METAL ION ACTIVATORS OF GLUTAMINE SYNTHETASE*

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Glutamine synthetase, like most other ATP dependent enzymes, is activated by magnesium ions. Manganous and cobaltous ions have also been reported to promote glutamine synthetase activity, but at rates lower than those for magnesium (Denes, 1954; Levintow, et al., 1955). Greenberg and Lichtenstein (1959) have shown that the pH optimum for Mn^{++} lies lower than that for Mg^{++} at equimolar concentrations, and that the optimal activity for Mn^{++} may equal or exceed that for Mg^{++}. A study was initiated in this laboratory to determine if other metal ions stimulated glutamine synthetase, and to examine the conditions under which such activations occur. In this communication, the effects of metal ions on the pH-activity profile of the enzyme are described.

EXPERIMENTAL. Highly purified glutamine synthetase was prepared from sheep or beef brains (Pamiljans et al., 1962). L - and D-glutamic acids were purchased from Mann Research Laboratories, Inc. The D- isomer yielded no detectable carbon dioxide in the presence of Clostridium welchii L-glutamic decarboxylase (Meister, et al. 1951). L-glutamine synthesis was measured in a system containing 50 μmole L-glutamate, 5 μmoles ATP,

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20 μmoles ammonium acetate, metal salts as indicated, and enzyme in a final volume of 1.0 ml. Controls contained no glutamate. Hydrogen ion concentration was controlled with acetate buffer in the pH range of 4 to 6, and tris-maleate or imidazole buffer in the pH range of 5.5 to 8.6. Buffers of higher pH were prepared with glycine. All buffers were 0.1 M with respect to the anion. No detectable change in rate of glutamine synthesis was observed when the determinations were carried out in more dilute buffer or after the addition of sodium chloride. In order to avoid major shifts in the pH of the complete system, glutamate, ATP, and ammonium acetate were adjusted to pH 5.5, 6.5, or 7.5, and added to the system in the appropriate pH range. Reaction was initiated by addition of enzyme. Extent of glutamine synthesis was determined by measuring inorganic phosphate released from ATP in 15 min at 37°. D-glutamine synthesis was measured in the same way, but because of the slower rate of reaction, a 1 hour incubation time was used. Glutamyl transferase activity was determined in a system containing 50 μmoles L-glutamine, 100 μmoles hydroxylamine adjusted to the appropriate pH, 0.5 μmole ADP, 10 μmoles 2-mercaptoethanol, 100 μmoles of buffer, and metal ions at various concentrations in a final volume of 1.0 ml.

Extent of glutamohydroxamate formation was measured by the method of Lipmann and Tuttle (1945).

RESULTS. As the concentration of Mg^{++}, Mn^{++}, or Co^{++} was increased, the pH optima for L-glutamine synthesis moved to more acid values with no decrease in maximum velocity. Table 1 shows the results.

The measured velocity at the pH optimum for a given metal concentration was assumed to be the maximum velocity, since a change in metal concentration in either direction at this pH caused a decrease in rate of release of inorganic

Table I.

Comparison of the catalytic effects of metal ions on glutamine synthetase activity

Cation	Conc'n	pH optimum		Vmax	
	mM	L-glu	D-glu	L-glu	D-glu
Mg^{++}	2.5	7.6	---	0.325	---
	5.0	7.6	7.6	0.475	0.220
	50	6.5	7.6	0.475	0.420
Mn^{++}	2.5	5.3	6.5	0.475	0.210
	5.0	4.8	5.7	0.475	0.210
	10	4.8	5.7	0.475	0.190
Co^{++}	2.5	6.1	---	0.240	---
	5.0	6.0	7.2	0.495	0.060
	50	5.2	---	0.495	---
Fe^{++}	5.0	5.2	*	0.120	*

* Activity was below detectable limits and pH optimum could therefore not be determined.

phosphate. The rates of glutamine synthesis for Mg^{++}, Mn^{++}, and Co^{++} at their respective pH optima and at 5mM final concentration of each metal were equal. Each of these metals could therefore replace the other in the glutamine synthesis system when due consideration was paid to the effects of pH on activity. Since ferrous ion is situated in the periodic table between manganese and cobalt, it was of interest to see if Fe^{++} also activated glutamine synthetase. The velocity of reaction with Fe^{++} was about one fourth that of Mg^{++} under similar conditions.

Increasing the ATP level at constant Mn^{++} had only a small effect on the pH optimum. With Mn^{++} constant at 2.5 mM, pH optima for 2.5, 5.0, and 10 mM ATP were 5.2, 5.4, and 5.6, respectively. The profound effects of Mn concentration on optimum pH of L-glutamine synthesis were therefore

not primarily due to the ratio of Mn to ATP. The possibility that the metal plays an activating role in addition to that in which ATP participates merits investigation.

The pH optima for D-glutamine synthesis were in general higher than for L-glutamine synthesis. Maximum reaction velocities were invariably lower for the D-than for the L-antipode. Ratio of rates of L-to D-glutamine synthesis varied with the metal. At 5 mM Mg^{++} or Mn^{++}, the ratio was 2.2; at 5 mM Co^{++}, 8.3; with Fe^{++}, D-glutamine synthesis was not detected even after 2 hr incubation. In all cases, glutamine synthesis was confirmed chromatographically (Monder and Meister, 1958). The results indicate that the metal ion influenced the relative stereospecificity of the synthetase reaction. Other cations, including Cu^{++}, Zn^{++}, Ni^{++}, Ba^{++}, Ca^{++}, Na^{+}, K^{+}, Cu^{+}, Fe^{+++}, and Al^{+++} were not activators of glutamine synthetase.

Unlike the synthetase reaction, glutamyltransferase was activated only by Mn^{++} or Mg^{++}. Other metals had no effect. The pH optimum for Mn^{++} was 5.5 at 1 mM Mn^{++}, and this was shifted to 5.2 at 5 and 20 mM Mn^{++} with a progressive decrease in overall activity. The pH optimum for 5 mM Mg^{++} was 7.0. At 5 mM metal concentration, transferase activity was 1.8 times higher for Mn^{++} than for Mg^{++} at their respective pH optima.

DISCUSSION. The basis for the catalytic effects of the activating metals on glutamine synthetase activity is obscure. Though Mg^{++}, Mn^{++}, and Co^{++} stimulated enzyme activity equally well, the latter two are transition metals and the former is not. Fe^{++}, the least effective of the four, lies between Mn^{++} and Co^{++} in the first transition series of the periodic table. Ni^{++}, though it resembles Co^{++} closely in ionic radius and charge, did not activate the enzyme. It is clear that the relative activating effects of these ions

do not follow those expected from the Irving-Williams series (Williams, 1959). The chemical and kinetic bases for their catlytic effects on glutamine synthetase activity remain to be clarified.

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