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MAMMALIAN CARBAMYL PHOSPHATE : GLUCOSE PHOSPHOTRANSFERASE AND GLUCOSE-6-PHOSPHATE PHOSPHOHYDROLASE: EXTENDED TISSUE DISTRIBUTION

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Summary

Carbamyl phosphate : glucose phosphotransferase and glucose-6-phosphate (Glc-6-*P*) phosphohydrolase activities have been demonstrated in pancreas, adrenals, brain, testes, spleen, and lung. Catalysis of these activities by classical multifunctional glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase; EC 3.1.3.9) has been firmly established for the first four of these tissues on the basis of characteristic catalytic properties of the transferase, pH—activity profiles, apparent K_m values for carbamyl phosphate and glucose, substrate specificity, susceptibility to inhibition by molybdate, and activation by deoxycholate. Additional such activity due to non-specific acid (and alkaline) phosphatase action also is indicated at very high glucose concentrations. The possible physiological significance of the newly-elucidated presence of glucose-6-phosphatase-phosphotransferase in these various tissues, in addition to previously extensively studied liver, kidney, and mucosa of small intestine, is discussed briefly.

Introduction

A variety of tissues (in addition to well-characterized liver, kidney, and small intestine) have been reported, at one time or another, to catalyze the hydrolysis of the important metabolite glucose-6-phosphate (Glc-6-*P*) (see Tables IV and V of ref. 1). However, in general it has not been established as clearly as desirable whether such hydrolyses involve specifically the enzyme D-Glc-6-*P* phosphohydrolase ("glucose-6-phosphatase", EC 3.1.3.9), or are ef-

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonate.

fected simply by non-specific acid (or alkaline) phosphatase(s) acting on the sugar phosphate ester at acid pH (see Nordlie, ref. 1).

Glucose-6-phosphatase of liver [1—8], kidney [1,2,9], and mucosa of small intestine [1,2,10] is now established to possess potent synthetic (e.g. carbamyl phosphate : glucose phosphotransferase, Eqn 1) as well as hydrolytic activity.

$$\text{Carbamyl-}P + \text{glucose} \rightarrow \text{glucose-6-}P + NH_3 + CO_2 \quad (1)$$

In this paper, utilization is made of this plurality of functions of this enzyme, and of distinguishing features characteristic of such phosphotransferase activity, to document the presence of relatively small but reproducible amounts of classical glucose-6-phosphatase-phosphotransferase in a number of mammalian tissues in addition to those previously characterized. Some physiological implications of the presence of this complex enzyme system in certain such tissues is discussed briefly.

Materials and Methods

Sources of substrates, buffers, and other chemicals were as previously described [3,5,6].

Young adult albino rats (approx. 250 g) purchased from Sprague—Dawley Inc., Madison, WI, served as source of testicles, brain, lung, and spleen. Pancreas of newborn calf and adult bovine adrenals were obtained frozen in solid CO_2 from Pel-Freez Biologicals, Inc., Rogers, AR, and were stored frozen at −20° C.

Homogenates of all tissues, in 0.25 M sucrose, were prepared just prior to use. Frozen tissues were cut up into small pieces and mascerated in a Waring blendor, operating at maximum speed, for 30-s intervals with intermitent 1-min periods of cooling in ice, until homogeneous. Small pieces of fresh tissues were ground in a Potter—Elvehjem homogenizer operating at 60 rev./min for 2 min at 0° C. All preparations were filtered through two layers of cheesecloth, and the filtrates rehomogenized at 0° C with 10 strokes of the Potter—Elvehjem homogenizer. Such preparations were diluted with additional ice-cold 0.25 M sucrose to 2 ml/g wet tissue (lung), 3 ml/g wet tissue (spleen, brain, adrenals), or 4 ml/g wet tissue (pancreas and testes). Except in those experiments as described in Fig. 3A where deoxycholate concentration was the experimental variable, nine parts of these homogenates were supplemented with one part of neutral 2% (w/v) sodium deoxycholate solution just prior to assay [11]. All operations were at 0° C. Aliquots, 0.1 ml, of such supplemented homogenates routinely were utilized per 1.5-ml assay mixture.

