A Functional Modeling Study of the CO Oxidation Site of Nickel CO Dehydrogenase

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Abstract: In a functional model study of carbon monoxide dehydrogenase (CODH), a homologous series of Ni(II) complexes with a biologically relevant O, N, S ligand set has been synthesized and characterized. In aqueous solution at room temperature, they are active for CO oxidation by methylviologen (= mv^{2+}) to produce CO_2. The key features of the reaction are pseudo-first-order dependence on catalyst, CO, H_2O, and mv^{2+}, a sigmoidal rate−pH profile with an inflection point at pH 7.6, and the absence of any H_2 as a product, although H_2 is the exclusive product of the related water gas shift reaction. The proposed mechanism, involving decarboxylation of a Ni−COO^- intermediate by mv^{2+} in the key step, accounts for all these features. As in CODH itself, CO oxidation is inhibited by both CN^- and MeI. O_2 is also a competent electron acceptor in this system because reduced mv^+ is air-sensitive.

A carbon monoxide dehydrogenase (CODH) from *Clostridium thermoaceticum* is the central enzyme which catalyzes both acetyl-CoA synthesis from CO, coenzyme A (CoASH), and a methylated corrinoid/iron−sulfur protein (CH_3-C/Fe−SP) (eq 1) and the reversible oxidation of CO to CO_2 (eq 2).[1] It is found

$$CH_3\text{-}X + CO + CoASH \rightarrow CH_3\text{-}CO\text{-}SCoA + X^- + H^+ \quad (1)$$

$X = C/Fe-SP$

$$CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^- \quad (2)$$

that nickel is essential for both activities. There are two essential nickel ions in the enzyme,[2] each of which is believed to be associated with one of the two reactions.[3] Treatment of CODH with 1,10-phenanthroline removes Ni from the first Ni site, which suppresses CoA synthase activity but not the CO oxidation. A variety of inhibitors for CoA synthesis do not inhibit CO oxidation.[3b,4] The first Ni site, believed to be responsible for the biosynthesis of acetyl-CoA,[3a] is also called the NiFeC center because the EPR signal associated with this site shows coupling to all three elements.[5a] The Ni is therefore believed to be associated with, but probably not part of, an Fe_xS_x cluster and contains bound CO in the EPR-active form.[5a] Very recently, good evidence has been obtained[5b] that CO binds to Fe, not Ni, at this site. The second Ni site, which may be a Ni−Fe cluster, seems to be responsible for CO oxidation.[3a] The CO binding site at this center (Ni or Fe) has not yet been determined. It has been suggested that the electron needed to reduce $[NiFeC]_{ox}$ comes from CO oxidation at the second Ni site. The two-site model is strongly supported by kinetic data.[5c]

Methylviologen (mv^{2+}) can act as an alternative electron acceptor from the second Ni (CO oxidation) site (eq 3). Using a suitable base, the rate of CO oxidation with mv^{2+} increases with pH with an inflection point close to pH 8.4, which indicates that a group with a pK_a of 8.4 may be involved in the reaction.[6a] The rate of CO oxidation by CODH follows simple Michaelis−Menten kinetics with a K_m(CO) of 0.025 mM[6b] and a K_m(mv^{2+}) of 0.4 mM.[6a]

$$CO + H_2O + 2mv^{2+} \rightarrow CO_2 + 2H^+ + 2mv^+ \quad (3)$$

Incubation of CODH with CN^- in the absence of CO resulted in a reversible loss of the CO oxidation activity;[3b,6] inhibition by cyanide is pH independent. Methyl iodide can also inactivate CO oxidation,[3b] and incubation with CO does not fully restore CODH activity; the mechanism of MeI inhibition is not understood.

