Exploring Substrate and Product Channels in CO Dehydrogenase II from *Carboxydothermus hydrogenoformans*

Lilith Domnik, Jae-Hun Jeoung, Jochen Fesseler, Holger Dobbek
Institut für Biologie, Strukturbiologie/Biochemie, Humboldt-Universität zu Berlin, Philippstr 13, Leonor-Michaelis-Haus, 10115 Berlin, Germany

Introduction

Rising of CO_2 levels in the earth's atmosphere demands the development of energy sources that are CO_2 neutral. Therefore understanding how microorganisms utilize C1-compounds will be of crucial importance in the future. The carbon monoxide dehydrogenase II (CODHII) of the thermophilic bacterium *Carboxydothermus hydrogenoformans* catalyses the reduction of CO_2 to CO and water using two protons and two electrons with a turnover rate of 15 s^{-1}.

$$CO_2 + 2e^- + 2H^+ \rightleftharpoons CO + H_2O$$

In order to guarantee an efficient supply of substrates and release of products controlled routes are needed within the enzyme [1, 2, 3]. Biochemical and structural studies on mutants have been applied to illuminate the putative proton and water transfer pathways. Exploiting the ability of xenon to bind hydrophobic protein cavities allowed determination of gas channels in CODHII.

Electron transfer pathway in $CODHII_{Ch}$

Two electrons need to be transferred to the C-cluster for the reduction of CO_2 to CO. The Proximity of metal clusters in $CODHII_{Ch}$ suggests an electron transfer pathway connecting the D-cluster to the B-cluster and C-cluster [1].

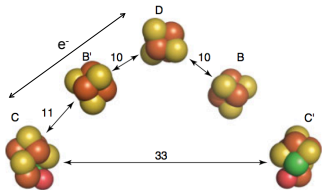
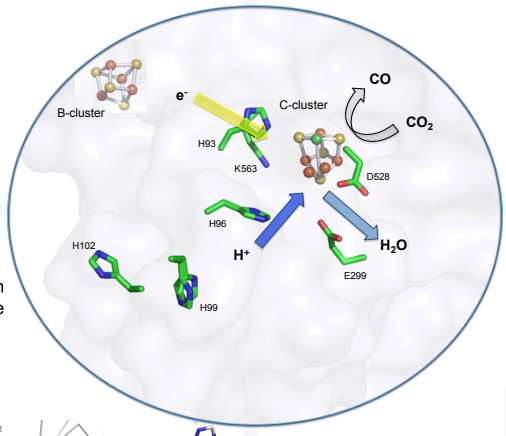


Fig. 1: The five metal clusters of $CODHII_{Ch}$ (green: nickel, orange: iron, yellow: sulfur, red: oxygen). Electron transfer probably proceeeds from cluster D over B' to C. The numbers given are the nearest Fe–Fe distances in angstrom [1].



Gas channels in $CODHII_{Ch}$

Cavity calculations suggested a hydrophobic CO_2/CO channel in the monofunctional $CODHII_{Ch}$ [2]. Application of xenon gas verified this assumption and showed xenon binding sites within the predicted CO_2/CO channel.

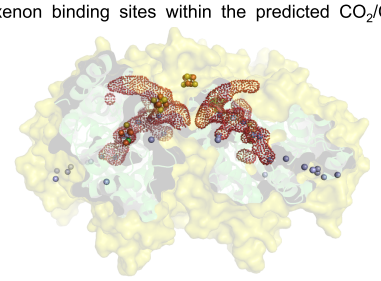


Fig. 2: CO channel system in $CODHII_{Ch}$. Red: calculated channel, blue: xenon sites.

Proton transfer network

Mutational studies on specific amino acid residues in CODH of *M. thermoacetica* suggested the existence of a proton transfer network within $CODH_{Mt}$ [3].

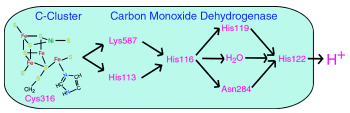


Fig. 3: Proposed proton transfer network in $CODH_{Mt}$ [3].