Protein concentrations and Glc-6-*P* phosphohydrolase activity were assayed as in previous studies [11]. Carbamyl-*P* : glucose phosphotransferase activity also was determined as in earlier work [5], except that Glc-6-*P* produced was measured by the Lowry cycling method [12]. This amplifying technique permitted accurate measurement of the relatively small amounts (in comparison with liver or kidney (see refs 1 and 2)) of phosphotransferase activity present in the tissues considered. Assay mixture composition and other details are given in Results and the legends to figures and tables.

Results

Activity levels in various tissues

Levels of carbamyl-*P* : glucose phosphotransferase and Glc-6-*P* phosphohydrolase activities of six tissues derived from rat or ox were measured at pH 5.5 in the presence of relatively high levels of substrates. Results obtained, presented in Table I, indicate significant levels of both types of activity, characteristic of multifunctional glucose-6-phosphatase-phosphotransferase [1,2,5,13, 14] in adrenals, pancreas, testes, brain, spleen, and lung. Phosphotransferase activity levels ranged from 36% of that observed for hydrolase (calf pancreas) down to 6% (rat spleen). With this last tissue, carbamyl-*P* : glucose phosphotransferase was comparatively high considered with respect to the other tissues, but the ratio value was low since Glc-6-*P* phosphohydrolase was even comparatively higher. Under the conditions specified in Table I, phosphotransferase activity of rat liver and kidney is, typically, 400 and 425 units/20 min per g wet tissue [1,2], respectively.

In supplementary studies, phosphotransferase activity could not be detected in either skeletal muscle or cardiac muscle minces which did, however, manifest small amounts of Glc-6-*P* phosphohydrolase activity.

The enzymic nature of Glc-6-*P* formation via phosphotransferase action was established in supplemental studies in which it was found that no Glc-6-*P* was formed when (a) enzyme was omitted from otherwise complete assay mixtures, or (b) boiled homogenates replaced the usual enzyme preparations. The linearity of Glc-6-*P* formation with duration of incubation and as a function of varied homogenate protein concentration likewise was demonstrated in preliminary studies with the various preparations.

Some catalytic characteristics of carbamyl-P : glucose phosphotransferase

Attempts to sub-fractionate tissue homogenates were either precluded

TABLE I

CARBAMYL-*P*: GLUCOSE PHOSPHOTRANSFERASE AND Glc-6-*P* PHOSPHOHYDROLASE ACTIVITY LEVELS IN VARIOUS TISSUES

Assay mixtures, pH 5.5, contained, in 1.5 ml, 20 mM acetate buffer plus 20 mM cacodylate buffer, 10 mM Glc-6-*P* (phosphohydrolase) or 10 mM carbamyl-*P* plus 180 mM glucose (phosphotransferase), and tissue homogenates as indicated in the text. Enzymic activity is expressed as μmoles Glc-6-*P* formed (phosphotransferase) or hydrolyzed (phosphohydrolase) per g wet tissue per 20 min.

Tissue	Enzymic activity		Transferase activity / Hydrolase activity
	Carbamyl-*P*: glucose phospho-transferase	Glc-6-*P* phospho-hydrolase	
Calf pancreas	0.50	1.39	0.36
Beef adrenals	0.62	3.64	0.17
Rat testes	0.53	5.16	0.10
Rat brain	0.19	1.82	0.10
Rat lung	0.57	5.87	0.10
Rat spleen	1.88	33.6	0.06

TABLE II

COMPILATION OF CATALYTIC CHARACTERISTICS OF CARBAMYL-*P*:GLUCOSE PHOSPHOTRANSFERASE ACTIVITY OF VARIOUS TISSUES

Data are compiled from Figs 1—3, and comparable studies with the various, indicated tissues. Experimental conditions and other details are as described in the legends to Figs 1—3. Carbamyl-*P* concentration was 10 mM when kinetics with respect to glucose were studied; glucose concentration was maintained at 90 mM when kinetics were considered with respect to carbamyl-*P*. The pH was 5.5 in all instances, except where pH optima were determined.

