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The non-specific nature of the nucleoside diphosphatase of rat liver microsomes

Recent work in this laboratory[1,2] has established that isolated rat liver nucleoli contain at least two nucleoside diphosphatases, an inorganic pyrophosphatase and glucose-6-phosphatase, which in several properties are like the analogous enzymes of microsomes[3–5]. Previous workers have detailed some of the properties of the microsomal nucleoside di- and triphosphatases[3] and the partial purification of the microsomal enzyme specific for IDP, GDP and UDP[6]. However, in comparing the phosphatases of nucleoli and microsomes, we have found the latter can dephosphorylate dGDP and dADP, an activity not previously reported.

Microsomes from livers of mature rats of the Sprague–Dawley strain were isolated from 10% homogenates (w/v) prepared in a medium containing 0.25 M sucrose–3 mM $MgCl_2$–5 mM triethanolamine (pH 7.0). The initial low-speed sediment (1000 × *g* for 15 min) was washed once in additional medium; the combined supernatants were then subjected to successive centrifugations at 2°: once for 20 min at 10 000 × *g* and twice for 20 min at 30 000 × *g*. Clean tubes were used for each centrifugation. The microsomes were isolated by centrifugation for 60 min at 105 000 × *g* and they were washed once in additional medium. The preparation was suspended in 0.25 M sucrose; 5 mM triethanolamine (pH 7.8) for use. Formation of P_i was determined by a modified Martin and Doty procedure[7]. Protein concentrations were determined by the biuret procedure[8].

Microsomes can dephosphorylate dGDP and, to a lesser extent, dADP, but no activity is demonstrable with dCDP and dTDP. For this reason we have studied microsomal activity by comparing GDP and dGDP as substrates. Microsomal preparations show a relative constancy in the respective specific activities supported by these two substrates; that supported by GDP ranges from 1.1 to 1.4 times that for dGDP. Variation of the pH of incubation has an identical effect upon P_i formation by microsomes with either substrate. The curves obtained with each substrate are superimposable upon that of NOVIKOFF AND HEUS[6] for the purified nucleoside diphosphatase which acts upon IDP, GDP and UDP. Other comparisons establish that microsomal activity with either substrate is affected identically by various conditions such as preincubation, sonication and/or the addition of Triton X-100 to the incubation medium (Table I). Lastly, incubations conducted with mixtures of GDP and dGDP indicate that there is no additivity in activity in the presence of both substrates (Table I); levels of such substrate mixtures higher than that of Table I give less activity than that obtained with either substrate alone, suggesting that substrate inhibition is occurring under such conditions. Reciprocal plots of reaction velocity *versus* substrate concentration yield the following constants for GDP and dGDP, respectively: v_{max} 1162 and 970 mμmoles/min per mg protein; K_m 1.63 and 1.65 mM. Assays of 5′-nucleotidase with both GMP and dGMP are negative and long-term incubations to reaction completion indicate that there is release of 1 mole of P_i per mole of either GDP or dGDP. Such results preclude any contribution to the measured activities by a nucleoside diphosphokinase or 5′-nucleotidase.

These results indicate the fundamental similarities in the properties of micro-

TABLE I

EFFECTS OF VARIOUS CONDITIONS ON THE HYDROLYSIS OF NUCLEOSIDE DIPHOSPHATES BY MICROSOMES

Conditions: Expt. 1: 0.28 mg protein; 30 μmoles DL-histidine (pH 7.4); 3 μmoles $MgCl_2$; 2.6 and 3.9 μmoles of ADP and dADP, respectively; incubated 10 min at 30°; 1.6 ml final volume. Expt. 2: 0.28 mg protein; 30 μmoles DL-histidine (pH 7.4); 3 μmoles $MgCl_2$; 2.5 and 3.9 μmoles of GDP and dGDP, respectively; incubated 5 min at 37°; 1.6 ml final volume. Expt. 3: 0.49 mg protein; 25 μmoles DL-histidine (pH 7.2); 3 μmoles $MgCl_2$; 2.6 and 4.0 μmoles of GDP and dGDP, respectively; incubated 17 min at 37°; 1.3 ml final volume.

