Phosphotransferase Activities of Kidney Glucose 6-Phosphatase*

(Received for publication, October 4, 1965)

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SUMMARY

Kidney glucose 6-phosphatase has been shown to catalyze inorganic pyrophosphatase and inorganic pyrophosphate-cytidine diphosphate-, cytidine triphosphate- and mannose 6-phosphate-glucose phosphotransferase reactions. The catalytic properties of phosphohydrolase and phosphotransferase activities of the kidney enzyme were found to be very similar to those of corresponding hepatic activities (11, 15). Formation and hydrolysis of glucose 6-phosphate by phosphotransferase and phosphohydrolase activities, respectively, of this enzyme are postulated to play a role in the transport of glucose across the kidney tubular cell of the diabetic animal.

The presence of glucose 6-phosphatase (β-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9; Reaction 1) has been observed in mammalian liver (1-4), kidney (2-7), and small intestine (8 10), although the last activity is masked by an inhibitor in certain species (9). This enzyme from liver has been shown (11-14) to catalyze the hydrolysis of inorganic pyrophosphate (Reaction 2) and the synthesis of glucose 6-phosphate via the pyrophosphate-glucose phosphotransferase and mannose-6-P-glucose phosphotransferase reactions (Reactions 3 and 4). Recent work in our laboratory (15) has revealed the presence of nucleoside 5'-triphosphate- and diphosphate-glucose phosphotransferase activities (Reactions 5 and 6) in rat liver and kidney. These activities in rat liver microsomes resembled the PPi-glucose phosphotransferase and glucose-6-P hydrolase activities in a number of respects (13, 14).

Glucose-6-P + H2O → glucose + P1

(1)

PPi + H2O → 2 P1

(2)

PPi + glucose → glucose-6-P + P1

(3)

Glucose-6-P + mannose = mannose-6-P + glucose

(4)

Nucleoside 5'-triphosphate + glucose →

glucose-6-P + nucleoside 5'-monophosphate

(5)

Nucleoside 5'-diphosphate + glucose →

glucose-6-P + nucleoside 5'-monophosphate

(6)

Since it has been suggested that glucose-6-P phosphohydrolases of liver, kidney, and intestine are under common genetic control (7, 10), comparative studies of properties of the various phosphotransferase activities of this enzyme in these tissues have been undertaken to gain insight into their possible physiological roles. Results of investigations of the catalytic properties of such enzyme activities in rat kidney microsomes are reported in this paper. Marked similarities between the kidney and hepatic enzymes were found. Evidence for the catalysis of the kidney nucleotide- and PPi-glucose phosphotransferase reactions by classical glucose 6-phosphatase is presented.

EXPERIMENTAL PROCEDURE

Experimental methods and sources of chemicals were as described previously (11, 12, 15). In addition, in certain instances glucose production was assayed with glucose oxidase (“Glucostat Special”; Worthington). In this case, reactions were terminated with 6 N KOH solution. Kidney microsomes were isolated and dispersed with deoxycholate (0.2%, w/v) as previously described for liver (11, 12). Protein fractions precipitating between 10 and 15% ammonium sulfate saturation (10), having specific activities in the range 0.21 to 0.33, were used. All data have been reported as specific activity per mg of protein.

1 The method is described in literature accompanying the enzyme reagent.
2 One unit of enzymic activity is that amount catalyzing the hydrolysis of 1 μmole of glucose-6-P per min under the conditions described in the legend to Fig. 2. Specific activity is the activity per mg of protein.
3 In contrast with the liver activities, which precipitated principally between 40 and 50% ammonium sulfate saturation (11), the bulk of the kidney activity sedimented between 10 and 15% saturation of the salt. Since the experimental results discussed indicate marked similarities in catalytic properties of various hepatic and kidney phosphotransferases and phosphohydrolases, the variations in precipitation patterns apparently reflect differences in the physicochemical nature of the enzyme-containing submicrosomal particles produced after deoxycholate dispersion of microsomal suspensions from these two tissues, or differences in the environment from which the enzyme was fractionated.

