A PHOSPHATASE SPECIFIC FOR NUCLEOSIDE DIPHOSPHATES*

by

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The enzyme preparation from pork kidney mitochondria which catalyzes the phosphorylation of GDP§ or IDP by P_i—a process linked to the breakdown of succinyl CoA[1]—has recently been reported[2] to contain a myokinase-like activity[3,4,5] which permits transphosphorylations between the following pairs of nucleotides:

(1) $\quad GTP + AMP \rightleftharpoons GDP + ADP$

(2) $\quad ATP + GMP \rightleftharpoons GDP + ADP$

(3) $\quad ITP + AMP \rightleftharpoons IDP + ADP$

This same preparation also possessed high myokinase (adenylate kinase) activity[6] with ADP as substrate. During the course of several unsuccessful experiments to demonstrate myokinase activity with GDP or IDP as substrate, it was found that the added nucleoside diphosphate was quantitatively converted to the corresponding monophosphate derivative. These studies have revealed the presence of a specific phosphatase which hydrolyzes GDP and IDP§§

(4) $\quad GDP \longrightarrow GMP + P_i$

The nucleoside diphosphatase (NDPase) preparation used in this study was obtained from a 0.02 *M* $KHCO_3$ extract of a mitochondrial acetone powder prepared from pork kidney cortex by fractionating first with ammonium sulfate (add 30 g solid ammonium sulfate per 100 ml extract; then add 20 g to supernatant solution) and finally with ethanol (10% to 30% v/v; 0.02 *M* potassium phosphate buffer, pH 5.8; $-3°$ to $-14°$).

The GDP was purified from yeast[1]; and GMP was obtained from pure GDP by enzymic hydrolysis with the specific phosphatase as well as by mild acid hydrolysis. Some samples of the inosine nucleotides were prepared according to the method of KLEINZELLER[7] while other samples were supplied by Sigma Chemical Co. The UMP and UDP were prepared from commercial UTP (Pabst) by partial acid hydrolysis. Each was subjected to final purification by ion-exchange and paper chromatography[8]. Purity was evaluated by spectral characterization, phosphate analysis and paper chromatography.

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§ The following abbreviations will be used: adenosine mono-, di-, and triphosphates, AMP, ADP, and ATP; guanosine mono-, di-, and triphosphates, GMP, GDP, and GTP; uridine mono-, di-, and triphosphates UMP, UDP, and UTP; inosine mono-, di-, and triphosphates, IMP, IDP, and ITP; inorganic phosphate, P_i, coenzyme A, CoA; and tris(hydroxymethyl)aminomethane buffer, THAM.

§§ Dr. J. L. STROMINGER and Dr. L. A. HEPPEL have also found in calf liver a similar enzyme which hydrolyzes UDP and IDP to the monophosphate derivative (personal communication). The enzyme described in this paper hydrolyzes UDP (see Table I) and the preparation of STROMINGER and HEPPEL hydrolyzes GDP.

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The nucleotides GDP, IDP and UDP were incubated separately with enzyme. Aliquots were removed at intervals for quantitative determination of P_i and nucleoside mono- and diphosphates. The nucleotides were resolved by chromatography on paper with *iso*butyric acid-ammonia-water solvent[8] and each ultraviolet-absorbing region isolated and eluted by immersion in *M* phosphate buffer (pH 6.8) for 36 hours at 38°[9]. Nucleotide concentration was determined spectrophotometrically at 260 mμ. For comparison, AMP, ADP, ATP, GMP, IMP, ITP, UMP and UTP were incubated simultaneously under similar conditions. The results are presented in Table I and show the essential stoichiometry of P_i released, the increase in nucleoside monophosphate and the decrease in nucleoside diphosphate. Release of P_i from the other nucleotides was considerably slower. In particular it was noted that the substrate for myokinase, ADP, was not a substrate for this phosphatase.

