Nickel-Containing Hydrogenases and CO Dehydrogenases

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Nickel-containing enzymes have attracted attention as biological catalysts involved in several reactions that are of scientific importance. These enzymes are usually isolated from bacteria and algae. Four types of Ni-containing enzymes are now known [1]: ureases, methyl-coenzyme M reductases, hydrogenases and carbon monoxide dehydrogenases. The latter two types will be discussed in detail.

Hydrogenases catalyze the reversible oxidation of H_2 to H^+. Several Ni-containing hydrogenases have been characterized, and they differ considerably in their active site composition [2]. They consist of two subunits of approximately 60 and 30 KDa and contain one or several [4Fe-4S] clusters plus one Ni atom. Hydrogenases are typically isolated in air as a mixture of inactive forms with distinct EPR spectra. The so-called Form A is only slowly reactivated after incubation under H_2. Form B is also inactive but is readily activated upon exposure to H_2. Enzyme reactivation under H_2 leads to a new EPR-detectable species (Form C). These EPR signals are attributed to the Ni center by ^{61}Ni substitution experiments [3]. EPR, ENDOR spectroscopy and XAS have been used to investigate the Ni active site environment of the hydrogenases [4]. It has been suggested that the Ni site features a distorted pseudo-octahedral structure. EXAFS data indicate that the Ni center is surrounded by 2 or 3 S ligands at an average Ni-S distance of 2.21 Å and 2 to 4 N or O ligands at 2.06 Å. A Ni-Fe distance >4 Å is also indicated [5]. A tentative catalytic and activation scheme has been proposed showing the involvement of the redox centers in the hydrogenases [6]. Two Ni compounds with S_4N_2 and S_2N_3 coordination spheres have been synthesized as structure models of these hydrogenases [7].

The anaerobic CO dehydrogenases (CODH) catalyze the following reactions:

$$CO + H_2O \rightleftharpoons CO_2 + 2\,H^+ + 2e^- \qquad (1)$$

$$CH_3\text{-}[Co] + CO + CoASH \longrightarrow H\text{-}[Co] + CoASCOCH_3 \qquad (2)$$

[Co] = corrinoid protein, CoA = coenzyme A

Reaction 2 is a sum of the final steps in CO fixation via acetyl-CoA synthesis. CODH is the central participant in this reaction [8]. The pathway responsible for reaction (2) involves several additional enzymes. The mechanism shown in Fig. 1 has been proposed by Wood et al. [9]. CODH from *C. thermoaceticum* has been determined to have an $(\alpha\beta)_3$ hexameric protein subunit structure, where each $\alpha\beta$ unit consists of 2 Ni, 11 Fe, 1 Zn and 31 S atoms [10]. The as-isolated CODH from *C. thermoaceticum* is EPR silent. Incubation under CO leads to an axial EPR spectrum with g values of 2.08 and 2.02. This EPR signal arises from a species containing Ni, Fe and the carbon from CO, as demonstrated by the hyperfine broadening of the signals when either ^{61}Ni, ^{13}C, or ^{57}Fe is present [11]. The complex responsible for this EPR signal has been referred to as a NiFeC complex. The CODHs from other bacteria exhibit similar but not identical EPR spectra. EPR, Mössbauer and ENDOR data [12] on the CO-reduced form of the enzyme indicate a Ni-Fe-C assembly of probable stoichiometry $Ni_1Fe_{3\text{-}4}S_{\geq 4}C_1$. EXAFS results on the CO-free, EPR silent form of the enzyme reveal a sulfur-rich nickel coordination environment, possibly planar NiS_4 with Ni-S

distance of 2.16 Å. A combination of S/O/N ligands may also exist [13]. A N, O, S-ligated Ni complex and complexes with composition of $[Ni(NCH_2CH_2SR)L]^+$ (L = Cl, H, CO; R = i-Pr, t-Bu) have been used as models of CODHs [14].

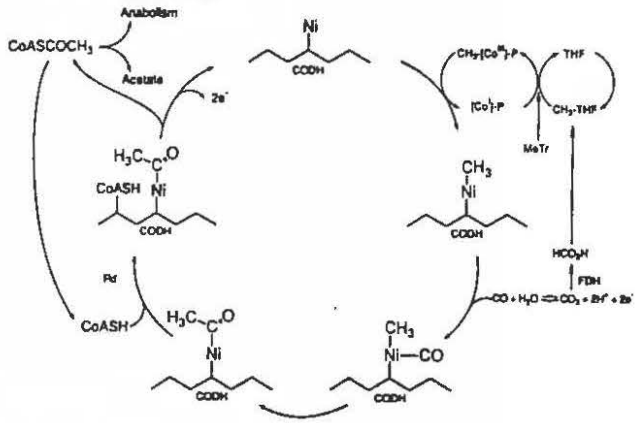

Figure 1. Proposed reaction pathway of Ni-CODH as an acetyl-coenzyme A synthase: CoA = coenzyme A, FDH = formate dehydrogenase, MeTr = methyltransferase, [Co]-P = corrinoid protein, Rd = disulfide reductase, THF = tetrahydrofolate.

Only preliminary results have been obtained in this area. The exact electronic and molecular structures of these enzymes remain unresolved and the catalytic roles of the active sites need to be established.

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