A second type of CODH exists, for example in *Rhodospirillum rubrum*, which has CO oxidation activity only[11] and does not catalyze acetyl CoA synthesis. This seems to contain a Ni site resembling the second (CO oxidation) Ni site in the *C. thermoaceticum* enzyme. EPR studies shows an EPR signal, assigned to the second type of nickel site, which disappears

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when the *R. rubrum* enzyme is treated with CO.[7,8] EXAFS data suggest the presence of a N- (and/or O-) and S-donor environment for this Ni site.[9,10]

It is not yet clear whether the catalytic activity for CO oxidation is associated with a Ni ion in the enzyme and, if so, what the mechanism is. We have therefore screened a series of Ni complexes to look for CO oxidation activity and report here our success in the case of compound **1**.

Most of the rare examples of Ni(II)-CO complexes found in the literature are five-coordinate,[12] for reasons we have discussed.[13] Vibrational spectroscopy[14] shows that the enzyme binds CO $\{\nu(CO) = 1995\ cm^{-1}\}$ at an Fe site of the NiFeC cluster, the site responsible for acetyl-CoA synthesis, not CO oxidation. No detectable intermediate having a Ni−CO group has been found for the CO oxidation site in the enzyme, however.

In this paper we report a series of Ni(II) complexes which, at room temperature and in an aqueous medium, can catalyze CO oxidation with electron transfer to methylviologen. A mechanism is proposed in which CO interacts with the Ni(II) complex and then undergoes nucleophilic attack by H_2O which leads to CO_2 formation and methylviologen reduction to mv^+. CN^- and MeI can inhibit the model CO oxidation reaction. This seems to be the first functional model for the second Ni site of CO oxidation using the biologically relevant metal and ligands and has been briefly reported as a communication.[15a] The reverse reaction ($CO_2 \rightarrow CO$) has been well documented in different systems, e.g., by Beley *et al.*[15b]

Experimental Section

Nickel(II) acetate tetrahydrate, 4-methyl-3-thiosemicarbazide, 4,4-dimethyl-3-thiosemicarbazide, 2′-hydroxy-5-methylacetophenone, and 2′-hydroxy-4′,5′-dimethylacetophenone were purchased from Aldrich. Spectrochemical grade solvents were used without further purification. The ligands and Ni complexes were prepared as reported previously.[15,16]

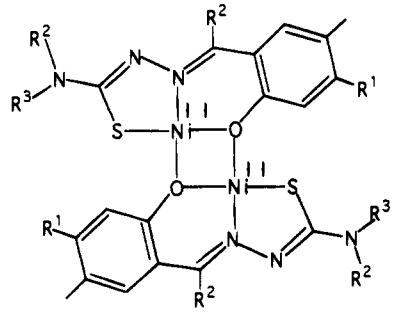
Catalytic Studies. CO was passed (50−70 mL/min) at room temperature through a solution of **1** (0.010 g), NaAcO (0.10 g), water (1.0 mL), and mv^{2+} (0.10 g) in CH_2Cl_2/MeOH (1:1, 60 mL). The formation of CO_2 was measured by passing the CO exit stream through an aqueous solution of $Ca(OH)_2$. The $CaCO_3$ precipitate was determined by weighting, and its identity was confirmed by IR. To quantify the proton release, the pH change observed (pH meter) was compared with the pH change found for the identical solution when titrated with 0.01 N HCl. The turnover numbers of the CODH reactions were calculated in moles per mole of Ni. Control reactions, run under the same conditions but omitting **1** and with free $tmtssH_2$ (tmtss = tetramethylsalicylaldehyde thiosemicarbazone) and with $Ni(AcO)_2$ or Ni metal alone, showed no CODH activity. No formate was ever detected among the products.

Spectroscopy. IR spectra were recorded on a Nicolet 5-SX FT-IR system. Pellets for the IR measurements were made in dehydrated KBr. Proton NMR measurements were conducted on a Bruker WM-250 spectrometer.

