Phosphohydrolase and Phosphotransferase Activities of Intestinal Glucose 6-Phosphatase*

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ABSTRACT: Rabbit intestinal microsomal glucose 6-phosphatase has been shown to possess inorganic pyrophosphatase- and inorganic pyrophosphate-(and nucleoside 5′-di- and triphosphate-) glucose phosphotransferase activities. All these activities responded in a parallel fashion in studies of: (a) subcellular location, (b) distribution along the length of the small intestine, and (c) stability to partial thermal inactivation. Activation energies for all were quite similar. Inhibitions by molybdate and by citrate, which previously has been shown to inhibit activities of the rat liver enzyme in a highly pH-dependent manner, were studied at pH 6.0. K_i values for each of these compounds, determined with the various activities, agreed closely. K_m values for pyrophosphate in both phosphohydrolase and phosphotransferase reactions were nearly identical, as were K_m values for glucose in reactions in which either pyrophosphate or nucleotide served as phosphoryl donor. K_m values for the various substrates also were determined with microsomal preparations from rabbit liver and kidney and were found to correspond well with their counterparts evaluated for the intestinal enzyme.

The common genetic identity of glucose 6-phosphatases from these various tissues is supported by these observations, as well as by a variety of other similarities noted in catalytic properties of the rabbit intestinal enzyme and those previously described for liver and kidney activities of rat. The presence in rat intestine of an inhibitor of glucose 6-phosphate phosphohydrolase has been confirmed, and inhibition of phosphotransferase activity by this same, heat-labile factor also has been observed.

Classical liver and kidney microsomal glucose 6-phosphatases (D-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9; reaction 1) have been shown to catalyze inorganic pyrophosphate hydrolysis (reaction 2) and the synthesis of glucose 6-phosphate by transfer of a phosphoryl group from pyrophosphate to glucose (reaction 3) (Nordlie and Arion, 1964; Stetten and Taft, 1964; Nordlie and Soodsma, 1966). A variety of nucleoside di- and triphosphates, from which CDP[1] was selected for the present studies, also serve as phosphoryl donors for glucose 6-phosphate formation catalyzed by by this enzyme (reaction 4) (Nordlie and Arion, 1965; Nordlie and Soodsma, 1966).

$$\text{glucose-6-P} + H_2O \longrightarrow \text{glucose} + P_i \quad (1)$$

$$PP_i + H_2O \longrightarrow 2P_i \quad (2)$$

$$PP_i + \text{glucose} \longrightarrow \text{glucose-6-P} + P_i \quad (3)$$

$$\text{CDP} + \text{glucose} \longrightarrow \text{glucose-6-P} + \text{CMP} \quad (4)$$

Since properties of the various activities of liver and kidney glucose 6-phosphatases are very similar (Nordlie and Soodsma, 1966), and the common genetic identity of this enzyme in liver and kidney (Freedland, 1962), as well as intestine (Lea and Walker, 1964; Öckerman, 1964), has been suggested, intestinal mucosal preparations were studied to see whether such similarities of catalytic properties also exist with respect to glucose 6-phosphatase present in this latter tissue. Our original conclusions regarding the multifunctional nature of this enzyme are confirmed and extended by the results of these investigations, described in this paper, which indicate the catalysis of reactions 1–4 by glucose 6-phosphatase of rabbit intestinal mucosa. Catalytic properties of the various activities of the intestinal enzyme are described and compared with those of their counterparts in liver and kidney of rat, and in certain cases, rabbit.

Materials and Methods

Enzymic assays and sources of reagents were as described previously (Nordlie and Arion, 1964, 1966). Nucleotides were obtained from Calbiochem and assayed spectrophotometrically.[2] Reaction mixture compositions and further experimental details are given in the legends to tables and figures and in the text. It was

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[1] Abbreviations used are listed in *Biochemistry 5*, 1445 (1966).

[2] Pabst Circular OR-17, 1961, pp 5–30.

TABLE I: Distribution of Phosphohydrolase and Phosphotransferase Activities along Rabbit Intestine.[a]

Segment	Enzymic Activity[b]			
	Glucose-6-P Phosphohydrolase	PP_i Phosphohydrolase	PP_i–Glucose Phosphotransferase	CDP–Glucose Phosphotransferase
1	6.8 ± 0.6[c]	5.8 ± 0.3 (0.85)[d]	2.2 ± 0.2 (0.32)	0.68 ± 0.08 (0.10)
2	7.0 ± 0.8	6.1 ± 0.4 (0.87)	2.3 ± 0.3 (0.33)	0.73 ± 0.07 (0.10)
3	5.7 ± 0.7	5.5 ± 0.6 (0.96)	1.9 ± 0.3 (0.33)	0.60 ± 0.08 (0.11)
4	5.5 ± 0.4	5.3 ± 0.5 (0.96)	1.9 ± 0.2 (0.35)	0.60 ± 0.04 (0.11)

[a] Microsomal preparations from successive intestinal segments approximately 25 cm in length were assayed for the indicated activities with the reaction mixtures described in the legend to Figure 1. Segments are numbered as described in Results. Average values from studies with five animals are presented. [b] Enzymic activity is expressed in terms of 100 × μmoles of substrate hydrolyzed (phosphohydrolase) or 100 × μmoles of glucose-6-P formed (phosphotransferase) per min per mg of protein. [c] Mean activity values plus and minus standard errors. [d] Values in parentheses indicate the ratios of designated activity/glucose-6-P phosphohydrolase activity.

demonstrated in supplementary experiments that all activities measured were linear with respect to incubation time and protein concentration.

