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Glycerylphosphorylcholine Diesterase: Inhibition by Nucleotides* (33856)

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Glycerylphosphorylcholine (GPC) diesterase (glycerolphosphorylcholine glycerophosphohydrolase, EC 3.1.4.2), which catalyzes the following reaction: GPC + H_2O → L-α-glycerophosphate + choline is found at the end of the phosphatidylcholine degradative scheme. GPC, unlike lysolecithin is not capable of being acylated to form lecithin (1). Consistent with this data is our inability to demonstrate the synthesis of GPC from L-α-glycerolphosphate and choline. It therefore appears in view of available evidence that the sole purpose of GPC diesterase is to hydrolyze GPC.

The rate at which this enzyme operates with respect to the other degradative enzymes in the metabolism of lecithin in mammals could conceivably exert an influence on the rate of metabolism of lecithin due to its unique position in the degradative scheme.

Studies designed to test the effects of various biosynthetic and degradative intermediates in lecithin metabolism on GPC diesterase in our laboratory (2) showed ATP to be a strong inhibitor of the enzyme. The present communication reports the results obtained from studies on the nucleotide inhibition of GPC diesterase from rat kidney.

Materials and Methods. The ATP was purchased from Sigma. The GTP, UTP, and ITP were products of P. L. Biochemicals and all other nucleotides were obtained from Calbiochem. PP_i was the product of the Fisher Chemical Company. Sources of substrates and other reagents were those used in a previous publication (2).

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The pH of all solutions used was adjusted so that the final pH of the reaction mixtures was 9.2. The enzyme was prepared as previously described (2) and this type of preparation was used in all studies.

Two types of assays were routinely used for these studies and are based on the estimation of L-α-glycerolphosphate. Briefly, Assay I utilized a two-stage incubation with L-α-glycerolphosphate dehydrogenase being used to quantitate the total amount of glycerolphosphate released from the hydrolysis of GPC. This assay was employed during the investigation of the effects of pH and $MgCl_2$.

Assay II consisted of a single-stage incubation, including L-α-glycerolphosphate dehydrogenase as a coupling enzyme. NADH production was monitored continuously with a Beckman DK-2 recording spectrophotometer. This assay was used exclusively in the studies designed to test the affinity of nucleotides and PP_i on the diesterase. The concentrations of inhibitors used in experiments evaluated with Assay II were without effect on the coupling enzyme.

A more thorough description of the assays has been previously reported (2). Both types of assays were carried out at 30°.

Results. Figure 1A and B depict the type of inhibition produced by UTP and ATP. Lineweaver-Burk (3) plots similar to those observed for ATP and UTP inhibition were obtained for all other nucleotides tested and a compilation of the K_i values from these studies is presented in Table I. Of interest is the apparent selectivity of the inhibition; the purine triphosphates producing K_i values of approximately 1.5 m*M*, whereas K_i values obtained with the pyrimidine triphosphates were near 10 m*M*.

Since nucleotides were observed to inhibit the diesterase, PP_i and P_i were tested as possible inhibitors. PP_i was found to be a very potent inhibitor of GPC diesterase (K_i PP_i = 0.74 m*M*) whereas P_i was found to be a very weak inhibitor of the enzyme as 15 m*M* P_i produced only 15% inhibition. Inhibition by PP_i was observed to be competitive with respect to GPC. Deviations from classical Michaelis-Menton kinetics were noted with PP_i and these results are shown in Fig. 2. The reason for this apparent deviation is currently not clear.