Synthesis of Na[Ni(CN)tmtss]·2H₂O. [Ni(tmtss)]₂ (1 mmol) was treated with NaCN (1 mmol) in MeOH (20 mL, solvent not dried) and isolated in microcrystalline form by evaporation of the solvent to give the dihydrate (70%). Anal. Calcd for $C_{13}H_{15}N_4NaNiOS·2H_2O$: C, 41.30; H, 5.20; N, 13.77. Found: C, 41.25; H, 5.27; N, 13.66. ^1H NMR: δ 7.28 (s, 2H, Ph); 6.68 (s, 1H, Ph); 2.81 (s, 3H, Me); 2.59 (s, 3H, Me); 2.14 (s, 3H, Me); 2.12 (s, 3H, Me). The identity of the complex was also confirmed crystallographically.

Results and Discussion

The role of Ni in CODH remains problematic in part because the enzyme reaction of eq 3 has never been observed in a model system. A stoichiometric model system for the acetyl-CoA synthase activity of CODH has been reported,[17] however. A serious problem in looking for CO binding and reduction of Ni(II) is that CO complexes of Ni(II) are very rare. The thiolate environment in the enzyme probably makes the nickel softer and more easily able to bind CO, but in model compounds, at least, the presence of thiolates almost always leads to S-bridging, which permanently removes the open sites that would otherwise be available to bind CO. We have suggested[16] that iminothiolates are the best ligands to use in such a situation because they provide an S-donor environment but have a very low bridging tendency.



1

(a: $R^1 = R^2 = Me$, $R^3 = H$;
b: $R^1 = H$, $R^2 = R^3 = Me$;
c: $R^1 = R^2 = R^3 = Me$)

We have therefore screened a variety of nickel(II) complexes with combinations of O-, N-, and S-donor ligands and have found a series of complexes of type **1** that show CO oxidation activity. The structure[16] of $[Ni(C_{12}H_{13}N_3OS)]_2$ (**1b**), shows that the complex is weakly bridged through the O rather than S, showing that the bridging tendency of the iminothiolate S-donor is indeed very low. The two halves of **1b** are folded relative to each other with a dihedral angle of 135.0(1)°. The avoidance of S-bridging by iminothiolates has been rationalized.[16]

Catalytic Studies. The oxidation of CO with mv^{2+} in an aqueous solution (eq 4) is catalyzed by all the complexes of type 1. The methylviologen (mv^{2+}) acts as electron acceptor and is reduced to the highly colored cation mv^+ with its characteristic UV−vis spectral peak at 610 nm; sodium acetate acts as a base to carry away protons produced in the reaction. The CO_2 was detected by precipitation of $CaCO_3$ from $Ca(OH)_2$ solution, and the protons were detected by following the pH change (pH meter). All the products were directly detected, and the catalytic reaction can be formulated as shown in eq 4. At room temperature and pH 9.5, the reaction rate observed is

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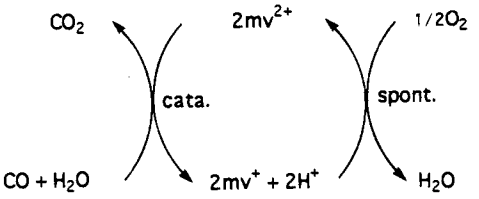
Scheme 1



1.01 ($CaCO_3$ precipitation) − 1.04 (pH meter) t.o./h (turn over/hour).

$$CO + H_2O + 2mv^{2+} + 2AcO^- \rightarrow CO_2 + 2mv^+ + 2AcOH \quad (4)$$

Like CODH itself, the Ni catalyst does not function effectively in neutral or acidic solution. A control reaction without sodium acetate leads to very slow reduction of mv^{2+} (<0.05 t.o./h). No other reducing reagents we tried (e.g., H_2, Ph_3SiH, Et_3SiH) were effective in producing the color change (formation of mv^+).

Aerobic CO Oxidation. The characteristic blue color of mv^+ radical cation makes the system a selective sensor for CO when the catalytic reaction is conducted under anaerobic conditions. Since **1** is relatively air-stable and mv^+ is rapidly reoxidized by air to mv^{2+}, the acetate-containing system can also be run aerobically for catalytic air oxidation of CO to CO_2 for which the observed rate at 25 °C and pH 9.5 was 0.94 t.o./h (Scheme 1).