Supporting such a contention are the following observations. Microsomes are extremely rich in lipids (17); phospholipids constitute approximately 94% of this material (18). Ensrister, Siekert, and Palade (10) have shown that glucose 6-phosphatase is concentrated in a “loose sediment” layer which they obtained by centrifugation of deoxycholate-dispersed microsomes at 105,000 X g for 60 min. The ratio of phospholipid to protein in this fraction also was approximately double that of the untreated microsomes. Although similar data regarding composition of submicrosomal...
FIG. 1. Effects of pH on glucose 6-phosphatase (•), PP↓ glucose phosphotransferase (○), CTP-glucose phosphotransferase (△), and CDP-glucose phosphotransferase (□) reactions. Sodium acetate (open symbols) and sodium cacodylate (closed symbols) buffers were used. Reaction mixtures (1.5 ml) included 15 μmoles of phosphate substrate; transferase assay mixtures were supplemented with 270 μmoles of D-glucose. The P↓ assay was used to measure glucose-6-P hydrolysis; glucose-6-P formation was assayed spectrophotometrically. Reaction mixtures were prepared in duplicate, and pH was measured in one series with the aid of a Beckman expanded scale meter.

been adjusted to 0.052 unit of activity per 1.5 ml of reaction mixture. Composition of various assay mixtures are described in the tables and legends to figures. Incubations were performed for 5 min at 30° with shaking. Under these conditions, reactions were in all instances linear with time and protein concentration.

RESULTS

Preliminary Studies

In initial studies similar to those described earlier (11, 15) for the hepatic enzyme, it was found that kidney glucose 6-phosphatase, inorganic pyrophosphatase, and phosphotransferases were quite similar to their hepatic counterparts. All activities were present predominantly in the isolated microsomal fraction of the cell. All were activated by addition of sodium deoxycholate (0.2%, w/v). Activities sedimented together in constant proportions during fractional ammonium sulfate precipitation from deoxycholate-dispersed microsomal preparations. Activities were parallelly inhibited by ammonium molybdate in the range 10−6 to 10−3 M. Because of these similarities between hepatic and kidney enzymes, and among the various kidney activities themselves, the following studies were made (a) to afford a basis for further comparison of hepatic and kidney enzymes, and (b) to determine whether all activities were catalyzed by kidney glucose 6-phosphatase.

Effects of pH

The effects of pH on the various phosphatase and phosphotransferase activities are shown in Fig. 1. The curves obtained with kidney preparations are very similar to corresponding plots for the hepatic activities (11, 15). Maximal activity was observed at the following pH values: glucose 6-phosphatase, 6.5; CTP- and CDP-glucose phosphotransferase, 5.2; PP↓-glucose phosphotransferase, 6.

Thermal Inactivation

Phosphatase and phosphotransferase activities of the hepatic enzyme all have been shown to be equally labile to mild heating in the absence of substrates (11, 15). Similar studies on heat stability, described in Fig. 2, were carried out with kidney preparations. All activities were found to be equally thermally labile in the range 20-40°.

Kinetic Studies

Glucose 6-Phosphatase, Inorganic Pyrophosphatase, and PP↓-Glucose Phosphotransferase—When two or more compounds serve as alternate substrates for one enzyme, each will function as a competitive inhibitor of reactions involving the others (23). Furthermore, K↓ values for each compound will be identical with

4 Previously (11), we have pointed out that identical thermal lability of the variety of phosphohydrolase and phosphotransferase activities in enzyme preparations prepared by ammonium sulfate fractionation of liver microsomes is consistent with, but does not in itself prove, the common identity of these activities, since "... it is possible that [several] enzymes might show identical susceptibility to thermal inactivation, especially if they were associated in a common particulate body." This conclusion holds also for the kidney activities.
corresponding $K_m$ values when a single enzyme is involved. Hence, inhibition kinetics is a useful tool in determining whether a number of similar reactions are catalyzed by a single enzyme, or by a group of unseparated enzymes. We have previously used the kinetic approach in establishing the common identity of liver microsomal glucose 6-phosphatase, inorganic pyrophosphatase, and PP$_i$-glucose phosphotransferase. Results of similar studies of these kidney microsomal activities are described in Figs. 3a to 6. All data are presented as conventional double reciprocal plots (24). $K_m$ and $K_i$ values calculated from data in the figures are compiled in Table I. Additional specific details regarding evaluation of kinetic parameters are given in an earlier paper (11) and in Dixon and Webb (25).