Since several separate enzyme preparations employed in the various studies described differed slightly in activity, all data presented in this paper have been normalized in terms of the inclusion of 0.0085 unit[3] of PP_i–glucose phosphotransferase activity/1.5-ml reaction mixture. Activity values recorded in all figures and tables thus may be compared directly with one another on this common basis.

Young adult male New Zealand white rabbits (2.0–2.6 kg) were purchased from the Gopher State Caviary, St. Paul, Minn. The animals, fasted for 23–26 hr, were killed by jugular laceration. The proximal 100 cm of small intestine was rapidly excised and cooled in ice. Short segments of this preparation were placed on a cold plate, cut open longitudinally, rinsed with cold 0.25 M sucrose, and blotted with filter paper to remove debris. The mucosa was collected by gentle scraping with a stainless steel spatula, suspended in 90 ml of 0.25 M sucrose, and weighed by difference.

Subcellular fractionation entailed modifications of a general method which has been used for human intestine (Öckerman, 1965), and another procedure developed by Hübscher *et al.* (1965) specifically for mammalian intestinal preparations. The mucosal scrapings were homogenized for 30 sec at 0° in a Potter-Elvehjem homogenizer operating at 600 rpm, filtered twice through cheesecloth, and supplemented with 0.25 M sucrose to a final volume of 8 ml of homogenate/g of wet mucosa. The nuclear fraction was sedimented from the homogenate by centrifugation for 10 min at 640 rpm (6500*g* min) in a Model PR-2 International refrigerated centrifuge equipped with a high-speed attachment. The resulting supernatant solution was decanted and spun in the same centrifuge at 3000 rpm for 10 min (140,000*g* min) to remove the mitochondrial fraction. The microsomal fraction was sedimented from the remaining supernatant solution by centrifugation at 30,000 rpm for 45 min ($3.5 \times 10^6 g$ min) in a Spinco Model L ultracentrifuge equipped with a no. 30 head. The resulting supernatant portion was labeled the soluble fraction. In subcellular distribution studies, the nuclear and mitochondrial fractions were washed three and two times, respectively, in 0.25 M sucrose. In all other studies, the homogenate was centrifuged as described for the mitochondrial fraction, the sediment was washed once in 40 ml of 0.25 M sucrose, and the combined supernatant fractions were centrifuged as described above to sediment the microsomes. The nuclear, mitochondrial, and microsomal fractions were resuspended in approximately 4, 1.5, and 1.5 ml, respectively, of 0.25 M sucrose per g of wet mucosa.

By the use of a method similar to that developed by Ganoza (1964) for liver glucose 6-phosphatase, a modest (approximately 1.5-fold) enrichment of intestinal enzyme was achieved. The microsomes were made 0.1 M with respect to $MgSO_4$. Precooled (to −16°) acetone was added to constitute 15% by volume. This preparation was stirred at 0° for 3 min before centrifugation at −5° for 5 min at 3000 rpm (70,000*g* min) in the Model PR-2 centrifuge described above. The sediment, which contained most of the glucose 6-phosphatase activity, was resuspended, to one-half the original volume of the microsomes, in 0.25 M sucrose which in some experiments contained 1 mM EDTA, a compound found in supplemental studies not to alter intestinal glucose 6-phosphatase activity under these conditions. This preparation was employed in certain indicated experiments.

Rat intestinal homogenates and microsomal suspensions were prepared in a manner analogous to that utilized for rabbit tissues. Scraped rat mucosa was homogenized as described above and was suspended in a final volume of 8 ml/g of wet mucosa.

[3] One unit of PP_i–glucose phosphotransferase activity = 1 μmole of glucose-6-P formed/min at 30° in a reaction mixture (pH 6.0) containing, in 1.5 ml, 3.3 mM sodium PP_i, 180 mM α-D-glucose, and 40 mM sodium cacodylate; specific activity = units/mg of protein.

Results and Discussion

Distribution of Hydrolase and Phosphotransferase Activities in Intestine. Small intestines were removed from rabbits and divided into 25-cm segments, beginning with the proximal end of the gut. Microsomal suspensions were prepared from each segment, and enzymic activities depicted in reactions 1–4 were assayed with each preparation (Table I). Though histological classification of rabbit intestinal subdivisions is not clearly defined (Craigie, 1951), these segments correspond approximately to the following portions of the intestine: segment 1, duodenum and some jejunum; segment 2, primarily jejunum; segment 3, primarily ileum; and segment 4, ileum. The activity ratios for all four activities remained quite constant from segment to segment, although a modest decrease in absolute levels of activity was noted in the more distal intestinal segments. On the basis of these experiments, the proximal 100-cm portion of small intestine was routinely employed as the enzyme source in the studies described below.