Relation to the Water Gas Shift. The CO/H_2O reaction of eq 2 is reminiscent of the well-known water gas shift reaction (WGSR, eq 6) except that the products is $CO_2 + 2H^+ + 2e^-$ in the former and $CO_2 + H_2$ in the latter. Our catalyst, like CODH, lacks the ability to reduce protons to H_2 which, if it occurred, would lower the yield of electrons in the reaction.

$$CO(g) + H_2O(l) \rightarrow CO_2(g) + H_2(g) \quad (5)$$

Kinetic and Mechanistic Studies. Since CO oxidation gives both protons and carbon dioxide, monitoring the pH change (pH meter) or CO_2 formation ($CaCO_3$ precipitation) are both convenient ways to measure the rate and are in good agreement with each other (1.04 vs 1.01 t.o./h). The air sensitivity of $mv^{\bullet+}$ makes quantitation of this product by UV/visible spectroscopy much less reproducible than that of the protons and CO_2. Recrystallization of **1** caused no change in the reaction rate, and neither $Ni(AcO)_2$ nor Ni metal mediated the reaction. No significant change was observed by ^1H NMR after a solution of **1** (CH_2Cl_2 or MeOH or DMSO) was stored at room temperature for over 48 h.

Other Metals. Since the CO oxidation center in CODH is an Fe−Ni cluster, there is a possibility that Fe not Ni is the active site for CO binding and oxidation. We therefore looked at the analogues of compound **1** with Fe(II) and other metals. The CO activation reaction was not catalyzed by complexes of the same ligand with any closely related transition metal (Co^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{2+}). It has been previously reported that cobalt, zinc, and iron inhibit the activation of *apo*-CODH (*R. rubrum*) by Ni^{2+}.[11b] Ni^{2+} is unique in its ability to catalyze the CODH reaction both with our ligand system and in the protein.

Kinetics. Figures 1−5 show the results of kinetic studies of CO oxidation catalyzed by complex **1a**. The reaction is first-order in the catalyst and each of CO, H_2O, and mv^{2+}. The curvature of Figures 1−3 shows saturation behavior, and the kinetic data can be successfully fitted to the Michaelis−Menten

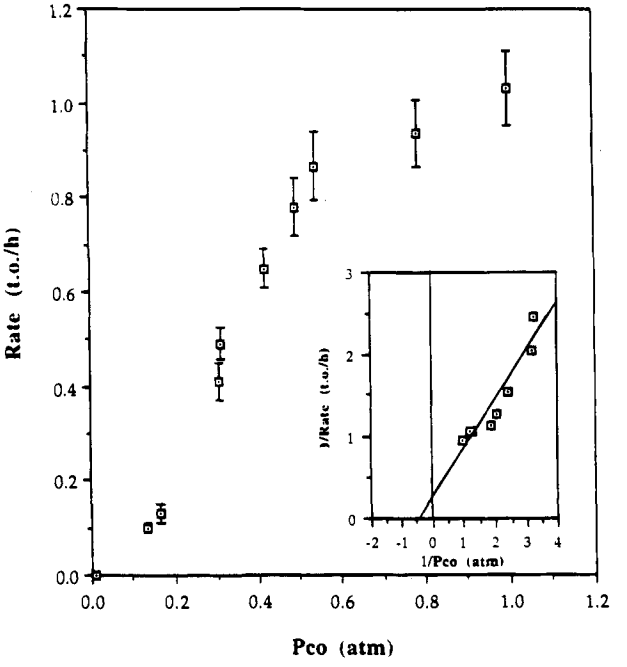


Figure 1. Dependence of the kinetics of CO oxidation on the carbon monoxide partial pressure. Inset: double reciprocal plot.