Properties of these three activities were very similar to those previously found for the hepatic activities. Glucose-6-P served as a competitive inhibitor of the PP$_i$ phosphohydrolase reaction (Fig. 3a), and PP$_i$ for glucose 6-phosphatase (Fig. 3b). The $K_m$ value for glucose-6-P agreed well with the $K_i$ value determined for this compound as an inhibitor of PP$_i$ hydrolysis (Fig. 3a). Similarly, $K_m$ (Fig. 3a) and $K_i$ values (Fig. 3b) for PP$_i$ were nearly identical. These values also were in good agreement with $K_m$ (PP$_i$) evaluated for the PP$_i$-glucose phosphotransferase reaction (Fig. 5). Mannose has been shown to participate as a substrate with glucose-6-P in a reversible transphosphorylation (Reaction 4) which is catalyzed by liver glucose 6-phosphatase (12); both forward and reverse reactions shown in Equation 4 have also been found to be catalyzed by the kidney preparations. $K_m$ (mannose) for this reaction was equal to $K_i$ for this compound as an inhibitor of the PP$_i$-glucose phosphotransferase reaction catalyzed by the liver enzyme (11). This same relationship was observed with the kidney microsomal preparation (Fig. 4). Our previous conclusion that liver glucose 6-phosphatase also catalyzes PP$_i$ hydrolysis and PP$_i$-glucose phosphotransferase thus appears to be applicable also to the kidney enzyme.

CDP and CTP-Glucose Phosphotransferase Activities—Nucleo-

**Fig. 3.** a, effect of PP$_i$ concentration on the rate of PP$_i$ hydrolysis in the absence (●) and presence (○) of 3.16 μmoles of glucose-6-P. Reaction mixtures, pH 5.5, contained 60 μmoles of cacodylate buffer and indicated concentrations of PP$_i$ in 1.5 ml. In the absence of glucose-6-P, PP$_i$ hydrolysis was measured as one-half the change in $P_i$ concentration. In the presence of glucose-6-P, PP$_i$ hydrolysis was calculated as $(Δ$ μmoles of $P_i - Δ$ μmoles of glucose)/2. b, effect of substrate concentration on rate of glucose-6-P hydrolysis in the absence (●) and presence (○) of 3 μmoles of PP$_i$. Other conditions were as in a. Glucose-6-P hydrolysis was measured by assaying for glucose formation.

**Fig. 4.** a, effect of mannose concentration on the glucose-6-P-mannose phosphotransferase reaction. Assay mixtures, pH 5.5, contained in 1.5 ml, 60 μmoles of cacodylate buffer, 15 μmoles of glucose-6-P, and indicated concentrations of 2-mannose. Man- nase phosphate formation was calculated as $Δ$ μmoles of glucose $-Δ$ μmoles of $P_i$. b, effect of glucose concentration on the PP$_i$-glucose phosphotransferase reaction in the absence (●) and presence (○) of 270 μmoles of 2-mannose. Assay mixtures, pH 5.5, contained, in 1.5 ml, 60 μmoles of sodium acetate buffer, 15 μmoles of glucose-6-P, and indicated concentrations of PP$_i$, and estimated concentrations of 2-glucose. Glucose-6-P formation was measured spectrophotometrically.

**Fig. 5.** Effects of phosphoryl donor concentrations on rates of phosphotransferase reactions. Assay mixtures, pH 5.5, contained in 1.5 ml, 60 μmoles of sodium cacodylate buffer, 270 μmoles of 2-glucose, and indicated amounts of PP$_i$ (○), CDP (●), or CTP (X). Glucose-6-P formation was measured spectrophotometrically.
Fig. 6. Effect of glucose concentration on the CDP-glucose (a) and CTP-glucose (b) phosphotransferase reactions in the absence (○) and presence (●) of 270 μmoles of D-mannose. Assay mixtures, pH 5.5, contained, in 1.5 ml, 60 μmoles of sodium cacodylate buffer, 4.65 μmoles of CDP (a), or 5.00 μmoles of CTP (b), and indicated concentrations of α-glucose. Glucose-6-P production was measured spectrophotometrically.

