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THE EFFECT OF Zn^{2+} ON THE INHIBITION OF Fru-P_2ase BY AMP

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SUMMARY: In the absence of chelating agents, the sensitivity of rabbit liver fructose 1,6-bisphosphatase to inhibition by AMP is increased by approximately 10-fold, apparently related to the presence of bound Zn^{2+}. At pH 7.5, 10 μM AMP causes virtually complete inhibition and nearly full activity is restored by the addition of chelating agents such as EDTA or histidine. These properties provide a mechanism for the induction of Fru-P_2ase activity under gluconeogenic conditions.

INTRODUCTION: Fructose 1,6-bisphosphatase (Fru-P_2ase, EC 3.1.3.11) requires a divalent cation, either Mg^{2+} or Mn^{2+}, as originally demonstrated by Gomori (1). Its activity is also enhanced by the addition of mercaptoethanol or low concentrations of a chelating agent such as EDTA, which is usually included in the assay mixtures (2-6). We have recently shown that the requirement for EDTA is due to the presence of Zn^{2+}, which binds tightly to the enzyme even at low concentrations and inhibits its activity (7-9). The specificity of Zn^{2+} binding to the enzyme (7), the high-affinity of Zn^{2+} for the inhibitory sites (8,9) and the activation of the enzyme by physiological concentrations of histidine (10) suggest that the enzyme-Zn^{2+} complex may play a role in the regulation of Fru-P_2ase activity in vivo. We have previously shown that in the absence of chelating agents the inhibitory binding sites are partially filled by Zn^{2+} (9), and it was of interest to study the effects of AMP, the allosteric regulator (2), on the enzyme assayed under these conditions. As reported here, we find the enzyme assayed in the absence of chelating agents

1/ Abbreviations: Fru-P_2, fructose 1,6-bisphosphate; Fru-P_2ase, fructose 1,6-bisphosphatase.

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to be much more sensitive to the inhibitory effects of AMP, as compared with the enzyme assayed in the presence of EDTA. Thus agents that remove Zn^{2+} also help to release the enzyme from the inhibitory effects of AMP.

MATERIALS AND METHODS

Fru-P_2ase was purified as previously described (9). Na_4Fru-P_2 and AMP were purchased from Sigma Chemical Co., St. Louis, Mo. Other chemicals were reagent grade and were used without further purification. Fru-P_2ase activity was measured as described in the figure legends and tables. The protein concentration was determined by absorbance at 280 nm using a value of 0.73 for the absorbance of a solution containing 1 mg/ml.

RESULTS

Effect of chelating agents on the inhibition of Fru-P_2ase by AMP. The K_i for AMP was found to be increased by approximately six- to eight-fold by the addition of EDTA or histidine (Fig. 1 and Table I). Thus, 15 μM AMP, which inhibited the activity of the Zn^{2+}-free enzyme by only 50%, caused almost complete inhibitior (>90%) of the enzyme assayed without chelating agents.

Because of the increased sensitivity of the enzyme-Zn^{2+} complex to inhibition by AMP, the effect of chelators on the recovery of Fru-P_2ase activity was greatly enhanced. The addition of EDTA or histidine to assay mixtures containing 10 μM AMP increased the Fru-P_2ase activity by 12-fold, as compared with the 2.5-fold increase observed when chelators were added in the absence of AMP (Fig. 2). The maximum activity reached in the presence of AMP reached 60% of that observed in its absence.

Effect of Zn^{2+}. We have previously estimated that in the presence of 2 mM $MgCl_2$ the concentration of Zn^{2+} required for half-saturation of the high-affinity inhibitory sites on rabbit liver Fru-P_2ase is approximately 0.38 μM (9). We therefore tested the effect of AMP and chelating agents in the presence of this concentration of added Zn^{2+} (Table II). In the absence of AMP, the activity was increased by nearly 5-fold by the addition of chelating agents; the corresponding increase observed in the presence of 10 μM AMP was approximately 40-fold and again the specific activity was nearly 60% of the maximum observed when the chelating agents were added in the absence of AMP.