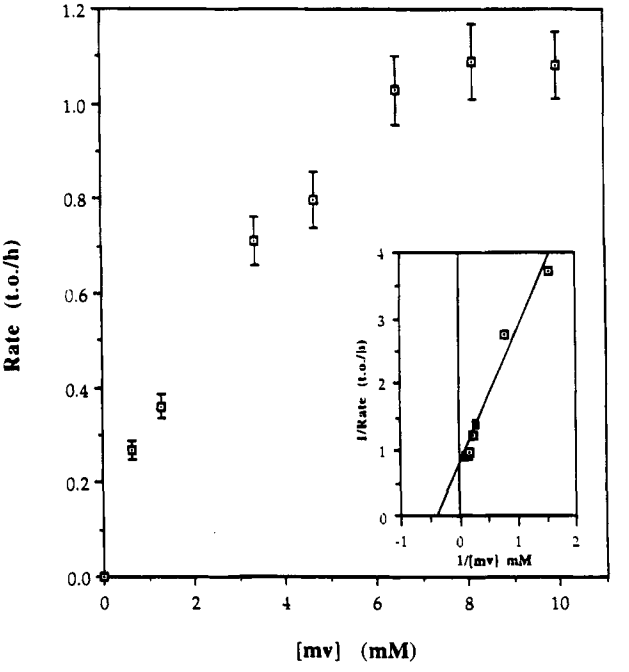


Figure 2. Dependence of the kinetics of CO oxidation on the methylviologen concentration. Inset: double reciprocal plot.

model (eq 6) by means of double reciprocal plots, which are

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \quad (6)$$

linear within experimental error. K_m and k_{cat} values (Table 1) were calculated from fits to the data. For which the rate is

$$V = V_{max}\frac{[S]}{[S] + K_m}$$

where $V_{max} = k_3[E_t]$ and $K_m = (k_3 + k_2)/k_1$. Molecular weight measurements (vapor pressure) show that although the average molecular weight of **1b** in a dichloromethane solution (567 ± 15) lies between that for the dimer and the monomer, being

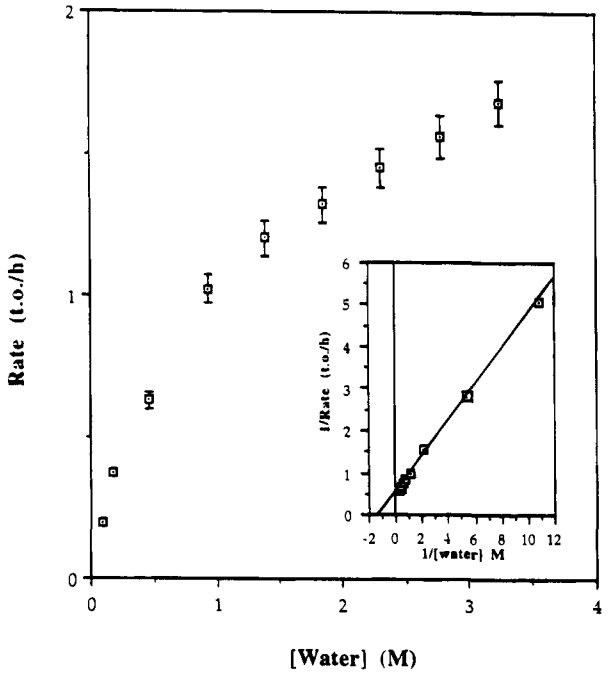


Figure 3. Dependence of the kinetics of CO oxidation on the water concentration. Inset: double reciprocal plot.

