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Structural Basis of Cyanide Inhibition of Ni, Fe-Containing Carbon Monoxide Dehydrogenase

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Nickel-containing carbon monoxide dehydrogenases (CODHs) catalyze the reversible oxidation of CO to CO_2 ($CO + H_2O \rightarrow CO_2 + 2e^- + 2H^+$). The active site of CODHs contain a [$NiFe_4S_4OH_x$] cluster known as C-cluster.[1,2] Reversible CO oxidation at C-clusters involves three oxidation states, C_{red1}, C_{int}, and C_{red2}.[3] The C_{red1} state is generated at redox potentials below −200 mV, is paramagnetic ($S = {}^1/_2$), and contains a high-spin Fe^{2+} called ferrous component II (FCII).[4] Studies employing ENDOR spectroscopy[5] and X-ray crystallography[2] identified water or hydroxide (OH_x) binding to FCII in the C_{red1} state recognizing the structural Fe_1 as FCII. Incoming substrate CO is believed to bind to an open coordination site of the Ni ion. A nucleophilic attack on nickel-bound CO by FCII-bound OH_x forms a carboxylate intermediate which is stabilized by both metals.[2]

Several analogues of CO and CO_2 have been reported to bind to the C-cluster and inhibit CO-oxidation, among which cyanide (CN^-) has been used as an isoelectronic analogue of CO.[6−9] CN^- has been characterized as a reversible slow binding inhibitor of CODHs from *Moorella thermoacetica* ($CODH_{Mt}$),[4,5,8−10] *Methanosarcina barkeri*,[6,7] *Rhodospirillum rubrum* ($CODH_{Rr}$),[11] and *Carboxydothermus hydrogenoformans* ($CODHII_{Ch}$).[12,13] CN^- binds specifically to the C-cluster in the C_{red1} state, generating a characteristic $CN-C_{red1}$ EPR signal.[3] CN^- inhibition of CODHs can be reversed by addition of substrate CO or CO_2 in the presence of reductant.[10−12] Due to the different signals observed upon CN^- binding to the C-cluster, its binding site has been interpreted as either the nickel ion or an iron ion, specifically FCII. DeRose et al. observed that CN^- treatment removes a strong coupling interaction of an OH_x group bound to FCII and suggested that CN^- and the OH_x ligand may bind to the same Fe ion of the C-cluster of $CODH_{Mt}$[5]. In contrast, CN^- binding on a Ni-deficient form of $CODH_{Rr}$ was not observed until the Ni-deficient form was reactivated by adding exogenous nickel.[11] The authors concluded that CN^- is a Ni-specific slow binding inhibitor. Recently, X-ray absorption spectroscopy on $CODHII_{Ch}$ identified CN^- binding to the Ni site[12].

To provide further complementary insight into CN^- binding to the C-cluster, we determined a crystal structure of $CODHII_{Ch}$ soaked with KCN at 1.36 Å resolution (Table S1, −320 mV+CN state, see Supporting Information, SI). Electron density maps clearly revealed a diatomic ligand bound to the Ni ion (Figure 1A). The electron density of the ligand agrees well with two light atoms originating from a CN^- molecule as shown in Figure 1A, whereas interpretations with single atoms resulted in clear difference densities.

The CN^- ligand binds to an empty coordination site of the Ni ion with a Ni−C distance of 1.79 Å and a S_5−Ni−C angle of 175° completing the typical square-planar coordination geometry of Ni(II) (Figure 1B). The observed C−N bond length of 1.15 Å is commonly found in nickel−cyanide complexes.[14] The CN^- ligand is further stabilized by implied hydrogen-bonding interactions between its nitrogen atom and His_{93} and Lys_{563} (Figure 1).