Studies designed to test the effect of pH on

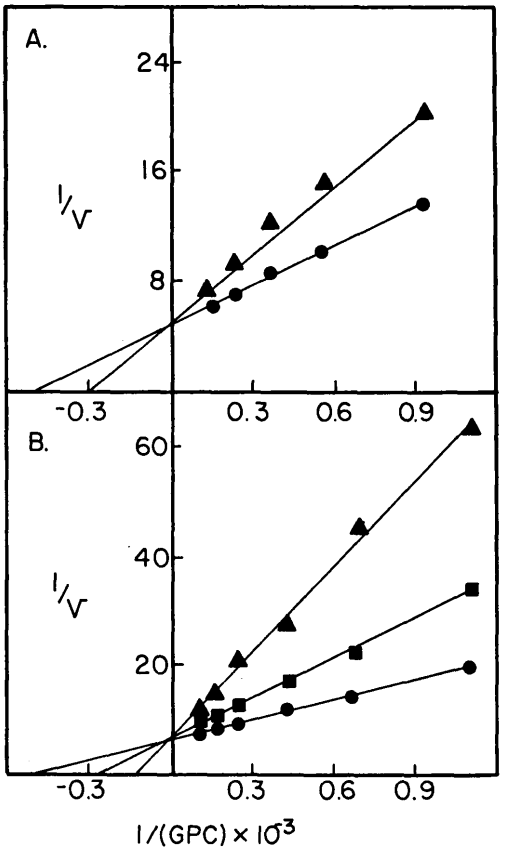

FIG. 1. Kinetics of pyrimidine and purine nucleotide inhibition of GPC diesterase: (A) pyrimidine (UTP) inhibition; reaction mixtures contained in a final volume of 3.45 ml, 0.15 mg of L-α-glyPO₄ dehydrogenase (sp act = 120 EU/mg), 900 μmoles of hydrazine–glycine buffer, pH 9.2, 2.5 μmoles of NAD, the indicated varied amounts of GPC, GPC diesterase (0.10 mg of protein) in the absence (●) and presence (▲) of 9.98 m*M* UTP. (B) purine (ATP) inhibition; reaction mixtures and conditions were the same as in (A), except that enzymic activity was determined in the absence (●) and presence of 1.45 m*M* ATP (■), and 5.53 m*M* ATP (▲). NADH production was monitored continuously with assay II in a Beckman DK-2 recording spectrophotometer. Kinetic parameters calculated from these data are (A) K_{GPC} = 2.0 m*M*, K_i UTP = 12.8 m*M*; (B) K_{GPC} = 2.1 m*M*, K_i ATP = 1.6 m*M*.

TABLE I. Compilation of K_i Values for Various Nucleotides.[a]

Nucleotide	Nucleotide concentration (mM)	K_i (mM)
Purines		
ATP	1.45, 5.53	1.6
ADP	22.8	31
AMP	22.6	119
GTP	1.94, 4.87	1.8
GDP	9.28	29
GMP	16.4	137
ITP	1.87, 4.69	1.3
IDP	16.7	16
IMP	12.0	118
Pyrimidines		
CTP	9.98	9.3
CDP	11.3	8.0
CMP	13.8	73
UTP	9.98	12
UDP	10.3	14
UMP	8.4	71

[a] Reaction mixtures and conditions were those as described in the legend for Fig. 1.

nucleotide inhibition are presented in Fig. 3. The greatest amount of inhibition was observed at pH 8.5, which is slightly below the pH optima of the enzyme (pH 9.2). As the pH approaches neutrality a progressive decrease in inhibition is seen and the same effect is also observed as the pH becomes more alkaline.

Since ATP is a chelator of Mg^{2+} ions, the effect of $MgCl_2$ on ATP inhibition was investigated, the results of which are shown in Fig. 4. As shown in Fig. 4, $MgCl_2$ alleviates the inhibition produced by ATP. $ZnCl_2$ was not effective in reversing the inhibition produced by ATP.

Since 5'-AMP is known to be effective in alleviating nucleotide inhibition of another enzyme, that being phosphofructokinase (4), similar studies were conducted on GPC diesterase. Neither 5'AMP or cyclic AMP were effective in this manner on the diesterase.

Discussion. The normal level of ATP in rat kidney has been reported to be 0.9 mmoles/kg of kidney (5). Assuming the content of intracellular water in rat kidney to be 70% as determined by Mannery and Hastings (6), the normal level of ATP in the kidney