Catalytic characteristic	Source of enzyme			
	Ox		Rat	
	Pancreas	Adrenals	Testes	Brain
pH optimum with 60 mM glucose present	5.6	5.7	5.5	5.6
$K'_{m,Glc}$ (mM)	60.0	40.0	50.0	65.0
$K''_{m,Glc}$ (M)	1.0	1.0	1.3	1.0
$K_{m,carbamyl\text{-}P}$ (mM)	0.8	0.6	1.3	2.5
Concn of sodium molybdate producing 50% inhibition (M)	$7 \cdot 10^{-5}$	$1 \cdot 10^{-3}$	$3 \cdot 10^{-5}$	$9 \cdot 10^{-5}$
Concn of deoxycholate producing maximal activation (%, w/v)	0.2	0.25	0.25	0.15

(frozen preparations) or largely unsuccessful due to the relatively small amounts of tissues and comparatively low activity levels involved, and problems generally inherent in fractionating such difficult non-hepatic tissues as lipid-rich brain and endocrines. Accordingly, it was decided to gain further insight regarding the nature of carbamyl-*P* : glucose phosphotransferase activity, whether it was due to multifunctional glucose-6-phosphatase-phosphotransferase or possibly to the action of non-specific acid or alkaline phosphatases which possess weak phosphotransferase activity in certain cases [15—18], by carrying out the following studies (Figs 1—3; Table II) with tissue homogenates. Typical results obtained are presented in the figures. Results of each of a variety of studies are illustrated with data from a single tissue, for brevity. Identical studies were in each case performed with homogenates of pancreas, adrenals, testes, and brain; strikingly similar patterns of results were obtained with all. Experimental findings with the four tissues are summarized in Table II.

Effects of pH on activity with high and low glucose concentration. In the presence of 180 mM glucose and 10 mM carbamyl-*P*, a relatively sharp, reproducible activity peak was noted at pH 5.4 with testicular (and other) homogenates (Fig. 1). Upward inflections additionally were noted at extremes of pH considered, pH 5 to 4 and pH 8 to 8.5. When the glucose level was lowered to 60 mM, these inflections at alkaline and quite acid pH were not apparent, and a pH—activity profile quite closely resembling that previously noted with carbamyl-*P* : glucose phosphotransferase activity of liver microsomal [1,5,19,20] or nuclear membrane [2,21—23] glucose-6-phosphatase-phosphotransferase, with a maximum at pH 5.5, was observed.

Effects of substrate concentration. The kinetics of the carbamyl-*P* : glucose phosphotransferase reaction were considered experimentally at pH 5.5 (where activity was maximal; see Fig. 1) with regard to both carbamyl-*P* and glucose. $K'_{m,\ carbamyl\text{-}P}$ values were determined with the various tissue pre-