TABLE I
Compilation of Michaelis and inhibitor constants from data in Figs. 2 to 6

<table>
<thead>
<tr>
<th>System studied</th>
<th>Data given in Fig.</th>
<th>Kinetic constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPi hydrolysis</td>
<td>3a</td>
<td>$K_m (\text{PPi})$</td>
</tr>
<tr>
<td>PPi inhibition of glucose-6-P hydrolysis</td>
<td>3b</td>
<td>$K_i (\text{PPi})$</td>
</tr>
<tr>
<td>PPi-glucose phosphotransferase</td>
<td>5</td>
<td>$K_m (\text{PPi})$</td>
</tr>
<tr>
<td>Glucose-6-P inhibition of PPi hydrolysis</td>
<td>3b</td>
<td>$K_m (\text{glucose-6-P})$</td>
</tr>
<tr>
<td>Glucose-6-P-mannose phosphotransferase</td>
<td>4a</td>
<td>$K_m (\text{mannose})$</td>
</tr>
<tr>
<td>Mannose inhibition of PPi-glucose phosphotransferase</td>
<td>4b</td>
<td>$K_i (\text{mannose})$</td>
</tr>
<tr>
<td>Mannose inhibition of CDP-glucose phosphotransferase</td>
<td>6a</td>
<td>$K_i (\text{mannose})$</td>
</tr>
<tr>
<td>Mannose inhibition of CTP-glucose phosphotransferase</td>
<td>6b</td>
<td>$K_i (\text{mannose})$</td>
</tr>
<tr>
<td>CDP-glucose phosphotransferase</td>
<td>5</td>
<td>$K_m (\text{CDP})$</td>
</tr>
<tr>
<td>CTP-glucose phosphotransferase</td>
<td>5</td>
<td>$K_m (\text{CTP})$</td>
</tr>
</tbody>
</table>

a $V_{max}$ for hydrolysis of PPi (Fig. 3a) and glucose-6-P (Fig. 3b) were 0.38 and 0.29 μmole per 5 min per 1.5 ml of reaction mixture, respectively.

d-tide-glucose phosphotransferase activities (Reactions 5 and 6) of liver have been found to be similar to the three activities discussed above, both in catalytic properties (15) and in responses to hormonal manipulations (21). These activities in kidney also appear to be catalyzed by glucose 6-phosphatase, for in addition to parallel thermal inactivation with other activities attributed to this enzyme (Fig. 2), the nucleotide-glucose phosphotransferase activities also were inhibited competitively, with respect to glucose, by mannose (see Fig. 6). Also $K_i$ values for the sugar inhibitor, calculated from data in Fig. 6, agreed very closely with the value for $K_m$ (mannose) in the glucose-6-P-mannose phosphotransferase reaction (Fig. 4a) and with the $K_i$ (mannose) value obtained for mannose inhibition of the PPi-glucose phosphotransferase reaction (Fig. 4b).

Combined Substrate Studies

As an additional, independent check on the validity of the results of the kinetic studies, experiments were performed in which the initial rates of hydrolysis (Table II) and phosphotransferase reactions (Table III) were determined with nonsaturating levels of substrates included in reaction mixtures singly and in pairs. Experimental values recorded in the tables agreed well with those calculated by substitution of average values for kinetic parameters (Figs. 3 to 6; Table I) into Equations 7 to 9.

$$V_0 = \frac{V_{max}}{1 + [a] \frac{1 + [b]}{K_i}}$$

(7)

$$V_0 = \frac{V_{max}}{1 + [b] \frac{1 + [a]}{K_i}}$$

(8)

$$V_t = V_a + V_b$$

(9)

These rate equations (23) describe the situation in which Compounds a and b serve as alternate substrates for a single enzyme. $V_a$ and $V_b$ are maximal reaction velocities for reactions of Compounds a and b, respectively; $v_a$ and $v_b$ are corresponding initial reaction velocities, and the subscript t refers to the total reaction of a plus b.

