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THE INTERACTION OF POLYPHOSPHOINOSITOLS WITH AMP DEAMINASE

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Polyphosphoinositols coupled to epoxy- activated Sepharose retained chicken liver AMP deaminase in a similar manner as phosphocellulose. After elution from polyphosphoinositol-Sepharose, in contrast to inositol-Sepharose and phosphocellulose, low Km AMP deaminase from the chicken liver exhibited markedly elevated $S_{0.5}$ value. Several commercialy available polyphosphoinositols were tested with rat liver AMP deaminase and only 1,3,4,5 IP_4 was found to stimulate the enzyme. This is the first report on the effect of naturally occuring polyphosphoinositol derivative on the soluble enzyme.

AMP deaminase is a highly regulated cytoplasmic enzyme which controls AMP catabolism in various types of cells (1-3). In general, it is activated by ATP and inhibited by GTP and Pi (2, 4, 5). In some animal and human tissues there are more than one forms of this enzyme (6-9). For example, in rat small intestine there is a low Km form which is relatively insensitive to modifiers, and a high Km form which is strongly regulated by effectors (9). In other tissues either a low Km or high Km form is present exclusively (6-8). The very tight binding of most vertebrate AMP deaminases to phosphocellulose is widely known and this feature has been commonly and effectively used during enzyme purification (10). In the case of frog liver AMP deaminase, the required eluting concentration of potassium chloride needs to be as high as 2.0 M (11), whereas enzymes

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from other sources usually require 0.2 - 1.0 M NaCl or KCl (6-9). This unique property indicates that the enzyme probably possesses high affinity for glucose-like, highly phosphorylated compound(s). Since the structure of glucose is very similar to inositol, this study was undertaken to test the interaction of chicken and rat liver AMP deaminase with various, commercially available, inositol phosphates.

MATERIALS AND METHODS

Randomly phosphorylated polyphosphoinositol-Sepharose was obtained by coupling 1.4 g of phytic acid with 1.4 g of epoxy-activated Sepharose at pH 9.0 and at 37^{o} C with gentle shaking for 20 h. After that time, an additional amount of 0.7 g of phytic acid was added together with 20 mg of $ZnCl_2$ and coupling continued for the next 6 h. The remaining active groups were blocked with 1.0 M ethanolamine. The gel was subsequently washed with 0.1 M phosphate buffer (pH 9.0), H_2O, 0.5 M KCl, 0.1 M phosphate buffer (pH 7.0) and 0.1 M KCl. An analogous procedure was used with the preparation of inositol-Sepharose. Low Km AMP deaminase from chicken liver was purified as described previously (12). AMP deaminase from rat liver was purified using two subsequent phosphocellulose chromatographies. The specific activities were 2.5 and 15.1 umoles/min per mg protein for the enzyme from chicken and rat liver, respectively.

AMP deaminase activity was assayed colorimetrically using phenol-hypochlorite reagent (13). Protein was determined by the Bradford assay (14).

Epoxy-activated Sepharose was obtained from Pharmacia (Sweden). Phytic acid, phospho-myoinositol derivatives; 2 IP_1, 4,5 IP_2, 1,4,5 IP_3 and AMP (free acid) were from Sigma (USA). 1,3,4,5 IP_4 and inositol pentaphosphate were from Calbiochem (USA). Phosphocellulose P-11 was from Whatman (U.K.).

RESULTS

Low Km AMP deaminase from chicken liver appeared to bind to polyphosphoinositol-Sepharose but not to inositol-Sepharose (Fig. 1). The enzyme was eluted using 0.45 M KCl. Similar binding properties and elution profile was obtained with rat liver enzyme (data not shown). A study on the kinetic properties of the enzyme before and after chromatography

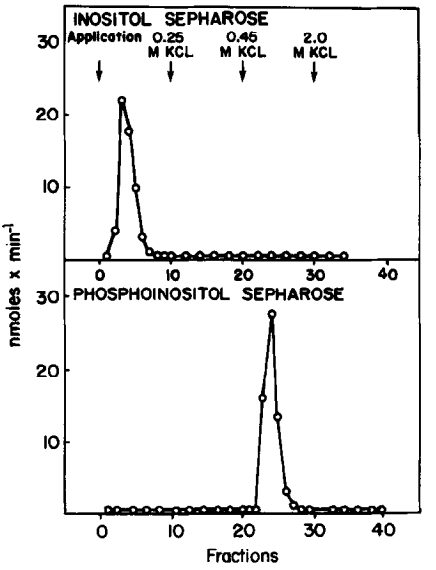

Fig. 1. Chromatography of low Km chicken liver AMP deaminase on polyphosphoinositol- and inositol-Sepharose. Resins were prepared as described in Materials and Methods. Approximately 3-6 umoles/min of enzyme activity was applied in each case. Recovery was 90 and 40-50 % in the case of inositol and polyphosphoinositol-Sepharose chromatography respectively. Column volumes were 5 ml and the flow rate was 20 ml per hour.