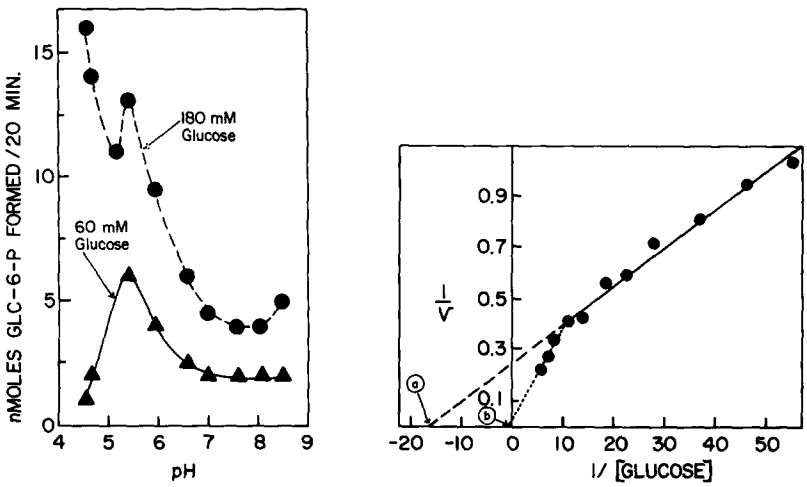

Fig. 1. Effects of assay pH on carbamyl-*P* : glucose phosphotransferase activity of rat testicular homogenate. Reaction mixtures contained, in 1.5 ml, 10 mM carbamyl-*P*, 60 or 180 mM glucose as indicated, 1.6 mg testicular homogenate protein, and buffer. Buffers were as follows: 40 mM acetate buffer at pH 4, 4.5, and 5; 20 mM acetate buffer plus 20 mM cacodylate buffer at pH 5.5; 40 mM cacodylate buffer at pH 6 and 6.5; 20 mM cacodylate buffer plus 20 mM HEPES at pH 7; 40 mM HEPES at pH 7.5. Incubations were for 20 min. The pH measurements were made with a Beckman research model meter with assay mixtures duplicating those utilized for measurement of enzymic activity.

Fig. 2. Effects of varied glucose concentration on carbamyl-*P* : glucose phosphotransferase activity of calf pancreatic homogenate. Assay mixtures, pH 5.5, contained, in 1.5 ml, 20 mM acetate buffer plus 20 mM cacodylate buffer, 10 mM carbamyl-*P*, indicated varied concentrations of glucose (from 18 to 180 mM), and 1.7 mg pancreatic homogenate protein. Velocity, v, is expressed as nmoles of Glc-6-*P* formed during the 20-min incubation. Indicated by a and b, respectively, are $-1/K'_{m,Glc}$ and $-1/K''_{m,Glc}$. Numerical values for $K'_{m,Glc}$ and $K''_{m,Glc}$ thus determined are given in Table II.

parations in studies in which glucose was held constant at 90 mM and v determined as a function of carbamyl-*P* concentration which was varied between 1 and 10 mM. Assay mixtures, pH 5.5, contained 20 mM acetate buffer and 20 mM cacodylate buffer. Linear double-reciprocal plots [24] were obtained in all instances; $K'_{m,\ carbamyl\text{-}P}$ values, calculated as negative reciprocals of x-axis intercepts of extrapolations of such plots, ranged from 0.6 to 2.5 mM; values for the enzyme of the various tissues are given in Table II.

Typical results obtained in studies in which carbamyl-*P* level was maintained at 10 mM and v determined as a function of varied concentrations of glucose (18—180 mM) are presented in Fig. 2. Double-reciprocal plots [24] obtained from such studies with calf pancreas (Fig. 2), and with preparations from adrenals, testes, and brain as well, consist of segments of two straight lines, rather than one, suggestive of the involvement of two glucose-phosphorylating enzymic activities with widely differing apparent Michaelis constant values for glucose (see ref. 25, for example). Such apparent $K_{m,Glc}$ values, determined as negative reciprocals of x-axis intercepts of extrapolations of the two linear double-reciprocal plots [26], are approximately 60 mM (see a in Fig. 2) and 1000 mM (see b in Fig. 2). Similar biphasic double-reciprocal plots also were obtained in studies with adrenals, testes, and brain; the two apparent $K_{m,Glc}$ values in each instance were similar to those obtained with pancreatic preparation (see Table II). The smaller of these two values (recorded as $K'_{m,Glc}$

