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Inhibition of AMP deaminase by zinc ions

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AMP deaminase (EC 3.5.4.6) may be important as a regulatory enzyme in the purine nucleotide cycle [1, 2], which is related to amino acid metabolism and interconversion of adenine, inosine and guanine nucleotides [3–6], as well as in stabilizing the adenylate energy charge [7] in various tissues. In previous papers, we have reported some regulatory properties of chicken erythrocyte enzyme [8–12], and the inhibition of bovine brain and liver enzymes by fatty acyl-CoA [13].

Zinc deficiency produces a variety of metabolic responses, such as reduced aspartate and elevated ammonia levels in blood [14, 15]. Recently, the activity of the enzymes of the purine nucleotide cycle, AMP deaminase, adenylosuccinate synthetase (EC 6.3.4.4) and adenylosuccinase (EC 4.3.2.2), was reported to be elevated in zinc deficiency [16]. We now report the potent inhibition of AMP deaminase by zinc ions. The striking inhibitory action of zinc ions on enzyme activity may be responsible for the recently observed increase in blood ammonia in zinc-deficient animals when compared to controls.

AMP was a product of Kyowa Hakko Co. (Tokyo, Japan), and was converted to the Tris salt before use. All other chemicals were of reagent grade and were used without further purification.

The purification procedure of AMP deaminase used in this study has been described previously [8]. The enzyme activity was measured by estimating the production of ammonia colorimetrically, using Nessler's reagent [11].

Table 1 shows that AMP deaminase was markedly inhibited by Zn^{2+} and, to a lesser extent, by Co^{2+}. Trivalent lanthanides, which are known to be good substitutes for Ca^{2+} [17] or in some cases for transition metals [18], also inhibited the enzyme. The enzyme reaction was studied kinetically in the presence of various concentrations of AMP and Zn^{2+} or holmium ions (Ho^{3+}). Plots of reaction velocity as a function of AMP concentration gave sigmoid curves, as demonstrated previously [11]. With increasing concentrations of Zn^{2+} or Ho^{3+}, the AMP saturation curves became more sigmoidal in shape, suggesting that these ligands may be allosteric inhibitors (Fig. 1). Using double reciprocal plots, the maximum velocity was unchanged, though the apparent K_m values were raised drastically. The cooperative effect of AMP, analyzed in terms of Hill's coefficient, was such that this coefficient increased from 2 to 4 as these ligands were increased from 0 to 80 μM.

Table 1. Effect of various metals on the activity of AMP deaminase*

Compounds	Relative velocity†
No addition	1.00
Zn^{2+}	0.16
Co^{2+}	0.86
Ca^{2+}	1.02
Mg^{2+}	1.03
Ba^{2+}	0.99
Ho^{3+}	0.46
Er^{3+}	0.45
Eu^{3+}	0.50
Tb^{3+}	0.42

*Activity was determined in the presence of 13.5 mM AMP and 80 μM of each metal as the chloride salt.

†Enzyme activity without metal was expressed as an arbitrary value of 1.00.

The effect of increasing concentrations of Zn^{2+} or Ho^{3+} on enzyme activity was examined. Plots of reaction velocity against inhibitor concentration gave sigmoid curves (Figs. 2 and 3), suggesting the presence of cooperativity with respect to the binding of the inhibitors, Zn^{2+} and Ho^{3+}. The cooperativity of inhibitor binding was enhanced by increasing the AMP concentration. The results suggest that these metals act as allosteric inhibitors of the enzyme. This system lends itself to the convenient method of analysis described by Blangy *et al.* [19] using a function called the 'quotient function', which is the ratio of the amount of the enzyme existing in the *R* state to that in the *T* state. We may write

$$Q = \frac{R}{1-R} = \frac{v}{V'_{max} - v} = C \cdot \frac{1}{(1+\beta)^n}.$$

where V'_{max} is the maximum velocity which can be reached in the presence of a given concentration of the substrate and a saturating concentration of the activator (ATP), that is V''_{max} is the velocity obtained when the protein is entirely in the *R* conformation, and β is the normalized concentration of the inhibitor. It is, therefore, convenient to plot $n\sqrt{(V'_{max} - v)/v}$ against Zn^{2+} concentration, assuming that there are four independent sites for Zn^{2+} per molecule of the enzyme in the *T* conformation. As shown in the insert of Fig. 2, all the curves in Fig. 2 were converted to straight lines converging on the abscissa at the same point, $-K_{T(Zn^{2+})}$. This suggests that there are indeed four independent sites for Zn^{2+} per molecule. From these data, K_T for Zn^{2+} was calculated to be 20 μM. If a number of sites other than four is assumed for Zn^{2+}, the functions deviate from linearity and do not cut the abscissa

