Accessory *cox* genes and active site formation in CO dehydrogenase

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The molybdo-cupro-iron-sulfur-flavoprotein carbon monoxide dehydrogenase (CODH) from the aerobic bacterium *Oligotropha carboxidovorans* catalyzes the oxidation of CO to CO_2 [1]. The 277 kDa protein forms dimers of heterotrimers. The heterotrimers are composed of a molybdo-cupro protein (L subunit), an iron-sulfur protein (S subunit), and a flavoprotein (M subunit) [2]. The enzyme's active site, which is part of the molybdo-cupro protein, was shown to contain a novel [$CuSMo(=O)_2$] cluster. The two metal sites in this cluster are bridged by a sulfido ligand μ_2-S^{2-} [3, 4].

The subunits M, S, and L of CODH are encoded by the structural genes *coxM*, *coxS*, and *coxL* [5]. The structural genes are part of the *cox* gene cluster (*coxBCMSLDEFGHIK*) that resides on the bacterium's megaplasmid pHCG3 [6]. Current mutational studies suggest the accessory genes *coxD*, *coxE*, and *coxF*, which are located directly downstream of the *coxMSL* subcluster, to be essential for the formation of a functional [$CuSMo(=O)_2$]-containing active site in CODH.

Here, we report first results of an X-ray absorption spectroscopic (XAS) study of recombinant CODH and a series of CODHs isolated from mutant strains of *O. carboxidovorans*. XAS data were collected at the EMBL EXAFS beamline D2 using a 13-element fluorescence detector. Data reduction was achieved with the EXPROG program package [7]. For EXAFS data analysis the refinement program EXCURV98 [8] was used.

Recombinant CODH was expressed from a bacterial strain carrying only the structural genes *coxMSL*. In addition different CODHs from a series of mutant strains were analyzed. These proteins were isolated from strains in which one of the genes *coxD*, *coxE*, *coxF*, or *coxG* on the plasmid pHCG3 was specifically inactivated by insertion of a kanamycin-resistance cassette. Recombinant CODH as well as the proteins isolated from the *coxD*, *coxE*, and *coxF* mutants are catalytically inactive. However, a mutation in *coxG* leads to the production of a fully functional enzyme. First of all, these results show that the expression of the structural genes alone does not lead to a functional enzyme. In a crystallographic study it was shown that the three-dimensional overall structure of recombinant CODH is correctly assembled and that the structural defect must be restricted to the active site [9]. From this, one can conclude that the gene products CoxD, CoxE, and CoxF are involved in the biosynthesis of the functional [$CuSMo(=O)_2$]-active site present in the functional enzyme.

According to the Mo- and Cu-*K*-absorption edges in Figure 1 three different groups of metal sites can be distinguished: (A), (B), and (C)/(D). Recombinant CODH and CODH from the *coxD* mutant contain a mononuclear trioxo-Mo-center [Figure 1(A), see comment to the "oxo-edge feature" in the Figure legend]. The CODHs from the *coxE* and *coxF* mutants carry mononuclear dioxo-Mo-centers [Figure 1(B)]. The enzyme from the *coxG* mutant contains the same functional [$CuSMo(=O)_2$] cluster found in wildtype CODH [Figure 1(C), (D)].

These data are in agreement with biochemical analyses (not shown) and the analyses of the extended X-ray absorption fine structures (EXAFS) of the Mo- and Cu-*K*-edges (not shown). The results suggest that the binuclear active site in functional CODH is built up stepwise from an inactive mononuclear Mo-center: mononuclear trioxo $\rightarrow$ mononuclear dioxo $\rightarrow$ binuclear dioxo. The first step of the posttranslational biosynthesis of the active site seems to be assisted by CoxD.

The second step seems to require both of the gene products CoxE and CoxF. The XAS data presented here allow the first structural insight into the posttranslational biosynthesis of the active site cluster in CODH. A detailed understanding of the CoxDEF-assisted incorporation of copper as well as the bridging sulfur atom into the mononuclear trioxo molybdenum site (recombinant CODH) to form the active site cluster (*coxG* mutant and wildtype) must await further studies.

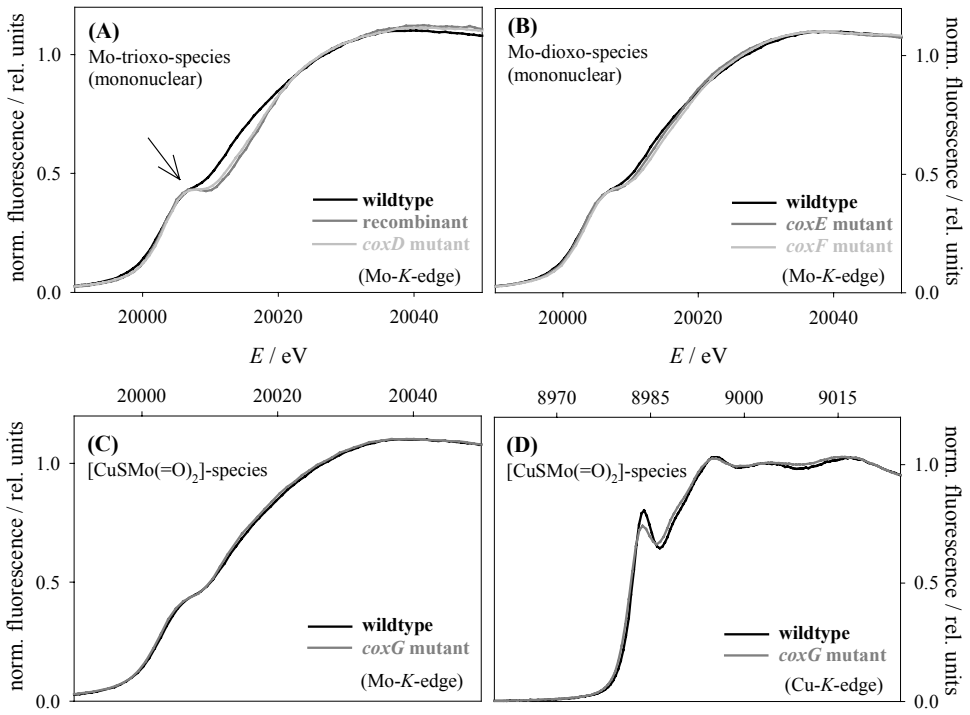

Figure 1: Mo- (A-C) and Cu- (D) *K*-edges of different CODH species (for details see text). The shoulder in the rising Mo-edge, the so-called "oxo-edge feature" (arrow in Figure 1(A)), is sensitive to the presence and number of terminal oxo ligands at the molybdenum ion [10]. The wildtype enzyme, which is used as a reference, has a $[CuSMo(=O)_2]$-containing, binuclear active site with a dioxo-Mo-center [3, 4]. Abbreviations: norm., normalized; rel., relative; *E*, photon energy.

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