Separate Identities of Glucose 6-Phosphatase–Phosphotransferase and Acid Phosphatase Activities. Since nonspecific acid phosphatases (typified by reaction 5) have been shown to be capable of catalyzing various phosphotransferase reactions in the presence of high concentrations of phosphoryl acceptor substrates (Morton, 1958a), it was necessary to demonstrate that reactions 2–4 were not catalyzed, under the experimental assay conditions employed, by this phosphatase which also is present in intestinal mucosa[4] along with specific glucose 6-phosphatase. Figure 1 depicts the results of subcellular distribution studies in which activities catalyzing reactions 1–4 (typified in the figure by PP_i–glucose phosphotransferase) were found predominantly in the microsomal fraction of intestinal mucosa. In contrast, nonspecific acid phosphatase, a lysosomal enzyme according to histological studies of intestine (Riecken and Pearse, 1965), sedimented predominantly with the mitochondrial fraction, which contains the lysosomes under the fractionation procedures used. These observations confirm earlier reports on the differing subcellular distribution of intestinal acid phosphatase and glucose 6-phosphatase (Porteous and Clark, 1965; Hübscher *et al.*, 1965), and further, indicate that pyrophosphatase and the two phosphotransferase activities follow the distribution pattern of the latter enzyme. With respect to each reaction, activity in the reconstituted homogenate accounted for all activity noted in the various isolated subcellular fractions. At pH 6.0

$$\beta\text{-glycerol-P} + H_2O \longrightarrow \text{glycerol} + P_i \qquad (5)$$

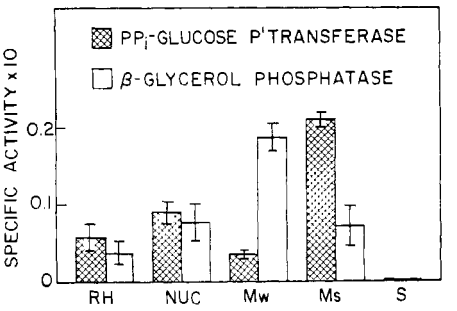

FIGURE 1: Enzyme activity distribution in subcellular fractions. Reaction mixtures (pH 6.0) contained, in 1.5 ml, 40 mM cacodylate buffer, 3.3 mM phosphate substrate, and 180 mM glucose (transferase). RH, NUC, Mw, Ms, and S refer to reconstituted homogenate, nuclear, mitochondrial, microsomal, and soluble fractions, respectively. Specific activity, defined in footnote 3, is plotted plus and minus standard deviation (indicate by vertical bars) on the basis of determinations made with five animals. Distribution patterns essentially identical with those presented for PP_i–glucose phosphotransferase activity also were obtained with glucose-6-P phosphohydrolase, inorganic pyrophosphatase, and CDP–glucose phosphotransferase activities.

in the presence of 3.3 mM phosphate substrates, the microsomal preparations hydrolyzed glucose-6-P at a rate nine times that of β-glycerol-P. Similarly, Clark and Sherratt (1967) previously have reported that 10% or less of the glucose-6-P hydrolysis in rabbit intestinal microsomes could be attributed to acid phosphatase.

Lability to mild heating in the absence of substrates is a characteristic of liver glucose 6-phosphatase (de-Duve *et al.*, 1949), inorganic pyrophosphatase, and PP_i–glucose phosphotransferase activities (Rafter, 1958, 1960; Nordlie and Arion, 1964, 1965). The renal enzymic activities also behave in a similar manner (Freed-

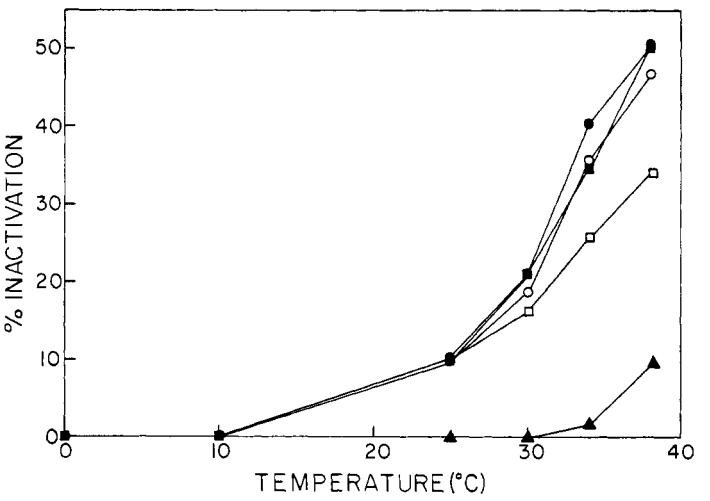

FIGURE 2: Thermal inactivation of glucose 6-phosphatase (○), inorganic pyrophosphatase (□), PP_i–glucose phosphotransferase (■), CDP–glucose phosphotransferase (●), and nonspecific acid phosphatase (β-glycerol phosphatase) (▲). Samples of partially purified enzyme preparation were preincubated for 5 min at the indicated temperatures, cooled, and aliquots were then incubated for 10 min in assay mixtures (pH 6.0) containing, in 1.5 ml, 3.0 mM phosphate substrate, 180 mM glucose (transferase), and 40 mM cacodylate.

