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

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**Introduction**

Rising of CO levels in the earth’s atmosphere demands the development of energy sources that are CO 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 to CO2 and water using two protons and two electrons with a turnover rate of 15 s⁻¹.

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\text{CO}_2 + 2\text{e}^- + 2\text{H}^+ \rightleftharpoons \text{CO} + \text{H}_2\text{O}
\]

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**

Two electrons need to be transferred to the C-cluster for the reduction of CO to CO2. The Proximity of metal clusters in CODHII suggests an electron transfer pathway connecting the D-cluster to the B-cluster and C-cluster [1].

**Gas channels in CODHII**

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

**Proton transfer network**

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

**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 Bis-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.

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**References**


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

**Water network in CODHII**

Close to the H2O/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].

**Mutational studies on CODHII**

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

**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

**Figure Captions**

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

Fig. 2: CD channel system in CODHII. Red: calculated channel, blue: xenon sites.

Fig. 3: Proposed proton transfer network in CODHII [1].

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

Fig. 4: Structure of CODHII H96D mutant.

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

Fig. 6: C-cluster, the active site of CODHII from *C. hydrogenoformans*. Two Positions are found for the dangling Fe-atom termed Fe2 and Fe3. Omit Fo – Fc map is shown as a mesh in green.