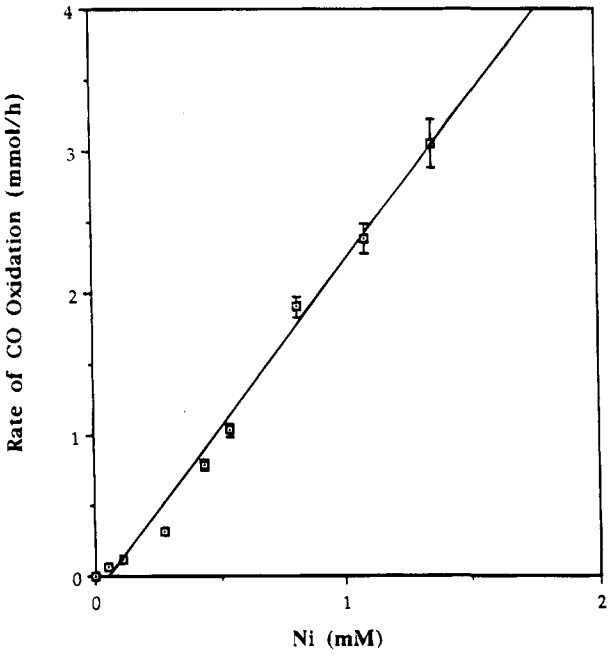


Figure 4. CO oxidation rate vs total Ni concentration.

close to that for a dimer, addition of MeOH gives a monomer (M_r 345 ± 10). That the reaction is essentially first-order (Figure 4) in total $[Ni]_0$ shows that the solvated monomer and not the dimer is kinetically significant under these conditions.

Other Solvents. To look at other solvents, we have also run our catalytic reaction in DMSO, CH_3CN, and DMF, in which the solvents can also split the O-bridge to give LNi(solv). Since methylviologen reacts directly with DMSO in solution, we were unable to measure a CO oxidation rate in this case. In acetonitrile, the rate of CO oxidation falls dramatically from 1.04 to 0.1 t.o./h, consistent with acetonitrile binding to Ni. Even 1 equiv of DMF in CH_2Cl_2 can split the dimer **1** (^1H NMR and molecular weight studies), but **1** is inactive for CO oxidation in DMF. The reason MeOH is the most successful solvent ligand may be that it is most easily displaced by CO (eq 7). We have shown the product as five-coordinate, because five-coordination is known to be especially favorable for the formation of Ni(II)–CO complexes.[13]

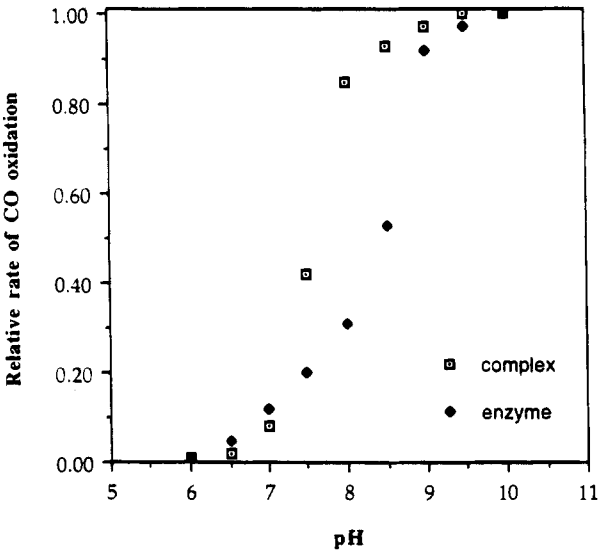


Figure 5. pH dependence of the rate of CO oxidation. The enzyme data were taken from ref 6a.

Table 1. Michaelis–Menten K_m and k_{cat} Values for CO Oxidation

	CO	H_2O	mv^{2+}
K_m	0.365 atm	0.889 M	0.00162 M
k_{cat}	1.10 h^{-1}	1.86 h^{-1}	1.07 h^{-1}
k_{cat}/K_m	3.01 atm/h	2.09 M/h	660.5 M/h

$$[(tmtss)Ni(solv)_2] + CO \rightarrow [(tmtss)Ni(solv)(CO)] + solv \quad (7)$$

pH Dependence. CO oxidation catalyzed by **1** is pH dependent. Although changes of pH in the range of pH 8–10 have only a slight effect on the reaction rate, the reaction slows sigmoidally at lower pH with an inflection point at pH 7.6 (Figure 5). Two plausible explanations can be advanced: one kinetic, the other thermodynamic. (1) It is possible that the mechanism depends on the deprotonation of a group with pK_a of 7.6. (2) In view of the pH dependence of $E°$ for eq 3, the driving force for the reaction should fall to zero at low pH. The aerobic CO oxidation of Scheme 1 has a high driving force at all pHs, however, and since this also shows sigmoidal behavior, we prefer the kinetic explanation (1, above) involving deprotonation. A similar sigmoidal pH dependence is also typical of CODH itself.[6a]