[4] Though intestinal alkaline phosphatase also can catalyze the same reactions (Morton, 1958b), the pH optimum (9.5) and glucose levels necessary for activity with this enzyme are much higher than those employed in the present studies. Furthermore, the former enzyme is easily separated from glucose 6-phosphatase of rabbit intestinal mucosa by subcellular fractionation procedures (Porteous and Clark, 1965; Hübscher *et al.*, 1965).

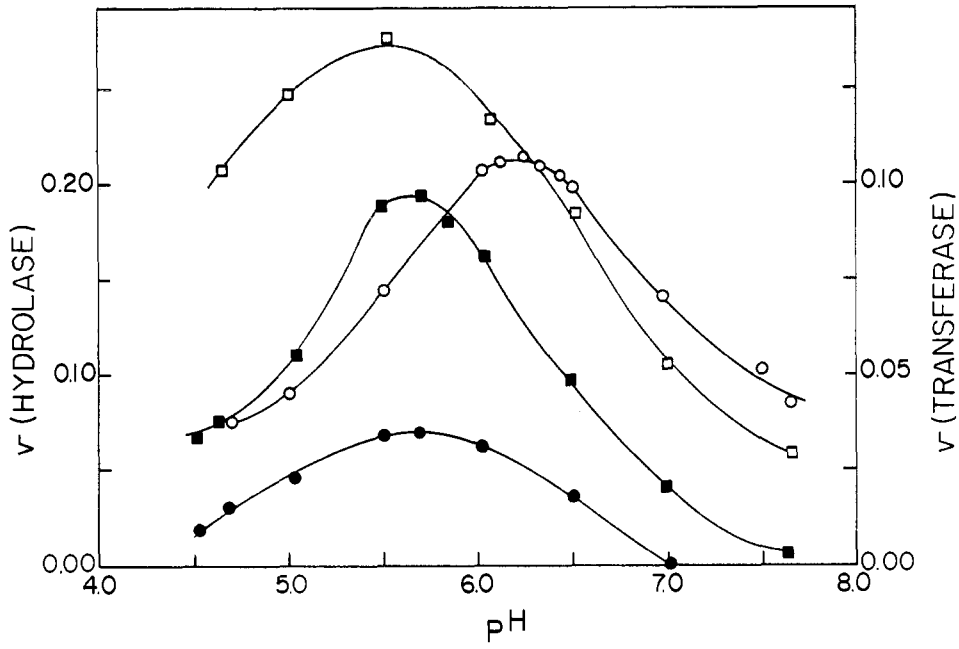

FIGURE 3: Effect of assay-mixture pH on glucose-6-P phosphohydrolase (○), inorganic pyrophosphatase (□), PP_i–glucose phosphotransferase (■), and CDP–glucose phosphotransferase (●) activities. Partially purified enzyme preparations were employed; incubation conditions were those described in the legend to Figure 2, except that assay mixture pH was varied as indicated. Left and right vertical axes indicate hydrolase and transferase activities, respectively. Activity, v, is expressed in terms of micromoles of glucose-6-P formed (phosphotransferase) or micromoles of substrate hydrolyzed (phosphohydrolase) per 10-min incubation at 30°. Assay mixtures were prepared in duplicate; activity was measured in one series and pH was determined with a Beckman expanded-scale meter in the second series.

land, 1962; Nordlie and Soodsma, 1966). Partial thermal inactivation also has been used as a criterion to distinguish human intestinal glucose 6-phosphatase from acid phosphatase (Öckerman, 1965). The results of studies of the relative thermal stability of a variety of rabbit intestinal mucosal activities are depicted in Figure 2. It is apparent that β-glycerol phosphatase (acid phosphatase) activity is much more stable to such heat treatment than are the other four activities studied.[5] It is concluded that the enzyme which hydrolyzes β-glycerol-P, acid phosphatase, is distinct from that catalyzing reactions 1–4 under the experimental conditions employed.

Catalytic Properties of Intestinal Glucose 6-Phosphatase–Phosphotransferase. The pH optima (Figure 3) for the four intestinal activities attributed to glucose 6-phosphatase agree closely with those reported for their counterparts in rat liver and kidney (Nordlie and Arion, 1964, 1965; Nordlie and Soodsma, 1966). Maximal activities were observed as follows: glucose-6-P hydrolase, pH 6.2; PP_i hydrolysis, pH 5.5; and PP_i- (or CDP-) glucose phosphotransferase, pH 5.7.