Expt. No.	*Substrate*	*Condition*	*mμmoles ΔP_i/min per mg protein*	
			—	*+0.75 mg Triton X-100*
1	ADP	—	14.2	7.8
	dADP	—	42.3	47.3
2	GDP	—	254	709
	dGDP	—	187	499
	GDP	Sonication*	464	626
	dGDP	Sonication*	340	445
3	GDP	—	94.5	304
	GDP	Preincubation** at 30°	101.3	
	GDP	Preincubation** at 60°	1.6	
	dGDP	—	64.3	197
	dGDP	Preincubation at 30°	69.1	
	dGDP	Preincubation at 60°	0	
	GDP + dGDP	—	72.5	

* Sonicated for five 10-sec intervals at 0° at peak output of a Bronson sonic oscillator.
** Incubation of microsomes for 30 min at pH 7.4 in absence of substrate.

somal GDPase and dGDPase. We conclude from them that the microsomal enzyme which hydrolyzes IDP, GDP and UDP is even less specific than thought heretofore and is also capable of hydrolyzing dGDP. Inasmuch as the cytoplasmic concentrations of 2-deoxyribonucleotides are quite small[9], it must be concluded that there is little functional significance in this property of microsomes. It represents an additional instance of the broad specificity which is an apparent characteristic of many phosphatases[10,11].

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Department of Biochemistry,
School of Medicine,
University of North Carolina,
Chapel Hill, N. C. (U.S.A.)

R. PENNIALL
J. P. HOLBROOK

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Demonstration of two forms of human pancreatic carboxypeptidase A

Carboxypeptidase A (EC 3.4.2.1), an exopeptidase from the pancreas, and different forms of its zymogen, procarboxypeptidase A, have been extensively studied in bovine and porcine pancreas[1–15]. Four forms of the bovine enzyme[10–12], carboxypeptidases A_α, A_β, A_γ and A_δ, and three forms of the porcine enzyme[7], carboxypeptidases A_1, A_2 and A_3, have been described, with differences occurring in their N-terminal sequence. Furthermore, allelomorphs of bovine carboxypeptidase A_α have been shown to contain either leucine or valine in their terminal hexapeptide[14,15]; either or both forms of the enzyme may be present in any one animal and the differences are believed to be the result of a generic trait. The presence of procarboxypeptidase A has also been demonstrated in human pancreatic juice[16], and in human duodenal juice, carboxypeptidase A activity has been determined as a measure of exocrine pancreatic function[17–20].

In the present investigation, it is demonstrated that carboxypeptidase A from human duodenal juice and homogenates of activated human pancreatic tissue also occurs in two forms with different electrophoretic mobility. Evidence is also presented which suggests that during the activation of procarboxypeptidase A by trypsin (EC 3.4.4.4), the slower migrating form of the enzyme appears first and is converted into the faster migrating form in the presence of endogenous endopeptidases.

Human duodenal juice was collected from healthy adults using a three-lumened duodenal tube[20] in order to avoid contamination of duodenal fluid with gastric contents. Carboxypeptidase A was measured by its activity towards *N*-carbo-β-naphthoxy-DL-phenylalanine[21]. Homogenates of human pancreatic tissue (7.5%, w/v) were prepared in 0.15 M NaCl containing 0.1% Triton X-100 (ref. 22). Activation of the zymogens was carried out at 37° by the addition of bovine crystalline trypsin. One ml of activation mixture contained 230 μg trypsin (Sigma), 2 μM $CaCl_2$, 125 μM NaCl, 0.9 mg Triton X-100, 4.5 mg protein[23] and 5 μM Tris buffer (pH 8.0). Trypsin and chymotrypsin (EC 3.4.4.5) activities in duodenal juice and in homogenates of pancreatic tissue were measured by the procedure of LUNDH[24]. Electrophoresis was carried out on cellulose polyacetate membranes (Sepraphore III, Gelman). The conditions of electrophoresis are described in the legend to Fig. 1. After electrophoresis,