energy ($2|D|$) of the Co^{2+} ion in a variety of binary and ternary complexes of CoLADH to assign the coordination number of the active site metal ion. The results are 9.3 cm^{-1} (CoLADH); 3.1 cm^{-1} (CoLADH-CF_3CH_2OH); 13 cm^{-1} (CoLADH-NADH-N,N-dimethylaminocinnamaldehyde); and 8.3 cm^{-1} (CoLADH-tetrahydroNADH), indicative of tetracoordinate sites, while the ZFS constants of the CoLADH-NADH, CoLADH-NADH-benzylalcohol, CoLADH-NADH-CF_3CH_2OH, and CoLADH-NAD^+–CF_3CH_2OH complexes are >20 cm^{-1}, indicative of pentacoordinate environments.

The results taken together indicate that the active site metal ion is pentacoordinate in catalytically competent reaction intermediates and is ligated by a neutral water molecule in the physiologically active ternary enzyme–NAD^+–alcohol complex. We suggest that the neutral metal-bound water molecule serves as the base catalyst for abstraction of the proton from the alcoholic hydroxyl group of the substrate. We present a mechanism for the catalytic action of LADH consistent with these observations and indicate how the metal-bound water molecule may modulate the Lewis acid reactivity of the active site metal to control the catalytic action of LADH. (Supported by NIH grant GM 21900).

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O31

Zn(II)-induced Cooperativity of *Escherichia coli* Ornithine Transcarbamoylase

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Ornithine transcarbamoylase (OTCase) catalyses the transfer of the carbamoyl group from carbamoyl phosphate to L-ornithine for the synthesis of L-citrulline in the urea cycle. The enzyme shares a common source of the carbamoyl group with aspartate transcarbamoylase (ATCase), which catalyzes a similar reaction in the pyrimidine biosynthesis pathway. Unlike the hexameric ATCase, anabolic OTCase is a trimeric molecule of 105,000 daltons and does not display sigmoidal substrate saturation curves.

The steady state reaction of OTCase purified from *E. coli* K-12 (*argR, argF*) [1] exhibits Michaelis–Menten kinetics for both substrates. Carbamoyl phosphate is the first substrate bound. However, when the competitive inhibitor Zn(II) is present, this anabolic OTCase expresses positive cooperativity towards its second substrate. The extent of cooperativity is a function of Zn(II) concentration. Steady state kinetic data yield a limiting Hill coefficient of 2.7 for L-ornithine at 0.3 m*M* Zn(II). The allosteric effect of Zn(II) on the enzyme is reversible and is not altered by the level of carbamoyl phosphate. At fixed substrate concentrations, initial velocity data obtained at 0–0.3 m*M* Zn(II) indicate cooperative binding of the metal ion to OTCase; a Hill coefficient of 1.7 ± 0.1 is found. These results suggest that conformational changes are only induced in the subunits of the enzyme by the metal ligand. Consequently, the positive cooperativity observed for L-ornithine is a manifestation of the allosteric effect of Zn(II). This phenomenon arises as a result of displacement of the metal ion from the enzyme by the substrate. The interpretation is further supported by a theoretical treatment based on equations derived for the two-state MWC model under the condition of competition between a substrate (non-cooperative) and an inhibitor (cooperative). Our study reveals that substrate cooperativity mediated indirectly via a competitive metal inhibitor is a special case previously unrecognized in enzyme allosteric control.

Because of the unique cooperative behavior of OTCase and its uncommon quaternary structure, it is of special interest to understand the mechanism through which subunit interactions are transmitted. For ATCase, the protein is composed of two trimeric catalytic subunits and three dimeric regulatory subunits; the catalytic trimer is devoid of cooperative action in the absence of the regulatory dimers [2]. In comparison and at least *in vitro*, Zn(II) appears to substitute functionally in lieu of a regulatory subunit in OTCase.

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