl-CoA Synthase. *Chem. Rev.* 2014; 114:4149–4174. [PubMed: 24521136]
17. Jeoung JH, Dobbek H. Carbon Dioxide Activation at the Ni Fe- Cluster of Anaerobic Carbon Monoxide Dehydrogenase. *Science.* 2007; 318:1461–1464. [PubMed: 18048691]
18. Jeoung JH, Dobbek H. Structural Basis of Cyanide Inhibition of Ni, Fe- Containing Carbon Monoxide Dehydrogenase. *J. Am. Chem. Soc.* 2009; 131:9922–9992. [PubMed: 19583208]
19. Jeoung JH, Dobbek H. n- Butyl isocyanide oxidation at the [NiFe₄S₄OH] cluster of CO dehydrogenase. *J. Biol. Inorg. Chem.* 2012; 17:167–173. [PubMed: 21904889]
20. Dobbek H, Svetlitchnyi V, Gremer L, Huber R, Meyer O. Crystal structure of a carbon monoxide dehydrogenase reveals a [Ni- 4Fe- 5S] cluster. *Science.* 2001; 293:1281–1285. [PubMed: 11509720]
21. Lindahl PA. Implications of a carboxylate- bound C- cluster structure of carbon monoxide dehydrogenase. *Angew. Chem., Int. Ed.* 2008; 47:4054–4056.
22. Svetlitchnyi V, Peschel C, Acker G, Meyer O. Two membrane- associated NiFeS- carbon monoxide dehydrogenases from the anaerobic carbon- monoxide- utilizing eubacterium *Carboxydotherrmus hydrogenoformans*. *J. Bacteriol.* 2001; 183:5134–5144. [PubMed: 11489867]
23. Ragsdale SW, Clark JE, Ljungdahl LG, Lundie LL, Drake HL. Properties of purified carbon monoxide dehydrogenase from *Clostridium thermoaceticum*, a nickel, iron- sulfur protein. *J. Biol. Chem.* 1983; 258:2364–2369. [PubMed: 6687389]
24. Bonam D, Ludden PW. Purification and characterization of carbon monoxide dehydrogenase, a nickel, zinc, iron- sulfur protein, from *Rhodospirillum rubrum*. *J. Biol. Chem.* 1987; 262:2980–2987. [PubMed: 3029096]
25. Bonam D, Lehman L, Roberts GP, Ludden PW. Regulation of Carbon Monoxide Dehydrogenase and Hydrogenase in *Rhodospirillum rubrum*: Effects of CO and Oxygen on Synthesis and Activity. *J. Bacteriol.* 1989; 171:3102–3107. [PubMed: 2498285]

