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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-$Zn(n)_2$LADH, 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 $Me(c)_2Zn(n)_2$LADH (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

$$M^{2+} + H_4Zn(n)_2\text{LADH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M^{2+}\cdot H_4Zn(n)_2\text{LADH} \xrightarrow{k_2} M(c)_2Zn(n)_2\text{LADH} + 4H^+$$

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 $H_4Zn(n)_2$LADH 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 $Co(c)_2Zn(n)_2$LADH and $Cd(c)_2Zn(n)_2$LADH 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 $H_4Zn(n)_2$LADH 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 $Zn(c)_2Co(n)_2$LADH, 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 $Co(c)_2Zn(n)_2$LADH 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

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

(which initiate sedimentation) and their reversals (*r*). Formation of particulate metals (*p*) can proceed by (microbially mediated oxidation) oxidation ($Fe(II) \rightarrow Fe(OH)_{3(s)}$, $Mn(II) \rightarrow MnOOH_{(s)}$), by incorporation into the biomass and by adsorption at inorganic particulates ($Fe(OH)_{3(s)}$, $MnOOH_{(s)}$, clay minerals). The actual extent of these reactions is dependent on the solution parameters (pH, pE, presence of ligands) *i.e.* the *chemical speciation of the metal in the aquatic environment.* Resolubilisation is caused by low redox potential ($Fe(OH)_{3(s)} \rightarrow Fe(II)$, $MnOOH_{(s)} \rightarrow Mn(II)$), by oxidation of the biological carrier and by desorption from inorganic particulate favored by low pH-values. In addition, metals such as Hg, Sn, Pb and Tl can be remobilized by biomethylation. The usual (biologically induced) distribution of pE and pH favors *p* over *r* near the surface and *r* over *p* near the bottom of the aquatic system. The resulting concentration gradients result in eddy-assisted back diffusion.

The marked increase in metal pollution (as documented by sedimentary record) gives rise to increasing concern for the *impact of metal ions upon the aquatic biosphere.* It is usually assumed that (some) metal ions are limiting at low concentrations and that most are toxic at higher concentrations. The sensitivity towards a given metal is largely dependent on the biological species. It is also generally agreed that availability of dissolved metals for phytoplankton is restricted to aquo ions. Hence, the biological impact of a given metal is again dependent on its chemical speciation. The situation is further complicated by the fact that some algae are able to release organic ligands (ferrichromes). Recent field studies seem to indicate that increase in metal concentration does not basically change the total amount of biota in a given aquatic system; the biological speciation is, however, greatly changed.

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The Electronic Structure of Iron Complexes of Bleomycin

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Bleomycin (BLM), a glycopeptide antibiotic, is activated in an Fe-dependent reaction and, when O_2 is present, catalyzes the cleavage of DNA. Drug activation takes place via two pathways. In the first, Fe(II)-BLM, an S = 2 species, and O_2 react to form a diamagnetic, ternary complex, 'oxygenated bleomycin'. The quadrupole splitting and isomer shift (ΔEq = 2.96 mm/sec, δ = 0.16 mm/sec) are suggestive of an electronic structure best described as low spin ferric bound to superoxide anion. Single electron reduction of oxygenated bleomycin yields 'activated bleomycin', a form of the drug that is kinetically competent to cleave DNA. As a second pathway for activation, Fe(III)-BLM reacts with peroxide; Fe(II)-BLM is not produced as an intermediate.

Activated bleomycin is an S = ½ species with a well resolved EPR spectrum (g = 2.26, 2.17, 1.94). When prepared with ^{57}Fe, the g = 1.94 EPR feature is split by 22 gauss, demonstrating that the electron spin resides primarily on the iron. When activated bleomycin is prepared from Fe(II)-BLM and $^{17}O_2$, the EPR spectrum is broadened, demonstrating the presence of at least a single oxygen atom derived from O_2.

Concomitant with DNA cleavage, activated bleomycin decomposes to form Fe(III)-BLM, an S = ½ species with EPR features (g = 2.45, 2.18, 1.89) and Mössbauer parameters (ΔEq = 2.85 mm/sec and δ = 0.20 mm/sec) closely resembling those of cytochrome P-450. Fe(III)-BLM, in the presence of phosphate or arsenate, or at pH below 4.5, becomes high spin with g_{eff} = 4.3 at 77 °K (E/D ~ 0.3). Yet, Fe(III) remains bound to the drug.

The bonding of iron to BLM is affected by the presence of DNA. Changes are observed in the g = 1.94 EPR feature for the activated complex, and in the hyperfine interaction with ^{14}N in the low spin ferric complex, as determined from differences in the electron spin echo spectrum. DNA also prevents the conversion of the iron in the low spin ferric complex to an S = 5/2 species by phosphate or arsenate.

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Chemical Aspects of Structure, Function and Evolution of Superoxide Dismutases

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The superoxide dismutases (SOD) constitute a class of metalloproteins having either Cu/Zn, Mn or Fe as their prosthetic group and their function is to dismute O_2^- to H_2O_2 and O_2. The distribution of the SODs has to be considered in the light of the acquisition of a permanent defence by organisms against any form of toxicity arising from the increase, by photosynthetic organisms, at atmospheric oxygen. Anaerobic sulphate reducing bacteria and fermenta-