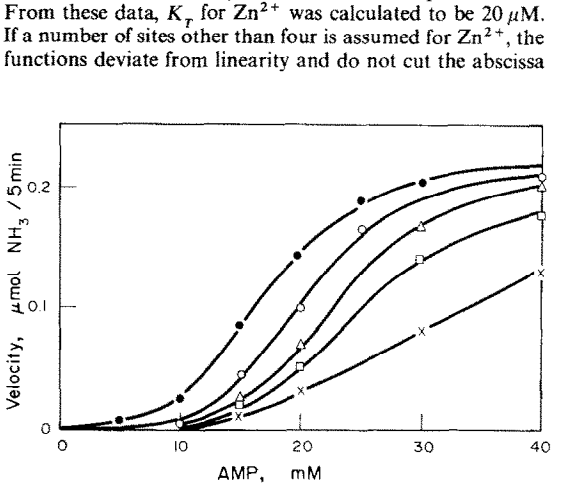

Fig. 1. Effect of AMP concentration on the velocity of the AMP deaminase reaction in the absence or presence of $ZnCl_2$ or $HoCl_3$. The reaction mixture contained 40 mM-Tris–HCl buffer, pH 7.1, 0.8 mg/ml of bovine serum albumin, various concentrations of $ZnCl_2$ and AMP in a final volume of 0.25 ml. The reaction was carried out at 37° for 5 min and was stopped by the addition of 0.1 ml of Nessler's reagent. The resulting color was measured at 430 nm in a final volume of 1 ml. The activity was determined in the absence (●) or presence of 10 μM (○) or 20 μM-$ZnCl_2$ (△), and 40 μM (□) or 80 μM-$HoCl_3$ (×).

at the same point. Thus, the most probable number of sites for Zn^{2+} is four. The number of binding sites and the dissociation constant of Ho^{3+} for the T state of the enzyme were also calculated from Fig. 3 to be 4 and 60 μM respectively (data not shown). AMP deaminases, purified from other tissues including chicken muscle, rat muscle and bovine brain, were also observed to be inhibited by Zn^{2+}.

The effect of various metals on AMP deaminase activity has not been examined in detail. The results presented here indicate that the zinc ion is a potent allosteric inhibitor of AMP deaminase; kinetic analysis has revealed that the number of Zn^{2+} binding sites is four, which is identical with the number of active or AMP binding sites of the enzyme [11]. AMP deaminase is known to be a zinc metalloenzyme [9, 20]. Intrinsic Zn^{2+} is assumed to be a general catalyst for hydrolytic reactions such as alkaline phosphatase produces, especially when it is coordinated with residues such as imidazole [21]. Added Zn^{2+} may bind to the active sites of the enzyme and compete with intrinsic Zn^{2+}, resulting in inhibition of the enzyme activity.

Zinc deficiency and zinc administration produce a variety of metabolic alterations of amino acids in various tissues; of particular interest are the reduced plasma aspartic acid levels and elevated ammonia levels in zinc-deficient animals [14, 15]. Recent observations on the increased activities of enzymes catalyzing the purine nucleotide cycle, which is involved in amino acid catabolism, in zinc-deficient animals [16] are also consistent with the aforementioned results. As described in this communication, AMP deaminase, as a regulatory enzyme in the purine nucleotide cycle, is inhibited by Zn^{2+} with a remarkably high affinity: the K_i value is within the range of physiological concentration of this metal in plasma. Thus, Zn^{2+} may be responsible for the control of AMP deaminase activity through a potent inhibition, and reversal of the zinc inhibition of the enzyme may contribute to the recently observed increase in ammonia and the decrease in aspartate in the blood of zinc-deficient animals.

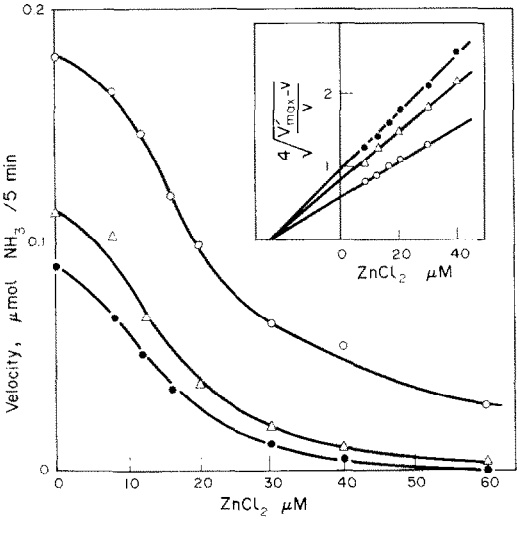

Fig. 2. Effect of $ZnCl_2$ on the velocity of the AMP deaminase reaction at various concentrations of AMP. The reaction mixture was similar to that in Fig. 1. The effect of $ZnCl_2$ was examined in the presence of 13.5 mM (●), 15 mM (△), and 17.5 mM-AMP (○). Insert: Variation of the 'quotient function' $\bar{Q}$ with respect to the inhibition by $ZnCl_2$ assuming four binding sites for this metal. The values of V_{max}^{T} taken in constructing this plot were determined in the presence of a saturating concentration of ATP (5 mM).

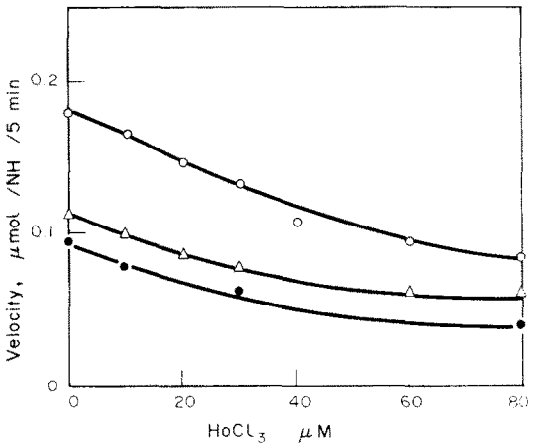

Fig. 3. Effect of $HoCl_3$ on the velocity of the AMP deaminase reaction at various concentrations of AMP. The reaction mixture was similar to that in Fig. 1. The effect of $HoCl_3$ was examined in the presence of 13.5 mM (●), 15 mM (△), and 17.5 mM-AMP (○).

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