As shown in Table 1, k_{cat} is very similar for all three substrates, indicating that all these data refer to the same reaction. The k_{cat}/K_m data are also listed. Methylviologen has the largest value, suggesting that the reduction of mv^{2+} to mv^+ is intrinsically much faster than CO binding and oxidation. The value for water is lowest, which suggests that the reaction of water with Ni–CO may be one of the turnover limiting steps.

Mechanistic Analogy with the WGSR. In the WGSR,[18] the basis for our proposed CO oxidation mechanism, CO binds to the metal and is activated for nucleophilic attack by water to give a metalacarboxylic acid intermediate (MCOOH). This intermediate then undergoes decarboxylation to give a metal hydride which is in turn protonated to yield H_2. In our case H_2 is not formed, but the kinetic data are consistent with a pathway

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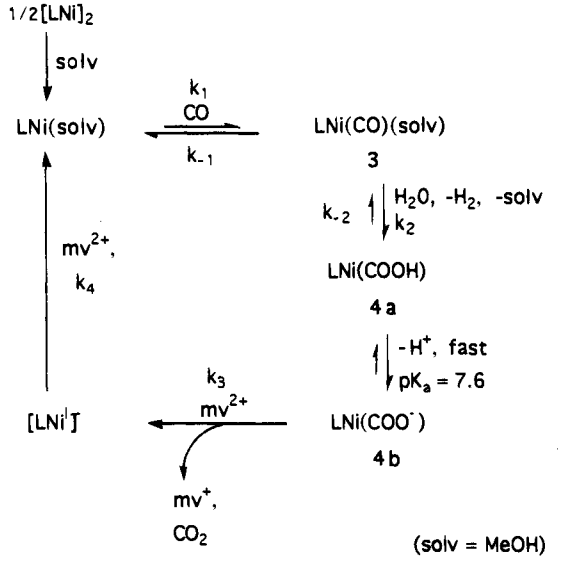
Scheme 2. A Proposed Mechanism for CO Oxidation



very similar to that for the WGSR, so we propose that a nickel hydride does not form in our CO oxidation, thus avoiding H_2 formation.

Proposed Mechanism. Although other mechanisms cannot yet be excluded, our results are most consistent with the mechanism of Scheme 2, which is also consistent with our current understanding of CO chemistry. If CO binds to a solvated monomeric form of the catalyst in the first step, the weak back donor power of Ni(II) should make the attached CO much more sensitive to nucleophilic attack by water to form a Ni−COOH intermediate (**4a**). If deprotonation of a pK 7.6 group is responsible for the pH dependence, the group in question may well be Ni−COOH. This allows the turnover limiting step to be the decarboxylation of **4b**, induced by oxidation of the Ni−COO^- group with mv^{2+} which would then give a Ni(I) species (**5**), a proton, and CO_2 (eq 8). In this way,

$$\mathbf{4b} + mv^{2+} \rightarrow \underset{\mathbf{5}}{[Ni^{I}(tmtss)(solv)]} + CO_2 + H^+ + mv^+ \quad (8)$$

the pH dependence, and the first-order dependence on CO, H_2O, and mv^{2+} can all be accounted for. The absence of H_2 as a product is also explained because, in contrast to the WGSR, no Ni(I)−H hydride species forms and so no protonation to give H_2 can take place. Methylviologen would then be expected to rapidly oxidize any Ni(I) intermediates such as **5** back to Ni(II) (eq 9).

$$\mathbf{5} + mv^{2+} \rightarrow \underset{\mathbf{2}}{[Ni^{II}(tmtss)(solv)_n]} + mv^+ \quad (9)$$