Analysis of the H96D mutant of $CODHII_{Ch}$ showed abolished activity (3 % wild-type activity) and a structurally invariable C-cluster suggesting the presence of a similar proton transfer network in $CODHII_{Ch}$.

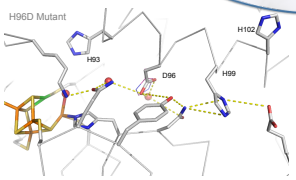


Fig. 4: structure of $CODHII_{Ch}$ H96D mutant

Water network in $CODHII_{Ch}$

Close to the H_2O/OH- ligand of cluster C a network of water molecules has been identified ending at a water/hydroxyl ligand on the Fe1 site, which may present a route for releasing water molecules from the C-cluster [2].

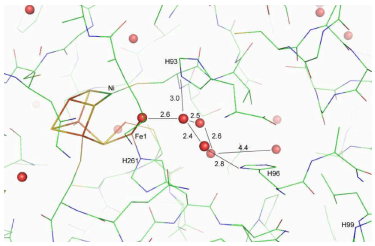


Fig. 5: Water network close to the H2O/OH- ligand of the C-cluster [2].

Optimization of CODHII expression

CODHII was heterologously expressed in *Escherichia coli* harbouring the ISC plasmid which encodes enzymes catalysing Fe-S cluster assembly. After purification CODHII showed a specific activity of 6840 U/mg at 70°C (CO oxidation activity). The active CODHII was crystallized in 0.1 M Bris-Tris pH 6.5, 0.2 M ammoniumsulphate, 14.5 % PEG3350 and 2 mM DT. Diffraction data was collected at beamline BL14.2 at BESSY II (Berlin, Germany) [4]. Structure was solved by molecular replacement and refined by Refmac 5 (ccp4i) at a resolution of 1.3 Å with metal occupancies of approximately 80 % at all clusters.

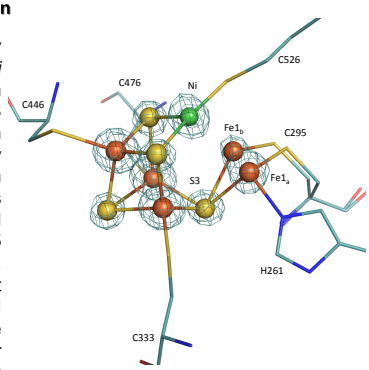


Fig. 6: C-cluster, the active site of CODHII from *C. hydrogenoformans*. Two Positions are found for the dangling Fe-atom termed $Fe1_a$ and $Fe1_b$. Omit $F_o - F_c$ map is shown as a mesh in green.

Mutational studies on $CODHII_{Ch}$

Various residues which are probably involved in substrate and product channeling in $CODHII_{Ch}$ were individually mutated using site-directed mutagenesis.
All of the mutants showed diminished activity in comparison to wildtype activity suggesting a participation of the respective residues in the corresponding pathways.

mutation	location	Expected effect	averaged % activity relative to WT
H93E		decrease in CO-binding velocity	33.6
H93A	active site	decrease in CO-binding velocity	11.2
K563A		destabilization of CO_2- binding	5.8
H93A + K563A		destabilization of CO-binding and reaction	0.98
H96D	H^+-channel	breakdown of H^+-relay	3.0
E299W	water channel	blockade of water channel, under turnover-condition, reaction velocity decreased	1.38

Future research focus

- X-ray analysis of the CODHII mutants allowing to exclude a loss of activity caused by breakdown of the C-cluster
- mutational analysis of the electron circuit
- Crystallographic analysis of crystals after reaction in organic solvent to identify water channels

References
[1] Jeoung JH and Dobbek H (2007) Handbook of Metalloproteins
[2] Jeoung JH and Dobbek H (2007) Science 318(5855):1461-4
[3] Kim EJ, Feng J, Bramlett MR, Lindahl PA (2004) Biochemistry 43(19):5728-34
[4] Mueller U, Darowski N, Fuchs MR, Förster R, Hellmig M, Paithankar KS, Pühringer S, Steffien M, Zocher G, Weiss MS (2012) Journal of Synchrotron Radiation 19:442-449

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