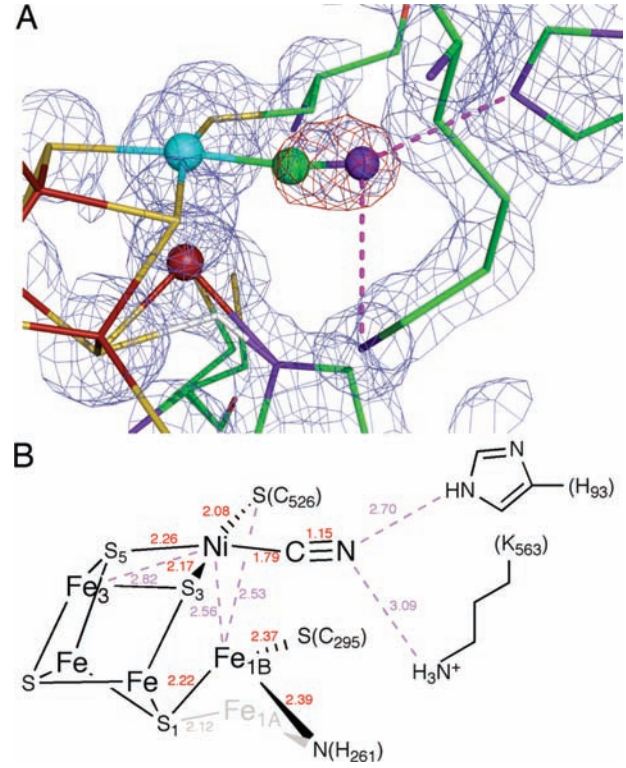

Figure 1. Binding of cyanide to the C-cluster of $CODHII_{Ch}$. (A) Electron density maps of the active site environment. $2F_{obs} - F_{calc}$ (blue) and $F_{obs} - F_{calc}$ omit (red) maps were contoured at 1.2 and 5.5 σ, respectively. The $F_{obs} - F_{calc}$ omit map was calculated after removing the CN^- ligand from the model. Ni (cyan), carbon (green), and nitrogen (violet) of CN^- and the major alternative position of Fe_1, termed Fe_{1B} (firebrick), are shown as spheres. Fe_{1A} (gray) is shown as a stick model. The occupancies of the CN^- ligand and selected atoms of the C-cluster are shown in Table S2. (B) Schematic representation of CN^- bound to the C-cluster. Bond lengths (red) and distances (dotted violet) are given in angstrom. For bond angles, see Table S3.

CN^- binding to the C-cluster also affects the coordination and position of Fe_1. In structures of the C_{red1} state two alternative positions for Fe_1 have been recognized of which the dominant position, termed Fe_{1A}, is coordinated by the OH_x ligand, while the weakly occupied position, termed Fe_{1B}, carries no OH_x ligand and is closer to the Ni ion and Sγ-Cys_{526}[2]. CN^- binding reverses the relative occupancies of the alternative positions of Fe_1 compared to the active state, and we estimate an occupancy of 10% for Fe_{1A} and 70% for Fe_{1B} (Table S2). The higher occupancy of Fe_{1B} also allowed modeling the position of the coordinating His_{261}, whose shift compared to the Fe_{1A} coordinating position also involves conformational changes for residues His_{261} to Ala_{284} (Figures 2 and S1). Given the relative occupancies we assume that all molecules

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in which CN^- is bound to the Ni ion contain Fe_1 in the B position and have no OH_x ligand.

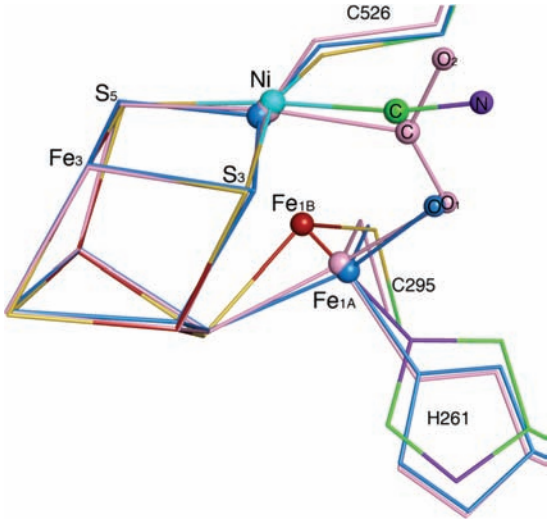

Figure 2. Superposition of the −320 mV+CN (element colors), −600 mV+CO_2 (pink, PDB ID: 3B52), and −320 mV (marine, PDB ID: 3B53) states. Only the major position of Fe_1, Fe_{1A}, for the CO_2 and OH_x-bound states and Fe_{1B} for the CN-bound state are shown.

Binding of CN^- to the C-cluster generates a similar coordination geometry at the Ni site as found in the CO_2 bound state (Figure 2). In both cases the additional ligand completes the square-planar coordination of Ni. The short Ni−Fe_{1B} distance (2.56 Å, Figure 1B) is similar to what has been observed in the reduced form of the Ni, Fe site of hydrogenase.[15] Previously, we proposed that Fe_{1B} is only occupied when the Ni ion is absent;[16] however this is not the case in the −320 mV+CN structure which shows similarly high occupancies of Ni and Fe_{1B} (Table S2). The short distance of 1.6 Å between the CN-carbon in the −320 mV+CN state and the OH_x ligand in the −320 mV state[2] in the superimposed structures (Figure 2) clearly indicates that a tight binding of CN^- in the square plane of the nickel ion is sterically hindered when the OH_x ligand is present.

