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A pyrophosphatase from mammalian tissues specific for derivatives of ADP*

During the course of an investigation designed to separate and characterize the enzymes in mammalian liver which catalyze the synthesis (Reaction 1) of nucleoside diphosphate sugars it was also observed that the crude extracts catalyzed the hydrolytic cleavage (Reaction 2) of the same nucleotides.

$$\text{Nucleoside triphosphate} + \text{sugar-1-}P \rightleftharpoons \text{Nucleoside diphosphate-sugar} + PP_i \quad (1)$$

$$\text{Nucleoside diphosphate-sugar} + H_2O \rightarrow \text{nucleoside monophosphate} + \text{sugar-1-}P \quad (2)$$

Crystallization of synthetase for UDP–glucose[1] and 400-fold purification of a synthetase for GDP–hexose[2] completely removed hydrolytic activity for these substrates. This communication is concerned with one of the hydrolytic enzymes, a pyrophosphatase, with unique specificity for nucleosides containing adenylic acid.

When glucose 1-phosphate was a product of the reactions, the enzyme was quantitated as described previously[1]. When adenylic acid was a product of the reaction, pyrophosphatase activity was measured by the use of AMP deaminase[3].

To identify the reaction products, the following solvent systems were used in descending paper chromatography on Whatman No. 1 paper: Solvent A, isobutyric acid–conc. NH_4OH–water, (52:32:16, v/v/v); Solvent B, acetone–35% chloroacetic acid, (60:40, v/v); Solvent C, ethyl acetate–acetic acid–water, (3:3:1, v/v/v). Chromatography on polyethyleneimine-impregnated paper with 0.3 M LiCl was also used[4]. Reducing sugars were detected on paper by silver nitrate[5] and phosphate-containing compounds were detected by the method of HANES AND ISHERWOOD[6]. One unit of enzyme is the amount which causes the formation of 1 μmole of Glc-1-P per min at 25° under the conditions of the assay.

Calf and rabbit liver contain appreciable amounts of the pyrophosphatase (2–3 units/g of fresh tissue); but it is also present in kidney, mammary gland and brain of both the rat and rabbit.

For extraction of the enzyme, tissues were rapidly removed from the animal and placed in ice. Each tissue was homogenized with 2 vol. of 0.1 M Tris buffer (pH 7.8). This extract was held at 0° for 15 min then 0.2 vol. of 2% protamine sulfate solution was added to the homogenate with stirring. After 10 min the suspension was centrifuged at 20 000 × g and held at 4°. $(NH_4)_2SO_4$ was added with constant stirring to the supernatant solution to reach 35% satn. After stirring for 10 min, the precipitate was removed by centrifugation. $(NH_4)_2SO_4$ was then added to the supernatant to bring it to 55% satn. After stirring 10 min, the precipitate was collected by centrifugation. It was dissolved in a minimum of deionized distilled water; then diluted to 60 mg of protein per ml with cold deionized water. This solution was quickly (less than 2 min) heated in a water bath to 60° and held at 60° for 2 min; then rapidly cooled in ice and centrifuged.

The supernatant solution was brought to 50% satn. with $(NH_4)_2SO_4$. After stirring for 10 min, the suspension was centrifuged and the precipitate was dissolved in the smallest possible amount of deionized water. This procedure resulted in about a 10-fold-purified preparation, which was used for the subsequent studies.

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TABLE I

SPECIFICITY OF ADP–SUGAR PYROPHOSPHATASE

Adenylic acid or Glc-1-*P* were measured as described in the text.

Substrate	*Activity relative to ADP-glucose*	*Chromatographic identification of products*
ADP-glucose	100	+
ADP–mannose	96	+
ADP-ribose	59	+
ADP-ribose nicotinamide (NAD)	3	+
ADP–riboflavin (FAD)	3	+
U,T,C,I and GDP-glucose	0	—

Of the various possible substrates tested the pyrosphophatase is most active toward ADP–glucose and ADP–mannose (Table I). The enzyme is not specific for the terminal sugar component of the nucleotide as mannose, ribose and substituted ribose derivatives such as NAD and FAD are also active. The adenine riboside, on the other hand, is a highly essential configuration for activity since other purine and pyrimidine nucleoside diphosphate sugars are almost inactive. Pyrophosphorylribose 5-phosphate has no activity for this pyrophosphatase.

The initial extracts also contain hydrolytic enzymes such as inorganic pyrophosphatase (EC 3.6.1.1), ATPase (EC 3.6.1.3) and adenylate kinase (EC 2.7.4.3). After fractionation of the ADP–glucose pyrophosphatase as described above, these enzymes are largely removed. Consequently hydrolytic activity towards either PP_i ADP or ATP in the final preparation is not high.

With ADP-glucose as substrate the 10-fold-purified enzyme preparation produced AMP and Glc-1-*P* in equimolar proportions in quantities approximating the amount of substrate added (Table II). These products were also qualitatively identified by the chromatographic procedures. ADP and ATP competitively inhibit the hydrolysis of ADP–glucose. The K_i values for ADP and ATP are $7.5 \cdot 10^{-5}$ M and $1.65 \cdot 10^{-4}$ M, respectively.

The pyrophosphatase requires a divalent cation for activity. Magnesium at 0.4 mM is the most active stimulant.

From LINEWEAVER–BURK[7] plots the K_m values for two of the most active

TABLE II

STOICHIOMETRY OF ADP–SUGAR PYROPHOSPHATASE REACTION

Adenylic acid and Glc-1-*P* were measured on separate aliquots of the incubation mixture by adding the various other reagents in excess. Substrate and product concentrations are given in μmoles/0.5 ml.

Substrate	*Products*	
ADP–glucose	*AMP*	*Glc-1-P*
0.170	0.164	0.167
0.085	0.086	0.085
0.054	0.050	0.052

substrates, ADP–glucose and ADP–ribose have been estimated as $2.9 \cdot 10^{-4}$ M and $1.3 \cdot 10^{-3}$ M, respectively. With ADP–glucose as substrate the pH optimum is between 7.8 and 8.4.

Rat skin, liver and kidney contain particulate or nuclear pyrophosphatases of broad specificity which are activated by magnesium[8]. Substrates which are hydrolyzed by the particulate pyrophosphatase from rat liver microsomes or nuclei are UDP–glucuronic acid, UDP–glucose, UDP–*N*-acetylglucosamine, GDP–mannose, NAD^+, NADH, $NADP^+$ and FAD[9]. Pyrophosphatases with broad specificity and activated by Mg^{2+} have also been extracted from various microbial sources[1,2]. Heating extracts for 5 min at about 58° is often required for full activity of the microbial pyrophosphatases.

The distinguishing property of the pyrophosphatase described herein is a unique specificity for adenosine substituted on one of the phosphate groups and either a glycosidic (glucose, mannose or ribose) or ester (NAD) linkage on the other phosphate group and stimulation by a divalent cation.

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Neuraminidase in human intestinal mucosa

Neuraminidase (*N*-acetyl neuraminate glycohydrolase, EC 3.2.1.18) is present[1,2] in animals, bacteria, myxo viruses and some tumor tissues. Although this enzyme has been detected in human plasma[1] and brain[1] no study has been undertaken with other human tissues. The present investigation was prompted by the reports[3] that while hepatic, renal and placental alkaline phosphatases (orthophosphoric monoester phos-