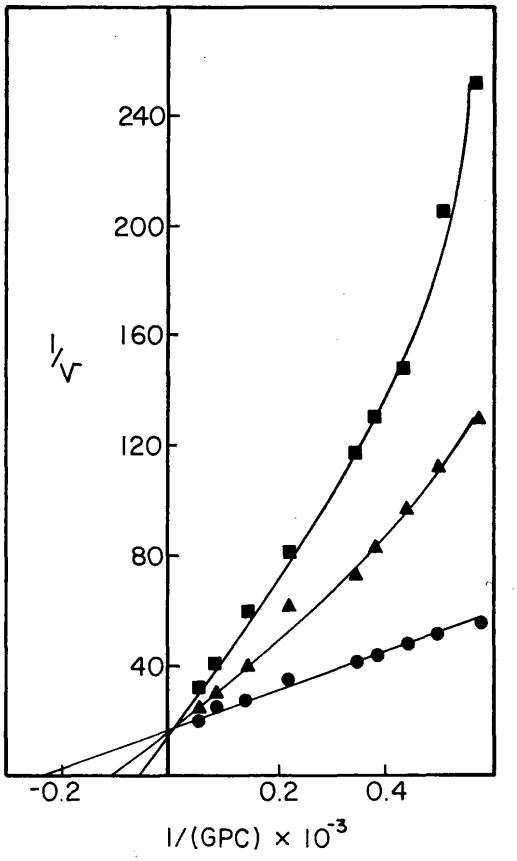

FIG. 2. Kinetics of PP_i inhibition: reaction mixtures contained in a final volume of 2.83 ml: 0.15 mg of L-α-glyPO$_4$ dehydrogenase, 720 μmoles of hydrazine-glycine buffer, pH 9.2; 2.5 μmoles of NAD, the indicated varied amounts of GPC and GPC diesterase (amount of protein not determined). Enzymic activity was measured with assay II in the absence (●) and presence of 0.90 mM (▲), and 1.79 mM PP_i (■). Assays were conducted in a Gilford recording spectrophotometer. Kinetic parameters evaluated from these data are K_{GPC} = 4.1 mM, K_i PP_i = 0.74 mM.

cells would be 1–2 mM. The data presented in Table I show that the K_i value for ATP is certainly within a range to be a physiologically significant inhibitor of the diesterase. The selectivity of the inhibition appears to be limited to the purine-type triphosphates and PP_i (see Figs. 1 and 2). The fact that PP_i is the more natural inhibitor (*e.g.*, the smallest K_i observed for the compounds tested) suggests that due to steric considerations the nucleotides are not able to bind as effectively

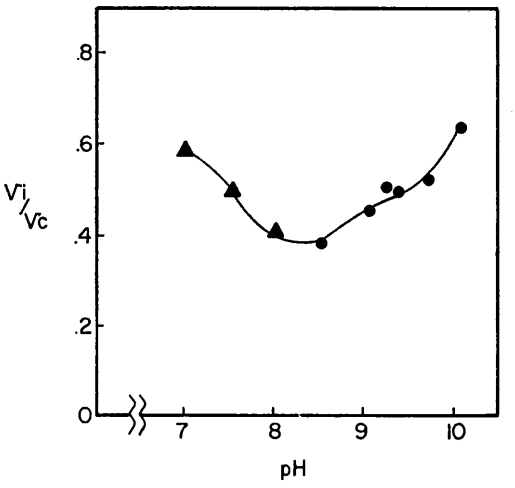

FIG. 3. Effect of pH on ATP inhibition: data are expressed as the ratio of the enzymic activity in the presence and absence of ATP at the indicated pH values. Reaction mixtures contained 100 μmoles of buffer—sodium phosphate pH 7.0–8.0(▲); sodium hydroxide–glycine buffer pH 8.5–10.1(●); 3.03 m*M* GPC, diesterase (0.06 mg of protein) and (when included) 2.32 m*M* ATP in a final volume of 1.65 ml. Assay I was used to measure enzymic activity; incubation was for 10 min at 30°.

as PP_i. It appears that the purine triphosphates molecular architecture is such that it will "fit" into the inhibitory site whereas the pyrimidine triphosphates' structure does not permit it to be as efficient in this manner.

As shown in Fig. 4, $MgCl_2$ alleviates the inhibition produced by ATP. At the highest concentration of $MgCl_2$ (2.4 m*M*) used, it was calculated that the ATP (2.0 m*M*) at pH 9.2 existed 100% as the Mg–ATP complex. This calculation was made assuming a stability constant of 4.04 (see Ref. 7). It therefore appears that the Mg–ATP complex will not bind to the enzyme in an inhibitory manner. The fact that both $MgCl_2$ ions and pH do affect the inhibition produced by ATP suggest that the inhibition is responsive to physiological changes within the cell.