26. Drake HL. Occurrence of nickel in carbon monoxide dehydrogenase from *Clostridium pasteurianum* and *Clostridium thermoaceticum*. J. Bacteriol. 1982; 149:561–566. [PubMed: 6895750]
27. Lu WP, Ragsdale SW. Reductive activation of the coenzyme A /acetyl- CoA isotopic exchange reaction catalyzed by carbon monoxide dehydrogenase from *Clostridium thermoaceticum* and its inhibition by nitrous oxide and carbon monoxide. J. Biol. Chem. 1991; 266:3554–3564. [PubMed: 1995618]
28. Amara P, Mouesca JM, Volbeda A, Fontecilla-Camps JC. Carbon Monoxide Dehydrogenase Reaction Mechanism: A Likely Case of Abnormal CO₂ Insertion to a Ni- H- Bond. Inorg. Chem. 2011; 50:1868–1878. [PubMed: 21247090]
29. Lindahl PA, Munck E, Ragsdale SW. Carbon monoxide dehydrogenase from *Clostridium thermoaceticum*. EPR and electrochemical studies in carbon dioxide and argon atmospheres. J. Biol. Chem. 1990; 265:3873–3879. [PubMed: 2154491]
30. Ha SW, Korbas M, Klepsch M, Meyer-Klaucke W, Meyer O, Svetlitchnyi V. Interaction of Potassium Cyanide with the [Ni- 4Fe- 5S] Active Site Cluster of CO Dehydrogenase from *Carboxydotherrmus hydrogenoformans*. J. Biol. Chem. 2007; 282:10639–10646. [PubMed: 17277357]
31. Gu WW, Seravalli J, Ragsdale SW, Cramer SP. CO-Induced Structural Rearrangement of the C Cluster in *Carboxydotherrmus hydrogenoformans* CO Dehydrogenase- Evidence from Ni K-Edge X- ray Absorption Spectroscopy. Biochemistry. 2004; 43:9029–9035. [PubMed: 15248760]
32. DeRose VJ, Telser J, Anderson ME, Lindahl PA, Hoffman BM. A Multinuclear ENDOR Study of the C- Cluster in CO Dehydrogenase from *Clostridium thermoaceticum*: Evidence for H₂O and Histidine Coordination to the [Fe₄S₄] Center. J. Am. Chem. Soc. 1998; 120:8767–8776.
33. Morris RH. Estimating the Acidity of Transition Metal Hydride and Dihydrogen Complexes by Adding Ligand Acidity Constants. J. Am. Chem. Soc. 2014; 136:1948–1959. [PubMed: 24410025]
34. Seu CS, Appel AM, Doud MD, DuBois DL, Kubiak CP. Formate oxidation via β -deprotonation in [Ni- (P^R₂N^{R'}₂)₂(CH₃CN)]²⁺ complexes. Energy Environ. Sci. 2012; 5:6480–6490.
35. Siegbahn PEM, Tye JW, Hall MB. Computational Studies of [NiFe] and [FeFe] Hydrogenases. Chem. Rev. 2007; 107:4414–4435. [PubMed: 17927160]
36. Seravalli J, Ragsdale SW. ¹³C NMR characterization of an exchange reaction between CO and CO₂ catalyzed by carbon monoxide dehydrogenase. Biochemistry. 2008; 47:6770–6781. [PubMed: 18589895]
37. Ho BK, Gruswitz F. BMC Struct. Biol. 2008; 8:49. [PubMed: 19014592]
38. Sucheta A, Cammack R, Weiner J, Armstrong FA. Reversible electrochemistry of fumarate reductase immobilized on an electrode surface. Direct voltammetric observations of redox centers and their participation in rapid catalytic electron transport. Biochemistry. 1993; 32:5455–5465. [PubMed: 8499449]
39. Krishnan S, Armstrong FA. Order-of-magnitude enhancement of an enzymatic hydrogen-air fuel cell based on pyrenyl carbon nanostructures. Chem. Sci. 2012; 3:1015–1023.
40. Stripp ST, Goldet G, Brandmayr C, Sanganas O, Vincent KA, Haumann M, Armstrong FA, Happe T. How oxygen attacks [FeFe] hydrogenases from photosynthetic organisms. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:17331–17336. [PubMed: 19805068]
41. Evans RM, Parkin A, Roessler MM, Murphy BJ, Adamson H, Lukey MJ, Sargent F, Volbeda A, Fontecilla-Camps JC, Armstrong FA. Principles of Sustained Enzymatic Hydrogen Oxidation in the Presence of Oxygen - The Crucial Influence of High Potential Fe-S Clusters in the Electron Relay of [NiFe]-Hydrogenases. J. Am. Chem. Soc. 2013; 135:2694–2707. [PubMed: 23398301]
42. Beley M, Collin JP, Ruppert R, Sauvage JP. Electrocatalytic Reduction of Carbon Dioxide by Nickel Cyclam²⁺ in Water: Study of the Factors Affecting the Efficiency and the Selectivity of the Process. J. Am. Chem. Soc. 1986; 108:7461–7467. [PubMed: 22283241]
43. Schmeier TJ, Dobereiner GE, Crabtree RH, Hazari N. Secondary Coordination Sphere Interactions Facilitate the Insertion Step in an Iridium(III) CO₂ Reduction Catalyst. J. Am. Chem. Soc. 2011; 133:9274–9277. [PubMed: 21612297]

44. Menon S, Ragsdale SW. Evidence That Carbon Monoxide Is an Obligatory Intermediate in Anaerobic Acetyl- CoA Synthesis. *Biochemistry*. 1996; 35:12119–12125. [PubMed: 8810918]
45. Crabtree, RH. The organometallic chemistry of the transition metals. 5th ed.. New York: Wiley; 2009.

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Figure 1. Structure of CODH II_{Ch} (PDB: 3B53) from *Carboxydothemus hydrogenoformans*. Two positions are shown for the “pendant” Fe atom that is thought to bind an oxygen atom during catalysis.