TABLE I

STOICHIOMETRY OF PHOSPHATASE REACTION

μmoles

Nucleotide added		*Analysis*	*Incubation time (min)*					
			10	*20*	*30*	*40*	*50*	*60*
GDP	7.1	$+\Delta P_i$	2.0	3.6	5.0	5.8	5.6	5.9
		$+\Delta$ GMP	1.8	3.2	3.8	4.4	4.4	4.8
		$-\Delta$ GDP	2.0	3.5	4.4	5.0	5.2	5.2
IDP	7.4	$+\Delta P_i$	2.3	5.1	6.3	6.2	6.9	6.7
		$+\Delta$ IMP	2.9	4.8	6.9	6.5	7.0	7.2
		$-\Delta$ IDP	3.1	5.2	6.6	6.8	6.8	6.6
UDP	3.4	$+\Delta P_i$	1.3	2.1	3.2	3.3	3.4	3.4
		$+\Delta$ UMP	1.2	2.6	2.6	2.7	3.1	2.8
		$-\Delta$ UDP	1.5	2.2	2.2	2.0	2.4	2.4
AMP	8.1	$+\Delta P_i$		0.1		0.4		0.6
ADP	8.0	$+\Delta P_i$		0.3		0.5		0.6
ATP	8.1	$+\Delta P_i$		0.4		0.9		1.1
GMP	1.7	$+\Delta P_i$						0.1
IMP	8.0	$+\Delta P_i$		0.8		0.5		1.1
ITP	7.4	$+\Delta P_i$		0.8		1.0		0.9
UMP	1.0	$+\Delta P_i$						0.1
UTP	1.8	$+\Delta P_i$		0.4		0.4		0.5

The reaction mixture consisting of 30 μmoles THAM buffer (pH 7.0), 10 μmoles $MgCl_2$, nucleotide and 0.48 mg enzyme in 1.0 ml was incubated at 38°. The initial aliquots were taken at zero time immediately subsequent to enzyme addition at 0°.

Several characteristics of the enzyme were revealed in separate experiments. At a concentration level of 3.3 μM/ml with IDP, GDP, UDP and ADP, the initial reaction velocities at 38° were, respectively, 36.5, 27.8, 24.0 and 0.7 μM P_i/h/mg enzyme (Fig. 1). Initial reaction velocities were linear with respect to enzyme concentration in the range examined (0.24 to 2.4 mg enzyme per ml). GDP incubated in a system with 10^{-2} *M* versene and with no added Mg^{++} was not hydrolyzed after one hour. Heating equivalent aliquots of the enzyme at an enzyme concentration of 2.4 mg/ml in THAM buffer (pH 7.0) at 40°, 50° and 60° for two minutes resulted in 7%, 31% and 96% loss in

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enzyme activity, respectively. Myokinase activity, with ADP as substrate, survived the 60° heat treatment, but no activity toward GDP or IDP could be demonstrated with this phosphatase-free preparation. The possibility remains that a myokinase for GDP or IDP would not remain stable under these conditions. The nucleotide product of enzymic hydrolysis of GDP was isolated in quantity and was identified with guanosine-5'-monophosphate*.

A possible function of NDPase may be that of shifting the equilibrium in synthetic reactions where nucleoside diphosphates are formed as products. One such example is the synthesis of succinyl CoA[1]:

(5) GTP + Succinate + CoA $\rightleftharpoons$ GDP + P_i + Succinyl CoA

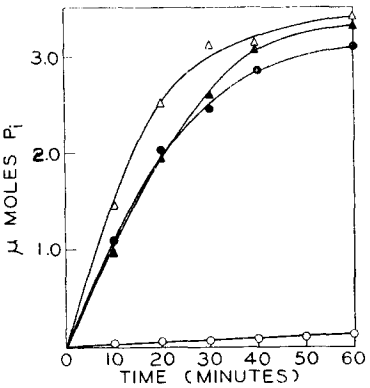

Fig. 1. Relative reaction rates of IDP (△), GDP (●), UDP (▲) and ADP (○). The reaction mixture consisting of 30 μmoles THAM buffer (pH 7.0), 10 μmoles $MgCl_2$, 0.24 mg enzyme and nucleotide in 1.0 ml was incubated at 38°. The μmoles of nucleotides added were: IDP, 3.34; GDP, 3.30; UDP, 3.35 and ADP, 3.20.

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SUMMARY

A new enzyme, nucleoside diphosphatase, which specifically hydrolyzes GDP, IDP and UDP, but not ADP, to the corresponding nucleoside monophosphates and inorganic phosphate has been identified in a preparation from pork kidney cortex. The enzyme is heat labile and requires the addition of Mg^{++} for activity.

RÉSUMÉ

Un nouvel enzyme, une nucléoside diphosphatase, qui hydrolyse spécifiquement le GDP, l'IDP et l'UDP, mais non l'ADP, en nucléoside monophosphate correspondant et phosphate minéral, a été identifiée dans une préparation de cortex reinal de porc. L'enzyme est thermolabile et n'est actif qu'en présence de Mg^{++}.

ZUSAMMENFASSUNG

Ein neues Enzym, Nucleosiddiphosphatase, das spezifisch GDP, IDP und UDP, aber nicht ADP, zum entsprechenden Monophosphat und anorganischen Phosphat spaltet, wurde in einem Präparat aus Schweinsnierenrinde festgestellt. Dieses Enzym ist hitzenbeständig und bedarf des Zusatzes von Mg^{++} Ionen für seine Wirksamkeit.

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