Figure 4 depicts the effects of assay temperature on activities 1–4 in the form of Arrhenius plots from which activation energies for each activity were calculated as described by Dixon and Webb (1964a). The following, quite similar values were obtained: glucose-6-P phosphohydrolase, 12.6 kcal/mol; PP_i–glucose phosphotransferase, 12.3 kcal/mol; CDP–glucose phosphotransferase, 12.4 kcal/mol; and inorganic pyrophosphate phosphohydrolase, 10.4 kcal/mol.[5] The first value agrees closely with that (12.5 kcal/mol) reported for liver glucose 6-phosphatase by Segal *et al.* (1958).

Enzymic activities were measured in the presence of several reagents possessing surface-active properties. Lysolecithin (0.08 mM) maximally stimulated phosphotransferase activities to 300%, pyrophosphatase to 250%, and glucose-6-P phosphohydrolase to 190% of untreated control values. These responses differ from those of guinea pig intestinal glucose 6-phosphatase, which has been reported to be inhibited by similar lysolecithin levels (James, 1965). Fresh microsomal suspensions supplemented to 0.1% (w/v) with sodium deoxycholate catalyzed both phosphotransferase activities to approximately 500%, pyrophosphatase to 250%, and glucose-6-P phosphohydrolase to 150% of control activity values observed in the absence of detergent. The somewhat lower levels of deoxycholate (0.1%, w/v) found necessary for optimal stimulation of intestinal activities than that (0.2%, w/v) needed for maximal response with the rat renal and hepatic activities (Nordlie and Snoke, 1967; Nordlie and Soodsma, 1966) may be a reflection of higher deoxycholate levels present in the enterohepatic circulation of the rabbit than in the rat (Ekdahl and Sjövall, 1955; see Bergström *et al.*, 1960); the freshly isolated rabbit intestinal enzyme employed

[5] The minor differences noted between inorganic pyrophosphatase activity and the other activities attributed to glucose 6-phosphatase appear to be due to the contamination of mucosal enzyme preparations by a second, specific, Mg^{2+}-stimulated, alkaline inorganic pyrophosphatase which displays a relatively small amount of activity at pH 6.0 in the absence of added Mg^{2+}.

in these studies may already have been partially modified through contact with this bile acid, *in vivo*.

The effects of palmityl-CoA in fresh intestinal microsomal activities, described in Figure 5a, resemble closely those previously observed with the hepatic enzyme (Nordlie *et al.*, 1967). This compound was found either to stimulate or to inhibit, in a concentration-dependent, activity-discriminating manner inasmuch as inclusion of $1\text{–}3 \times 10^{-5}$ M palmityl-CoA in the reaction mixtures inhibited glucose-6-P hydrolysis and activated the other activities. At higher levels ($3\text{–}12 \times 10^{-5}$ M) of palmityl-CoA, activation of all activities was observed.

Modification of enzyme activities of once-frozen microsomes by preincubation at 0° for 40 min with various concentrations of urea is depicted in Figure 5b. All activities were stimulated by urea levels below 3 M and were inhibited by exposure to higher levels (>6 M) of this compound. These observations may be interpreted to indicate that modest exposure to urea increases substrate accessibility to the enzymic active site, which is destroyed by more extensive exposure to urea. Thus, it appears likely that hydrogen and/or hydrophobic bonds are essential for the maintenance of the catalytic center of rabbit intestinal glucose 6-phosphatase and its associated activities.

Rather thorough kinetic studies of activities 1–4 were carried out at pH 6.0. Phosphohydrolase activities were assayed as functions of varied glucose-6-P or PP_i concentrations (1, 1.5, 2.0, 3.3, and 10 mM). In corresponding studies of phosphotransferase activities: (a) glucose concentrations routinely were held constant at 180 mM and activities were determined as functions of varied PP_i (1, 1.5, 2.0, 3.3, and 10 mM) or CDP (1, 1.5, 2.0, 3.0, 4.5, 6, 10.0, and 30 mM) concentrations or (b) phosphate substrate concentrations were maintained constant at 3.3 mM and activities were measured as functions of varied glucose concentrations (30, 45, 60, 90, and 180 mM). The resulting data were plotted in conventional double-reciprocal fashion (Lineweaver and Burk, 1934), and K_m values were calculated as the negative reciprocals of x-axis intercepts of extrapolations of the resulting straight lines (Dixon and Webb, 1964b). K_m values obtained are compiled in Table II. Supplemental experiments revealed that K_m values for the various substrates in the phosphotransferase reactions were independent of concentrations of second substrates, as previously has been noted for the liver (Arion and Nordlie, 1964; Nordlie and Arion, 1965) and kidney (Nordlie and Soodsma, 1966) enzymes. K_m values for the various substrates also were determined, under the conditions described above, with microsomal preparations (Nordlie and Arion, 1966) from rabbit liver and kidney. These values, also presented in Table II, were in close agreement for these two tissues as well as with corresponding values for the intestinal enzyme.