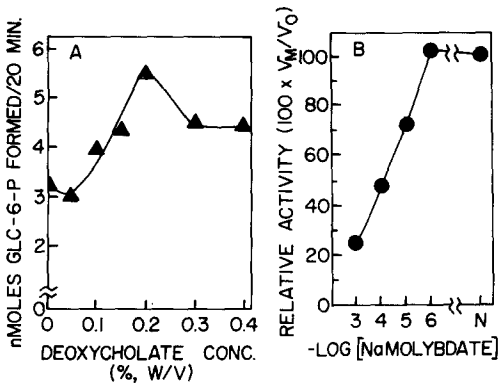

Fig. 3. Effects of supplemental deoxycholate on calf pancreatic carbamyl-*P* : glucose phosphotransferase (A), and of sodium molybdate on this same activity of rat brain homogenate (B). Assay mixtures, pH 5.5, contained, in 1.5 ml, 20 mM acetate plus 20 mM cacodylate buffer, 10 mM carbamyl-*P*, and 90 mM glucose plus 1.7 mg pancreatic homogenate protein (A) or 60 mM glucose plus 2.4 mg brain homogenate protein (B). Indicated levels of sodium molybdate were included in assay mixtures (B), while pancreatic homogenates were supplemented with sodium deoxycholate to the indicated final concentrations (w/v) prior to assay. All incubations were for 20 min. In B, v_M and v_O are reaction velocities observed in the presence and absence of molybdate, respectively.

in Table II) correlates well with the apparent K_m for glucose determined with phosphotransferase activities of glucose-6-phosphatase-phosphotransferase of liver and kidney [1,2]. K_m values of about 1 M or larger have been noted for glucose or other phosphoryl acceptor in transphosphorylation reactions which are catalyzed by certain non-specific acid and alkaline phosphatases from mammalian and microbial sources [15—18]. The present results (see $K''_{m,Glc}$ values in Table II) suggest that such activity may also be manifest in the presence of very high glucose levels with the tissues considered here.

Other characteristics. Activation by certain detergents including deoxycholate [1,2,9,10,22,27—29] and sensitivity to inhibition by molybdate [1—3,9,10,30,31] previously have been shown to be characteristic of phosphotransferase activities associated with well-characterized microsomal glucose-6-phosphatase of liver, kidney, and small intestine. Similar effects were noted with carbamyl-*P* : glucose phosphotransferase of pancreas, adrenals, testes, and brain. Representative illustrative results are presented in Figs 3A and 3B; results obtained with all tissues are summarized in Table II.

With all tissues, transferase activities also were observed with several phosphate compounds which, in addition to carbamyl-*P*, previously have been noted to function as substrates with hepatic, renal, and intestinal glucose-6-phosphatase-phosphotransferase [1—4,6—11,19]. For example, with pancreatic preparations at pH 5.5 with 10 mM phosphate substrate and 60 mM D-glucose activity noted relative to that with carbamyl-*P* was as follows: carbamyl-*P*, 1.00; PP_i, 0.34; phosphoenolpyruvate, 0.26; and CTP, 0.20.

Discussion

In the above studies, use is made of its comparatively low $K_{m,Glc}$ to study discriminately carbamyl-*P* : glucose phosphotransferase activity charac-

teristic of multifunctional glucose-6-phosphatase in the possible presence of other hydrolase and transferase activities. While non-specific acid and alkaline phosphatases from certain mammalian and bacterial sources have been found to catalyze transphosphorylation, such activity in general constitutes but a fraction of a per cent of these enzymes' hydrolytic capacity, and then only at molar or multimolar levels of phosphoryl acceptor [15—18]. K_m values for phosphoryl acceptors other than water routinely have been in the molar or near-molar range [15—18], in contrast with values of less than 90 mM with phosphotransferase activities of glucose-6-phosphatase [1,2,5,13,14,20].