Phospholipids are known to be components of various biological membranes and essential components of many enzymes (8–14). An uncontrolled breakdown of cellular lecithin would be of no great advantage to the overall metabolism of the cell. Therefore the regulation of this process is important. Since ATP is involved in the synthesis of precursors of lecithin (*e.g.*, phosphorylcholine, L-α-glycerolphosphate and diglycerides), it is conceivable that it may participate in regulating the breakdown of lecithin. It is possible that the inhibition of GPC diesterase by ATP may play a role in the overall control of lecithin metabolism. PP_i, a product of a synthetic reaction leading to the synthesis of lecithin, may also be involved in the regulation of this process, as it, too, is a potent inhibitor of GPC diesterase.

Summary. Glycerylphosphorylcholine diesterase (glycerolphosphorylcholine glycerophosphohydrolase, EC 3.1.4.2) from rat kidney was inhibited selectively by purine triphosphate nucleotides and inorganic pyrophosphate. Classical competitive inhibition for all compounds tested was observed. Of the purine nucleotides ATP, GTP, and ITP showed the greatest amount of inhibition, producing K_i values of approximately 1.5 m*M*. The purine diphosphates showed a lesser degree of inhibition, producing K_i values of approximately 20 m*M* and the corre-

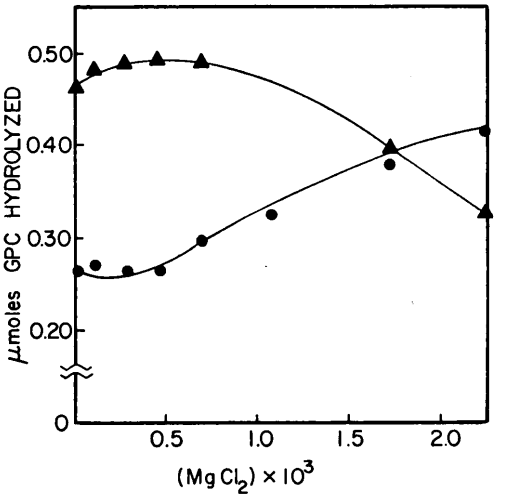

FIG. 4. Alleviation of ATP inhibition by $MgCl_2$: reaction mixtures contained 100 μmoles of sodium hydroxide–glycine buffer, pH 9.2; 4.0 m*M* GPC, GPC diesterase (0.06 mg of protein), the indicated varied amounts of $MgCl_2$ in the absence (▲) and presence of 1.90 m*M* ATP (●). The final volume of the reaction mixture was 1.75 ml; enzymic activity was measured with assay I and incubation was for 10 min at 30°.

sponding purine monophosphates gave very little inhibition, producing K_i values of approximately 125 mM. In the case of the pyrimidine nucleotides, both the cytidine and uridine triphosphates were not as effective as the purine triphosphates. The K_i values obtained with the pyrimidine triphosphates were approximately seven times greater than for the purine triphosphates. Inhibition by ATP was found to be pH dependent in the range studied, (pH 7.0–10.2) with the greatest amount of inhibition observed at pH 8.6. Michaelis-Menton kinetics were observed with all of the nucleotides studied. However, when considering inorganic pyrophosphate, sigmodial type kinetics were observed. Inorganic phosphate was without effect on the diesterase; 5′-AMP and cyclic AMP did not reverse ATP inhibition; $MgCl_2$ alleviated the inhibition produced by ATP.

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An Evaluation of the Effects of Polyvinylpyrrolidone on Blood Typing with Anti-Rh Serum: Importance of Albumin Concentration (33857)

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Polyvinylpyrrolidone (PVP) as an agent for increasing the sensitivity of agglutination tests evoked considerable interest more than a decade ago (1–5), though current reports have been limited largely to those dealing with automated agglutination techniques (*e.g.*, 6, 7). In view of recent interest in the use of PVP in manual blood typing, particularly as an additive to anti-Rh sera, we undertook an evaluation of the effects of this polymer in such typing systems.

Experimental Methods. Preliminary testing was done to determine whether, as suggested by various early reviewers (8–10), the danger of nonspecific results would be increased if, in an effort to increase the sensitivity of a test system, one simply added PVP to a commercial typing system. The PVP used was of two types, designated K-30 and K-90, which according to the manufacturer[2] had average molecular weights of 40,000 and 360,000, respectively. Two blood typing procedures were carried out under various conditions with red cells from 56 different donors and commercial Rh antisera of 4 different specificities, *viz.*, anti-Rh_0 (anti-D), anti-rh′ (anti-C), anti-rh″ (anti-E), and anti-hr″ (anti-e). Both were tube agglutination methods, one of which utilized a measured suspension of cells and the other of which used only an

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