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The Role of Zinc in Transcriptional Control of Xenopus 5S RNA Gene

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Zinc is essential to nucleic acid synthesis and function. While the universal presence of Zn in enzymes which bind DNA or RNA as template or substrate has been recognized, its participation in the function of a regulatory protein involved in nucleic acid synthesis is not known.

Transcription of Xenopus 5S RNA genes is dependent upon an intragenic DNA sequence of approximately 50 base pairs. A Xenopus transcription regulatory protein, factor A, has been found to interact specifically with this intragenic control region and direct accurate initiation of 5S RNA transcription by RNA polymerase III. Factor A also binds 5S RNA in Xenopus immature oocyte in the form of a 1:1 stable 7S particle, suggesting an autoregulatory mechanism for the transcription.

Analysis of highly purified preparations of the 7S particle by atomic absorption spectrometry led us to the discovery that it contains two moles of tightly bound Zn ions per mole of particle. The refractory nature of the Zn ions upon extensive dialysis against EDTA or 1,10-phenanthroline suggests that they are an integral part of the particle. However, Zn ions can be readily removed by the metal chelators after the particle has been treated with RNase to liberate factor A. Thus, the Zn ions in the 7S particle are most likely located at the contact domain between 5S RNA and factor A. Alternatively, they may be buried in factor A and become exposed to the chelator due to a conformational change of the protein following removal of 5S RNA from the particle.

Evidence that Zn is involved in the specific binding of factor A to the 5S RNA gene comes from inhibition studies with metal chelators. Both EDTA and 1,10-phenanthroline inhibit the specific binding as determined by the DNase I footprinting method. The concentrations required for a complete inhibition are 2 m*M* for EDTA and 0.15 m*M* for 1,10-phenanthroline. Inhibition of the specific binding of factor A to the 5S RNA gene is the result of Zn chelation from factor A by metal chelators. A direct proof for this conclusion is provided by the observation that the specific binding ability of EDTA-treated factor A can be restored by the addition of exogenous Zn ions (15 μM) prior to the footprinting reaction. In contrast to the specific binding, EDTA and 1,10-phenanthroline do not inhibit nonspecific binding of factor A to DNA as measured by nitrocellulose filter binding assay. These differential effects suggest that Zn may play a more specific role in the binding of factor A to the 5S RNA gene (*e.g.*, recognition of nucleotide bases in the control region) than simply providing a charge bridge between protein and DNA.

In an *in vitro* transcription system which synthesizes 5S RNA as well as tRNA, both syntheses are inhibited by high concentrations (>1 m*M*) of 1,10-phenanthroline, possibly reflecting the necessity of Zn in transcription reactions in general. An interesting finding is that at a low concentration (0.25 m*M*), 1,10-phenanthroline completely inhibits 5S RNA synthesis whereas tRNA synthesis is scarcely affected. Since factor A is the only addition necessary for 5S RNA synthesis in this system, the specific inhibition on 5S RNA synthesis is likely due to the failure of factor A binding to the 5S RNA gene via Zn chelation by 1,10-phenanthroline.

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The Catalytically Competent Coordination Environment of the Active Site Metal Ion of Liver Alcohol Dehydrogenase

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The coordination environment of the active site metal ion of horse liver alcohol dehydrogenase is investigated by EPR and steady-state kinetic methods with use of the native (ZnLADH) and the active site specific Co^{2+}-reconstituted enzyme (CoLADH) described by Zeppezauer and coworkers [1]. The pH dependence of kinetic parameters for the oxidation of benzylalcohol reveals two ionizations ($pK_1 \sim 6.7$; $pK_2 \sim 10.6$) that govern k_{cat} and belong to the ternary enzyme-NAD^+-alcohol complex and two ionizations ($pK'_1 \sim 7.5$; $pK'_2 \sim 8.9$) that govern k_{cat}/K_m and belong to the binary enzyme-NAD^+ complex. Only pK_2 and pK'_2 are substantially influenced by metal substitution. Comparable results are observed for the oxidation of isopropanol. We attribute these ionizations to metal-bound water that occur separately at the ternary and binary complex level in the course of alcohol oxidation.

In parallel studies from this laboratory [2, 3], we have demonstrated that the magnitude of the zero-field splitting (ZFS) of the high-spin Co^{2+} ion falls into three ranges according to coordination number: 0–13 cm^{-1} for tetracoordinate; 20–60 cm^{-1} for penta-coordinate; and 90–310 cm^{-1} for hexa-coordinate environments. We have determined the ZFS

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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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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