Figure 2.

Effects on CODH II_{Ch} electrocatalysis of injecting O₂-saturated buffer (0.5 mL) into the electrochemical cell (3.0 mL) under different conditions. The black line indicates times during which the electrode potential was poised at (a) +140 mV or (b) –260 mV, and the red line indicates times during which the electrode potential was poised at –760 mV for reductive reactivation. Experimental conditions: 25 °C, 0.2 M MES buffer (pH 7.0), rotation rate 2500 rpm and 100% CO. In panel c, black, red, and blue lines indicate times during which the electrode potential was poised at –260, +140, and –560 mV, respectively, while

the green dashed line indicates the film loss extrapolated from the first 10 min. The lower concentration of CO (5% CO in Ar in the white region) was used to accelerate formation of the inactive C_{ox} state in CODH at +140 mV. Oxygen-saturated buffer was injected at 1600 s. Experimental conditions: 10 °C, 0.2 M MES buffer (pH 7.0), rotation rate 2500 rpm, 100% CO (gray region), and 5% CO (white region).

Figure 3.

Experiment showing that cyanide protects CODH II_{Ch} against irreversible degradation by O₂. The black line indicates when the potential was poised at -260 mV, and the red line refers to a poise at -760 mV. The gray windows refer to periods during which buffer is exchanged. The green dashed line shows an extrapolation for film loss. Experimental conditions: 25 °C, 0.2 M MES buffer (pH 7.0), rotation rate 2500 rpm, and 100% CO.

Figure 4.

Cyclic voltammograms of the electrocatalytic activity of CODH I_{Ch} in the presence of different gases. The black, red, and blue lines indicate experiments carried out under 100% Ar, 100% N_2O , and 100% CO_2 , respectively. (a) Cyclic voltammetry performed with an electrode rotated at 1000 rpm. (b) Cyclic voltammetry performed with a stationary electrode. Experimental conditions: 25 °C, 0.2 M MES buffer (pH 7.0), scan rate 20 mV s⁻¹.

Figure 5.

Experiments in which CODH I_{Ch} on the PGE electrode was poised at (a) -209 mV , (b) -439 mV , and (c) -760 mV in the presence of N₂O. The white region indicates the presence of 100% CO (a, b) or 100% CO₂ (c); the blue and gray regions indicate the presence of 100% N₂ and 100% Ar, respectively; the red region shows when the atmosphere is 100% N₂O. The inset in Figure 5b shows an enlargement of the region of N₂O reduction at -439 mV . Experimental conditions: $25\text{ }^{\circ}\text{C}$, $0.2\text{ M MES buffer (pH 7.0)}$, and rotation rate 1500 rpm .

experiment

N₂ control

1 mM formate

Figure 6.

Formation of formate by CODH I_{Ch} adsorbed on a MWCT-modified PGE electrode at -760 mV. The upper panel shows the NMR spectrum of the solution after flowing CO_2 (40 scc/m) through the headspace for 3 days. Experimental conditions: 25°C , 0.2 M MES (pH 7.0 and 45.5% D_2O and 55.5% H_2O), electrode rotation rate 400 rpm. The middle panel shows the spectrum obtained for the control experiment conducted under N_2 . The lower panel shows the spectrum of 1 mM potassium formate in 100% D_2O under the same conditions.

Figure 7. Catalysis of H₂ evolution by CODH I_{Ch} and CODH II_{Ch} in the presence of 96% CO/4% CH₄. Aliquots of enzyme, 30 μ L of CODH I_{Ch} (15 mg/mL) and 20 μ L of CODH II_{Ch} (18 mg/mL) were added to 0.2 M MES buffer (pH 7.0) to give a final volume of 0.3 mL. Reactions were performed at 20 °C. See text for details.

Scheme 1.

Two Possible Mechanisms of Action of Ni-CODH Proposed by (a) the Groups of Dobbek¹⁷ and Lindahl^{21,32} (Involving a $Ni(0)$ Subsite for "Book-Keeping" Purposes) or (b) Fontecilla-Camps and Coworkers²⁸ (in Which a $Ni(II)-H$ Intermediate State Is Proposed)^a

^aB refers to the amino acid that provides or accepts a proton, possibly histidine or lysine.

The substrate analogue CN^- inhibits CO oxidation, whereas NCO^- inhibits CO_2 reduction.