Citrate previously has been shown (Nordlie and Lygre, 1966) to inhibit the various activities of liver glucose 6-phosphatase in a highly pH-dependent manner. The kinetics of inhibition of the four activities of intestinal glucose 6-phosphatase by this compound were studied at pH 6, where significant inhibition of the hepatic enzyme previously had been noted (Nordlie and Lygre, 1966). The kinetics of inhibition by molybdate, which also previously was found to inhibit potently the various activities of the enzyme of liver (Swanson, 1950; Rafter, 1960; Nordlie and Arion, 1964), were similarly investigated. In all instances, inhibitions were found to be competitive with respect to phosphate substrates, and noncompetitive with respect to glucose in the phosphotransferase reactions. Results of typical experiments, in which the inhibition of PP_i–glucose phosphotransferase by 6.7 μM ammonium molybdate was studied with respect to glucose and to PP_i, are described in Figure 6a,b, respectively. K_i values for both molybdate and citrate determined with the various activities also are presented in Table II. Of particular interest is the identity of K_{PP_i} or $K_{glucose}$ values calculated for the various reactions in which these compounds serve as substrates. Also, K_i values determined with respect to all activities and substrates studied agreed well in studies with both citrate and molybdate.

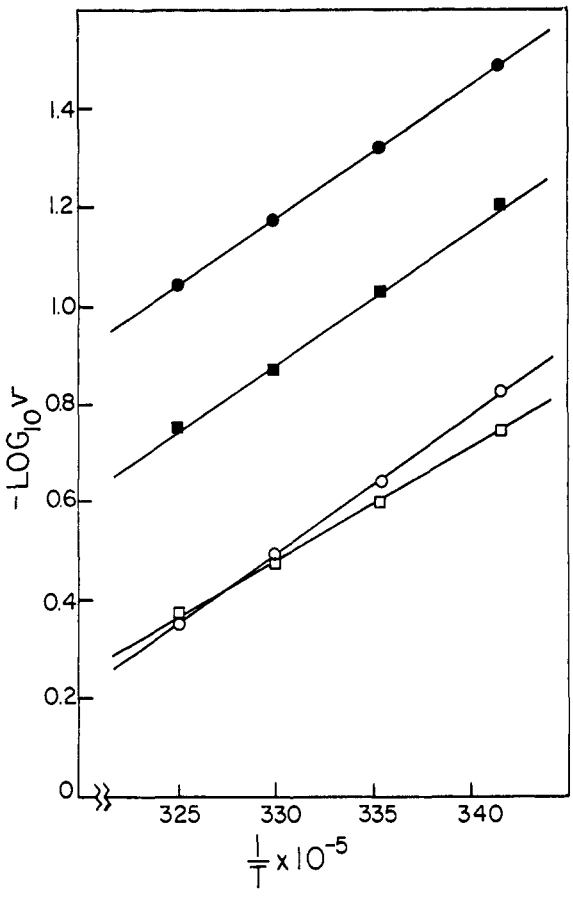

FIGURE 4: Effect of incubation temperature on glucose-6-P phosphohydrolase (○), inorganic pyrophosphatase (□), PP_i–glucose phosphotransferase (■), and CDP–glucose phosphotransferase (●) activities. The partially purified preparation was incubated at the indicated (in reciprocal form) temperatures with 40 mM cacodylate (pH 6.0), 20 mM phosphate substrates, and 180 mM glucose (transferase). Velocities, v, are as in Figure 3. Calculated energies of activation (kilocalories per mole) are as follows: glucose-6-P phosphohydrolase, 12.6; inorganic pyrophosphatase, 10.4; PP_i–glucose phosphotransferase, 12.3; and CDP–glucose phosphotransferase, 12.4.

Inhibition of Activities of Rabbit Glucose 6-Phosphatase–Phosphotransferase by a Factor from Rat Intestine.