The rate equation (eq 10), derived from this mechanism using the steady-state approximation, is consistent with the kinetic observations. In particular, if any two of the three cosubstrate concentrations ([CO], $[mv^{2+}]$, $[H_2O]$) are held constant, the equation reduces to the Michaelis−Menten form.

$$d(CO_2)/dt = \frac{k_1k_2k_3[Ni]_o[CO][H_2O][mv^{2+}]}{(a+b+c+d+e+f)} \quad (10)$$

where $[Ni]_o$ = total [Ni], $a = k_{-1}k_{-2}[H^+]$, $b = k_{-1}k_3[mv^{2+}]$, $c = k_1k_{-1}[CO][H^+]$, $d = k_1k_2[CO][H_2O]$, $e = k_1k_3[CO][mv^{2+}]$, $f = k_2k_3[mv^{2+}][H_2O]$. The catalytically active species may be a fraction, perhaps only a small fraction, of the total nickel, as is commonly the case for synthetic catalysts but unlike the situation for most enzymes.

Inhibition Studies. As in CODH itself, the CO oxidation activity can be inhibited by CN^-; the reaction rate falls to 3.0 $\times 10^{-2}$ t.o./h with 2.5 mM CN^-. The rate of inactivation by CN^- was pH independent in the range of pH 6.5 to 10.2. Inhibition is probably due to strong binding of the CN^- group, which blocks the Ni(II). CN^- is isoelectronic with CO and probably binds at the same site as CO but is more resistant to nucleophilic attack. Ludden *et al.*[19] have suggested that CN^- inhibits the enzyme by binding to the metal site that also binds CO. To look at this point, the Ni(II)−CN deactivation product was synthesized and isolated. Treatment of **1** in MeOH with sodium cyanide gives Na[Ni(CN)(tmtss)] (**6**), which has an IR band at 2118 cm^{-1} assigned as a terminal cyanide group.

Unlike CO, which binds weakly to Ni(II), CN^- binds so strongly to **1** that it breaks the O-bridge of the dimer to form a square-planar Ni complex, Na[Ni(CN)tmtss]·$2H_2O$, characterized by X-ray crystallography. MeI also weakly inhibited the CO oxidation activity (1.04 − 0.78 t.o./h with 50 mM MeI). The inactivation can be partially reversed by UV light, probably as a result of photochemical decomposition of MeI. No intermediates were isolated. The mechanism of the MeI inhibition is not understood.

Isocyanides and Hydrogen as Substrates. The complex is selective for CO and mv^{2+} and is not reduced with 2,6-dimethylphenyl isocyanide or H_2. Our system does not show any evidence of an interaction with H_2 such as formation of a dihydrogen complex. Isocyanides normally have very similar chemical properties to CO; however, RNC binds much more efficiently to Ni(II) than CO to give [LNi(CNR)]. The lack of activity may therefore result from the square-planar structure of the Ni(II) isocyanide compounds.

Conclusions

We have discovered functional models for the second Ni site of CODH. They have a biologically relevant N, O, and S ligand set, consistent with the result of EXAFS studies on the enzyme. In a CH_2Cl_2/MeOH/H_2O solvent system, **1** is an active catalyst for CO oxidation; methylviologen is reduced and CO_2 and protons are produced. The reaction rate was 1.04 t.o./h with NaAcO as base. Both CN^- and MeI inhibit CO oxidation, as is the case in the enzyme.

The proposed mechanism, involving decarboxylation of a Ni−COO^- intermediate by mv^{2+} in the key step, accounts for all the key features of the reaction: the pseudo-first-order dependence on catalyst, CO, H_2O, and mv^{2+}, a sigmoidal rate−pH profile, and the absence of any H_2 as a product, although H_2 is the exclusive product of the related water gas shift reaction (WGSR). This, we propose, is because the Ni−H species which is an intermediate in the WGSR is absent in our catalytic cycle. A Ni(II) center therefore remains a strong possibility for the CO oxidation site in CODH.

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