CN^- is isoelectronic to CO and acts as a competitive inhibitor of the CO-oxidation reaction by $CODHII_{Ch}$[12]. Recently we proposed a mechanism in which CO binds to the empty equatorial coordination site of the Ni ion placing it in an appropriate distance to the OH_x ligand of Fe_1[2]. In the −320 mV+CN structure CN^- occupies the suggested coordination site for CO and can thereby obstruct substrate binding, supporting the proposed mechanism.

Cyanide has been described as a slow binding inhibitor of CODHs.[5,6,11,12] This could mean that either CN^- reacts in a simple reversible slow binding step or a rapid reversible binding step is followed by a slow conformational change or isomerization reaction that leads to the tight and slow binding of the inhibitor.[17] The CN-bound structure observed supports a two-step mechanism in which the competitive aspect of the inhibition by CN^- is due to its reversible binding to the Ni ion in the [$NiFe_4S_4OH_x$] state of the C-cluster, while its slow binding inhibition is likely due to a conformational change of the protein during which the OH_x ligand of FCII is lost allowing CN^- to bind more tightly to nickel forming an inactive [$NiFe_4S_4CN$] state of the C-cluster. The observed structure and proposed binding model are in agreement with experiments observing CN-binding to the Ni ion of the C-cluster[11,12] as well as with the observed loss of an exchangeable proton from FCII in ENDOR spectra.[5] A terminal sulfido ligand bound to Fe_1 suggested to be present in the CN-inhibited state of $CODHII_{Ch}$[12] has not been observed.

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Supporting Information Available: Materials and Methods, crystallographic statistics, supporting figure and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ragsdale, S. W. *J. Inorg. Biochem.* **2007**, *101*, 1657–1666.
(2) Jeoung, J. H.; Dobbek, H. *Science* **2007**, *318*, 1461–1464.
(3) Lindahl, P. A. *Biochemistry* **2002**, *41*, 2097–2105.
(4) Hu, Z. G.; Spangler, N. J.; Anderson, M. E.; Xia, J. Q.; Ludden, P. W.; Lindahl, P. A.; Munch, E. *J. Am. Chem. Soc.* **1996**, *118*, 830–845.
(5) DeRose, V. J.; Telser, J.; Anderson, M. E.; Lindahl, P. A.; Hoffman, B. M. *J. Am. Chem. Soc.* **1998**, *120*, 8767–8776.
(6) Grahame, D. A.; Stadtman, T. C. *J. Biol. Chem.* **1987**, *262*, 3706–3712.
(7) Krzycki, J. A.; Zeikus, J. G. *J. Bacteriol.* **1984**, *158*, 231–237.
(8) Drake, H. L.; Hu, S. I.; Wood, H. G. *J. Biol. Chem.* **1980**, *255*, 7174–7180.
(9) Diekert, G. B.; Thauer, R. K. *J. Bacteriol.* **1978**, *136*, 597–606.
(10) Anderson, M. E.; Lindahl, P. A. *Biochemistry* **1996**, *35*, 8371–8380.
(11) Ensign, S. A.; Hyman, M. R.; Ludden, P. W. *Biochemistry* **1989**, *28*, 4973–4979.
(12) Ha, S. W.; Korbas, M.; Klepsch, M.; Meyer-Klaucke, W.; Meyer, O.; Svetlitchnyi, V. *J. Biol. Chem.* **2007**, *282*, 10639–10646.
(13) Seravalli, J.; Ragsdale, S. W. *Biochemistry* **2008**, *47*, 6770–6781.
(14) Sheridan, P. M.; Ziurys, L. M. *J. Chem. Phys.* **2003**, *118*, 6370–6379.
(15) Davidson, G.; Choudhury, S. B.; Gu, Z. J.; Bose, K.; Roseboom, W.; Albracht, S. P. J.; Maroney, M. J. *Biochemistry* **2000**, *39*, 7468–7479.
(16) Dobbek, H.; Svetlitchnyi, V.; Liss, J.; Meyer, O. *J. Am. Chem. Soc.* **2004**, *126*, 5382–5387.
(17) Szedlacsek, S. E.; Duggleby, R. G. *Methods Enzymol.* **1995**, *249*, 144–180.

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