On the basis of general correspondence of a variety of properties of carbamyl-*P* : glucose phosphotransferase of pancreas, adrenals, testes, and brain, apparent $K_{m,Glc}$ values determined with the hexose in the range 18—100 mM, pH—activity profiles with 60 mM glucose present, phosphoryl donor specificity, activation by deoxycholate and inhibition by molybdate, with those of this activity of previously well-characterized liver and kidney microsomal (and nuclear [22,23]) preparations, it is concluded that classical glucose-6-phosphatase-phosphotransferase (EC 3.1.3.9) is present in all*.

In addition, the present studies indicate the presence in these tissues of other enzyme(s) capable of catalyzing carbamyl-*P* : glucose phosphotransferase, but only at very high glucose levels.

These same conclusions also appear valid for spleen (supplementary studies), although data were not as clear cut with this tissue and hence are omitted from Table II. Due to unresolved experimental problems (irregular curvilinearity of double-reciprocal plots), it remains unclear whether or not carbamyl-*P* : glucose phosphotransferase activity of lung (Table I) is due to glucose-6-phosphatase.

Previous studies by Täljedal [32] and by Lazarus and Barden [33] serve to support the presence of specific Glc-6-*P* phosphohydrolase in pancreatic β-cells. The preliminary observation by Scott and Jones [34] of the occurrence of PP_i-glucose phosphotransferase activity in such cells further bears out this contention. The possibility that hydrolysis of Glc-6-*P* in brain [35] and tissues of the reticuloendothelial system, including spleen and lung [36], is catalyzed by glucose-6-phosphatase similar to the liver and kidney enzyme has been suggested on the basis of histochemical studies. A very recent preliminary report [37] suggests that a phosphoprotein of brain involved in sleep may be glucose-6-phosphatase.

The present manuscript includes the initial description** of the presence of carbamyl-*P* : glucose phosphotransferase characteristic of glucose-6-phosphatase in a variety of tissues in addition to liver, kidney, and intestine. Data presented here effectively support previous workers' conclusions regarding the presence of the enzyme in β-cells of pancreas, and appear to the authors to

* In studies of the sort described here, the possibility of course exists that the enzyme may not be located in the primary cell type of the tissues examined, but rather might conceivably be present in, for example, blood capillaries. While this possibility cannot be rejected a priori, the fact that transferase activities are organ and tissue specific and cannot, for example, be detected in cardiac or skeletal muscle preparations argues against this possibility.

** Preliminary reports of some of these observations have appeared in abstract form [38,39].

constitute the most definitive evidence yet obtained in support of the occurrence of specific, multifunctional glucose-6-phosphatase-phosphotransferase in the variety of other tissues considered.

Physiological significance

As with the enzyme of mucosal cells of small intestine [1,2,10], a role other than simply the hydrolysis of Glc-6-*P* as the terminal step in gluconeogenesis and glycogenolysis appears likely for this multifunctional enzyme in pancreas, testes, adrenals, and brain.

The possibility of a role for the enzyme in insulin-insensitive glucose transport is suggested on the basis of its multiplicity of functions, membraneous nature, strategic location, unique catalytic properties, similarities (see ref. 2) to the bacterial "phosphoenolpyruvate phosphotransferase" system of Roseman (see ref. 40), and patterns of response to diabetes and fasting as characterized for the hepatic enzyme [4,13,29,41]. Such a role for the enzyme in pancreatic β-cells already has been suggested ([32,34]; see also refs 1 and 2). A maintained, constant (or accelerated) rate of glucose uptake by the brain (for energy metabolism), adrenals and testes (for purpose of NADPH generation, via hexose monophosphate shunt activity, requisite to continuing steroidogenesis) also is essential.

It thus appears to us, finally, that our demonstration of the presence of the multifunctional catalyst in a variety of unique, new locations (tissues, this report, and mebranous structures, refs 21—23) must alter traditional perspectives regarding the physiological functions of this complex, multifunctional enzyme. Studies presently are underway in this laboratory directed at elucidation of such additional functions.

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