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Coordination Chemistry and Function of the Catalytic Metal Ion in Alcohol Dehydrogenase

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Coordination Chemistry

The catalytic zinc ion in horse liver alcohol dehydrogenase (HLADH) can be removed by treating a crystalline suspension of the enzyme with dipicolinic acid [1]. The resulting apo-zinc enzyme (H_4-$Zn(n)_2LADH$, where n denotes the noncatalytic metal ion) is inactive. It can be reconstituted with Zn^{2+}, Co^{2+} [1], Cd^{2+} [2], Ni^{2+} [3], Cu^{2+} [4], Fe^{2+} [5], and Pb^{2+} [5] to metallo LADHs of the general composition $Me(c)_2Zn(n)_2LADH$ (where c denotes the catalytic metal ions). Species containing Cu^{1+} and Fe^{3+} can be obtained via redox reactions. Catalytic activity is largely preserved with $M^{2+} = Co^{2+}$, Ni^{2+}, Cd^{2+}, Cu^{2+}; whereas the Cu^{2+} and Fe^{2+} enzymes show activities less than 5% that of the native enzyme and the Pb^{2+} enzyme less than 1% that of the native enzyme.

The process of reconstitution is biphasic according to the equation

$$M^{2+} + H_4Zn(n)_2LADH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M^{2+}\cdot H_4Zn(n)_2LADH$$

$$\xrightarrow{k_2} M(c)_2Zn(n)_2LADH + 4H^+$$

where k_2 is rate-limiting. The activation parameters as well as k_2 and $K = k_{-1}/k_1$ were determined for the insertion of Co^{2+} and Ni^{2+}. The process is characterized by an exceptionally high negative activation entropy indicating some flexibility in the empty catalytic site of $H_4Zn(n)_2LADH$ which leads to a sterically restrictive transition state for the metal insertion pathway. X-ray studies have shown that the tertiary structure of LADH is virtually unchanged upon removal of the catalytic metal ion. In both $Co(c)_2Zn(n)_2LADH$ and $Cd(c)_2Zn(n)_2LADH$ the coordination number four and the tetrahedral geometry remain unchanged as compared to the native enzyme. This shows that these (and probably several other) derivatives may serve as true models for the native enzyme. Another X-ray study has revealed that the change from the open to the closed conformation which is induced upon binding of NADH occurs in $H_4Zn(n)_2LADH$ as well as in the native enzyme which shows that the structural transition is independent of the presence of the catalytic metal ion.

Mechanistic Investigations

The use of metallo LADHs has helped to clarify several important mechanistic questions which had previously caused long-standing debates. First, NMR relaxation dispersion measurements on solvent and substrate protons have shown that no significant differences exist between the relaxation rates of the Co^{2+}, Ni^{2+}, Fe^{3+}, Zn^{2+}, and demetallized enzymes. This implies that previous models of outer-sphere substrate binding, based on NMR relaxation data obtained with Co_4LADH or $Zn(c)_2Co(n)_2LADH$, can be safely ruled out. Secondly, spectroscopic and kinetic investigations of the binding and turnover of chromophoric substrates (notably *trans*-4-N,N-(dimethylamino)cinnamaldehyde) to metallo LADHs containing Cd^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+} have provided strong evidence for inner-sphere coordination of the substrate's carbonyl group to the catalytic metal ion. Third, optical and NMR measurements on $Co(c)_2Zn(n)_2LADH$ have shown that at least three proton equilibria are linked to groups in the immediate vicinity of the metal ion. They include the free enzyme (pK ~ 9.5), the binary complex enzyme·NAD^+ (pK ~ 8) and the productive complex enzyme·NAD^+·ethanol (pK_a ~ 6.3). Probably none is due to the postulated ionization of the metal-bound water molecule.

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Metal Ions in Natural Aquatic Systems

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The *abundance* of metal ions in natural aquatic systems varies greatly from major species (Na^+, K^+, Ca^{2+}, Mg^{2+}) down to very minor components such as rare earth elements. Whereas most of the natural water systems are apparently homogeneous with respect to major components, the distribution patterns of minor elements reveal the impact of complex control mechanisms.

The *regulation of metal concentration* in aquatic systems is based on the reactions

$$\text{dissolved metal} \underset{r}{\overset{p}{\rightleftharpoons}} \text{particulate metal}$$