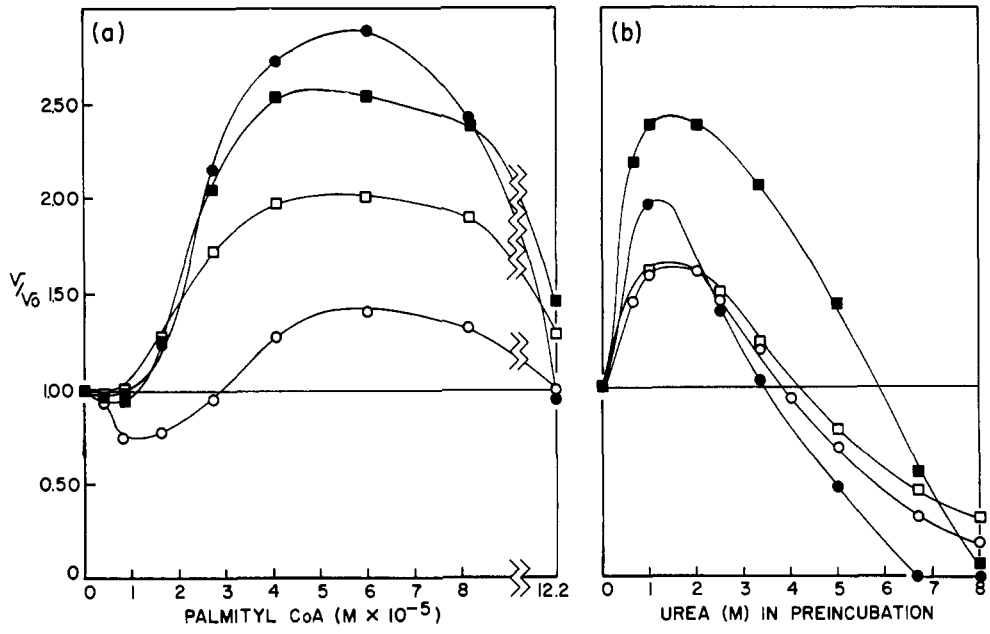

FIGURE 5: Effects of (a) palmityl-CoA and (b) urea concentrations on glucose-6-P phosphohydrolase (○), inorganic pyrophosphatase (□), PP_i–glucose phosphotransferase (■), and CDP–glucose phosphotransferase (●) activities. Palmityl-CoA concentrations indicated were included in assay mixtures whereas the enzyme was preincubated with the indicated concentration of urea for 40 min at 0° prior to assay. Reaction mixtures were those described in the legend to Figure 1. Other details are given in the text. Data are plotted as the ratio of activity (see Figure 3) noted in the presence (v) of palmityl-CoA or urea/activity observed in the absence (v_0) of palmityl-CoA or urea.

TABLE II: K_m Values and K_i Values for Citrate and Molybdate Determined with the Various Phosphohydrolase and Phosphotransferase Activities of Rabbit Glucose 6-Phosphatases.[a]

Activity	Reference Substrate	Rabbit Intestinal Enzyme			Rabbit Liver Enzyme, K_m(mM)	Rabbit Kidney Enzyme, K_m (mM)
		K_m (mM)	K_i (citrate) (M × 10^{-2})	K_i (molybdate) (μM)		
Glucose-6-P phosphohydrolase	Glucose-6-P	1.6	1.8	3.7	1.9	1.8
Inorganic pyrophosphatase	PP_i	2.1	1.5	2.4	2.1	2.1
PP_i–glucose phosphotransferase	PP_i	2.1	1.4	2.6	2.2	2.3
	Glucose	70.0	1.8	3.7	80.0	80.0
CDP–glucose phosphotransferase	CDP[b]	5.0	1.2	2.6	5.7	6.7
	Glucose	70.0	1.9	2.5	80.0	80.0

[a] Assay mixtures (pH 6.0) contained, in 1.5 ml, 40 mM sodium cacodylate buffer and varied substrate concentrations as described in detail in the text. In the inhibition studies, ammonium molybdate and sodium citrate levels were 6.7 μM and 13.3 mM, respectively. [b] Michaelis constants for other nucleotides, determined with the intestinal enzyme, are as follows: K_{CTP} = 4.5 mM, K_{ATP} = 4.1 mM, K_{ADP} = 7.1 mM, K_{GTP} = 7.5 mM, K_{ITP} = 4.3 mM, K_{IDP} = 7.6 mM, K_{UTP} = 6.1 mM, and K_{UDP} = 13 mM. Nucleotide concentrations were varied between 3 and 30 mM in these studies.

It has been reported that glucose 6-phosphatase is not demonstrable in rat intestine (Hers and deDuve, 1950; Ginsburg and Hers, 1960; Freedland, 1962). However, the observation that the presence of rat intestinal preparations reduced guinea pig intestinal glucose 6-phosphatase activity *in vitro* led Salomon *et al.* (1964) and James (1965) to propose that the enzyme is present in rat intestine, but is masked by the presence of some endogenous factor, possibly phospholipase A, a normal intestinal mucosal constituent (Epstein and Shapiro, 1959) which is particularly active in rat intestine (Salomon *et al.*, 1964). The results of our experiments described in Figure 7 confirm these earlier observations on the presence in rat intestinal mucosa of a factor which

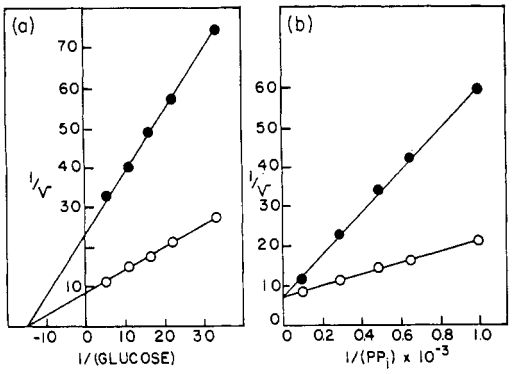

FIGURE 6: Kinetics of molybdate inhibition of PP_i–glucose phosphotransferase. (a) Inhibition with respect to glucose. Assay mixtures, pH 6.0, contained, in 1.5 ml, 40 mM cacodylate buffer, 3.3 mM PP_i, and indicated glucose concentrations. (b) Inhibition with respect to PP_i. Assay mixtures (pH 6.0) contained, in 1.5 ml, 40 mM cacodylate buffer, 180 mM glucose, and indicated levels of PP_i. Frozen microsomal suspensions served as enzyme source. In both instances, activities (v; defined as in Figure 3) were determined in the absence (○) and presence (●) of 6.7 μM molybdate. Kinetic parameters evaluated from these data and those obtained from similar studies with 13.3 mM citrate are compiled in Table II.