revealed a significant shift in $S_{0.5}$ (Table I). This was not observed with inositol-Sepharose nor with phosphocellulose.

Subsequent studies of the effect of myo-inositol phosphates on rat liver AMP deaminase revealed that among various commercially available compounds only 1,3,4,5 IP_4 exerted a significant effect (Table II). At 10uM concentration IP_4 stimulated the enzyme only by 20 - 30 % (data not shown). Various naturally occuring glucose and fructose phosphates were also tested but none of them significantly affected the enzyme activity.

DISCUSSION

The similar interaction of AMP deaminase with polyphosphoinositol-Sepharose and phosphocellulose and the strong activation exerted by IP_4 suggests searching for the explanation in structural similarities between IP_4 and phosphorylated glucose units in phosphocellulose. Indeed, the polymeric structure of cellulose allows

TABLE I

The substrate half saturation constant before and after passing through various columns

Type of resin	$S_{0.5}$ values (mM)	
	before	after
Phosphocellulose	1.4 ± 0.2	1.6 ± 0.2
Inositol-Sepharose	1.2	1.3
Phosphoinositol-Sepharose	1.3 ± 0.2	2.4 ± 0.3

Assays were performed at pH 7.2 in 50 mM cacodylate buffer, pH 7.2, 150 mM KCl, 1 mg/ml BSA and within 0.2 - 20 mM substrate concentrations. Substrate half saturation constants were derived using nonlinear regression method as described previously (9). Except for inositol-Sepharose (single experiment) data are mean from 3-4 chromatographies, ± SD.

positions 6 and predominantly 2 and 3 in the glucose moiety to be phosphorylated. Further comparison of the structures of glucose and inositol reveals that there is a structural analogy between positions 2

Table II

The effect of various phosphoinositol derivatives on rat liver AMP deaminase

Inositol derivative	Activity nmoles x min^{-1}	% of control
control	20.1 ± 1.8	100
2, IP_1	19.2 ± 2.0	96
4,5, IP_2	19.3 ± 1.3	96
1,4,5, IP_3	18.1 ± 1.2	90
1,3,4,5, IP_4	57.4 ± 5.1	286
inositolpentaphosphate	17.1 ± 1.4	85

Assay conditions; 50 mM cacodylate buffer, pH 7.2, 150 mM KCl, 1 mg/ml BSA and 1 mM AMP. Inositol phosphates were at 40 uM concentration. Data are mean from 3 measurements, ± SD.

and 3 in glucose and 3 and 4 in inositol. In both cases hydroxyl groups are at the similar configuration (15, 16). Therefore, if the above considerations are correct, the finding that only 1,3,4,5 IP_4 affected the enzyme activity may be explained by the fact that among various phosphoinositols tested, only this compound has phosphate esters bound in positions 3 and 4. Because of the relatively high concentration of IP_4 at which the effect was visible, it is difficult to discuss this finding in physiological terms. Unfortunately, the other physiologically occuring phosphoinositol derivative which possesses phosphate esters in positions 3 and 4 - 1,3,4 IP_3 was not available during this study. One can speculate however, that if 1,3,4 IP_3 is a physiological activator of AMP deaminase then the activation which was observed with IP4 migh have been due to the contamination by 1,3,4 IP_3. Also, the involvement of cyclic derivatives is possible. However, these intriguing possibilities require further studies.

The observation that chromatography of low Km AMP deaminase from chicken liver on polyphosphoinositol-Sepharose brought about a decrease of the substrate affinity could also be explained assuming that the low Km and high Km forms are interconvertible and differ in the content of a tentative, tightly bound activator which might be selectively removed during affinity chromatography. The other possibility is that interaction of the enzyme protein with the affinity resin itself alters the kinetic properties of the enzyme. A recent observation that a high protein diet causes shift of AMP deaminase from low Km to high Km form in the chicken liver would indicate that such transformation is possible (17).

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