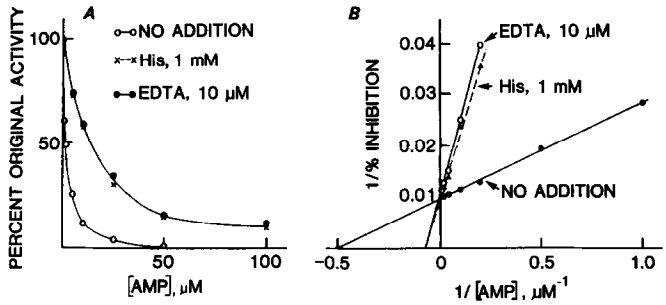

Figure 1: A. Effect of chelating agents on the inhibition of rabbit liver Fru-P_2ase by AMP. The assays were carried out at 23°C in 0.25 ml of 20 mM diethanolamine and 20 mM triethanolamine, pH 7.5, containing 2 mM $MgCl_2$, 0.1 mM Fru-P_2 and the indicated concentrations of AMP. Other additions were as shown on the figure. After 10 min the release of P_i was measured by the addition of 0.3 ml of the Tashima and Yoshimura reagent (11), followed by 0.45 ml of H_2O, and the absorbance was measured at 650 nm. B. Double reciprocal plot of the same data, calculated from the percent inhibition by AMP.

TABLE I

Values of K_i for the inhibition of Fru-P_2ase by AMP determined in the absence or presence of chelating agents

Conditions	K_i[a] (µM)
2 mM Mg	1.9
2 mM Mg + 1 mM Histidine	12.5
2 mM Mg + 10 µM EDTA	14.3

[a] Calculated from data in Fig. 1.

DISCUSSION

The results reported here suggest that Zn^{2+} may participate in the regulation of Fru-P_2ase by increasing its sensitivity to inhibition by AMP. In the presence of 10 µM AMP and low concentrations of Zn^{2+} (>0.4 µM) the catalytic activity is almost completely inhibited, but the same concentration of AMP causes only partial (40%) inhibition when the activity is assayed in the presence of chelating agents.

We have previously reported (10) that the concentration of histidine in liver increases markedly under gluconeogenic conditions. The concentration of this amino acid found in the livers of fasted rabbits was 1 mM, sufficient to reverse com-

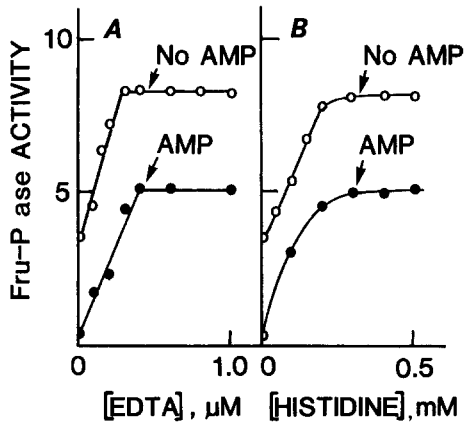

Figure 2: Effect of increasing concentrations of chelating agents on the activity measured in the presence or absence of AMP. The activities were assayed as described in the legend to Fig. 1, in the presence or absence of 10 μM AMP and the indicated concentrations of EDTA (A) or histidine (B).

TABLE II

Reversal of Zn^{2+} inhibition by chelating agents in the presence and absence of AMP

Additions to assay mixture		Fru-P$_2$ase activity[a] (μmol/min/mg protein)		Increase due to chelating agent	
Zn^{2+}	Chelator	-AMP	+AMP	-AMP	+AMP
0.4 μM	none	1.9	0.13	-	-
0.4 μM	10 μM EDTA	8.7	5.2	4.6X	40X
0.4 μM	1 mM Histidine	8.5	5.0	4.5X	38X

[a] Assayed as described in the legend to Fig. 1, in the presence of 0.4 μM added Zn^{2+}.

pletely the inhibitory effects of Zn^{2+}. Thus, in the presence of concentrations of AMP that are likely to be present in the cytosol of liver cells (12,13), changes in concentration of histidine become more significant and the enzyme activity may be regulated between fully-inhibited and partially activated states.

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