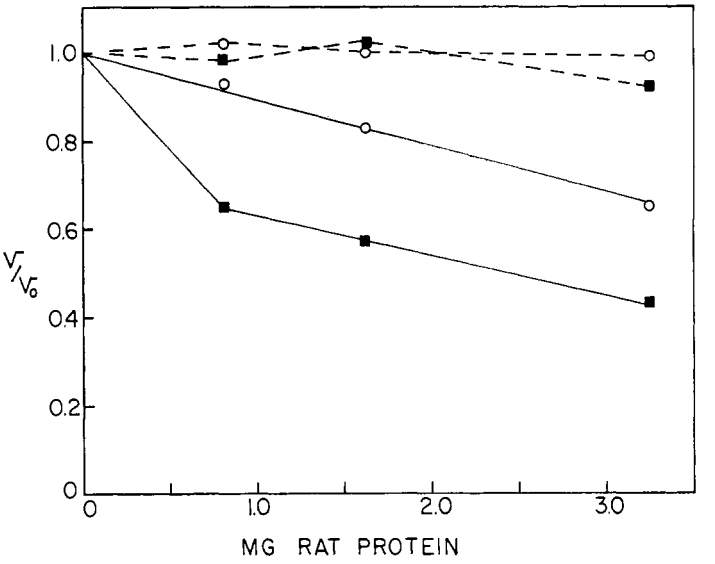

FIGURE 7: Effect of added rat intestinal homogenate on rabbit intestinal glucose-6-P phosphohydrolase (○) and PP_i–glucose phosphotransferase (■) activities. Reaction mixtures were those described in the legend to Figure 1. Rabbit intestinal microsomes were incubated in the presence of normal rat intestinal homogenate (solid lines) or homogenate boiled for 15 min at 100° (dashed lines). Values were corrected for the modest amount of glucose-6-P hydrolase and trace of phosphotransferase activity present in the normal (but not in the boiled) rat homogenate. Data are plotted as the ratio of activity (see Figure 3) measured in the presence of added rat homogenate (v)/absence (v_0) of rat homogenate.

lowers glucose-6-P phosphohydrolase activity of intestinal preparations from other species, and extend this effect to include inhibition of phosphotransferase activity of the rabbit intestinal enzyme. Inhibition, which was greater with respect to the latter activity, was destroyed by boiling the rat intestinal homogenate. Inhibition of these rabbit activities also was seen with rat intestinal microsomes in supplementary experiments. The different degrees of responses of the two activities to the inhibitor noted in these studies may well be related to other observations (see Figure 5a,b) which indicate that phosphotransferase activity of the enzyme is more sensitive than is glucose-6-P phosphohydrolase activity to factors which are capable of modifying the structure of proteins and lipoproteins.

The heat lability of this rat intestinal factor noted in our studies (see Figure 7) is consistent with its possible enzymic nature. Salomon *et al.* (1964) and James (1965) have suggested that phospholipase A may decrease glucose 6-phosphatase activity by breaking down lipoproteins of the endoplasmic reticulum of which the enzyme appears to be a part, and by generating lysolecithin, which they found to inhibit the enzyme of guinea pig intestine *in vitro*. However, we have observed (see text above) a rather marked stimulation of enzymic activity of rabbit intestine by the same concentrations of lysolecithin which the earlier workers reported to inhibit the guinea pig intestinal phosphatase. The precise nature of the mechanism of inhibition by rat intestinal preparations thus appears to remain unresolved at this time.

General Conclusions. It is concluded on the basis of the similarities in patterns of behavior of the various enzymic activities noted in the experiments described above that reactions 1–4 are catalyzed by a single enzyme, intestinal glucose 6-phosphatase, distinct from nonspecific acid phosphatase. The demonstration of the multifunctional nature of the rabbit intestinal enzyme, as well as the observed similarities in catalytic properties of these intestinal activities and the corresponding activities of rabbit (see Table II) and rat (see Nordlie and Arion, 1964, 1965; Nordlie and Soodsma, 1966; Nordlie *et al.*, 1967; also see discussion of individual experiments above) liver and kidney, support the common *genetic* identity of this enzyme in these various tissues, as previously has been suggested by other workers (Öckerman, 1964; Freedland, 1962; Lea and Walker, 1964; Field *et al.*, 1965).

The classical physiological role ascribed (Cahill *et al.*, 1959; Ashmore and Weber, 1959) to liver and kidney glucose 6-phosphatases, catalysis of the terminal step in gluconeogenesis, appears to be contraindicated in intestine due to the absence from this tissue of certain enzymes, *e.g.*, phosphoenolpyruvate carboxykinase (Utter, 1959) and pyruvate carboxylase (Utter, 1961), which probably are necessary for the over-all gluconeogenic process. Our present demonstration of the catalysis of phosphotransferase reactions by the intestinal enzyme suggests the possibility of a role for this multifunctional catalyst in a specialized mechanism for glucose absorption similar to that which recently has been postulated for the kidney enzyme (Nordlie and Soodsma, 1966; Soodsma *et al.*, 1967).

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