TABLE II
Rates of hydrolysis of PPi and glucose-6-P, individually and combined

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Calculated values</th>
<th>Experimental values</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPi utilized</td>
<td>Glucose-6-P utilized</td>
<td>PPi found</td>
</tr>
<tr>
<td>PPi</td>
<td>0.33</td>
<td>0.66</td>
</tr>
<tr>
<td>Glucose-6-P</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>PPi + glucose-6-P</td>
<td>0.23</td>
<td>0.983</td>
</tr>
</tbody>
</table>
Michaelis-Menten rate equation, into Equations 7, 8, and 9. Substituting substrate concentrations and initial reaction velocities described in Equations 1 to 6 by kidney glucose 6-phosphatase. Phosphoryl donors, either individually or in pairs as indicated, in 1.5 ml. Glucose-6-P formation was measured spectrophotometrically. Calculated values were obtained by substituting average \( K_m \) values from Table I, and apparent \( V_{\text{max}} \) values evaluated by substituting substrate concentrations and initial reaction velocities with individual substrates (from Table III) into the simple Michaelis-Menten rate equation, into Equations 7, 8, and 9.

<table>
<thead>
<tr>
<th>Phosphoryl donor</th>
<th>Glucose 6-P formation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated values</td>
</tr>
<tr>
<td></td>
<td>( \mu \text{mole/1.5 ml/5 min} )</td>
</tr>
<tr>
<td>PPi</td>
<td>0.206</td>
</tr>
<tr>
<td>CDP</td>
<td>0.090</td>
</tr>
<tr>
<td>CTP</td>
<td></td>
</tr>
<tr>
<td>PPi + CDP</td>
<td>0.210</td>
</tr>
<tr>
<td>PPi + CTP</td>
<td>0.213</td>
</tr>
<tr>
<td>CDP + CTP</td>
<td>0.120</td>
</tr>
</tbody>
</table>

**Discussion**

As was the case in earlier studies with the hepatic enzyme (11), the experimental results obtained in the present studies, considered in toto, strongly substantiate the catalysis of the reactions described in Equations 1 to 6 by kidney glucose 6-phosphatase. The reaction mechanism that we previously proposed (12) to describe the phosphohydrolase and transferase activities of glucose-6-phosphatase thus may be extended to cover the kidney activities, including those involving nucleoside tri- and diphosphate substrates. Freedland (7) has shown previously that kidney and liver glucose-6-P phosphohydrolases resemble one another closely relative to acid lability and values for \( K_m \) (glucose 6-phosphate). Results obtained in the present studies indicate similar agreement of \( K_m \) values for PPi, nucleotides, and sugar substrates with hepatic (15) and kidney activities.

The suggestion (7, 10) that glucose 6-phosphatase in various tissues is regulated by a common gene is substantiated by the recent findings that both liver (15, 27) and kidney (27) phosphotransferase and phosphohydrolase activities are significantly elevated in diabetic and depressed by insulin administration. We have demonstrated that, in the diabetic animal, liver phosphotransferase activity of glucose-6-phosphatase is capable of phosphorylating glucose at a rate more than 10 times that of hepatic glucokinase (15). The synthetic process was markedly favored by elevation both in amount of enzyme and in blood glucose levels accompanying the diabetic state. On this basis, we postulated that the phosphotransferase activity of this hepatic enzyme plays a physiologically significant, compensatory role for glucose phosphorylation in the absence of glucokinase in untreated diabetes. The present findings with the kidney enzyme suggest that the latter, multifunctional enzyme may constitute an additional part of this compensatory mechanism. The following factors bear on the conclusion that glycosuria rather than hyperglycemia is considered to be the most significant single lesion of carbohydrate metabolism in the untreated diabetic. "Without glycosuria diabetes would not be a deficit state" (28). Cahill et al. (29) cite the work of Issekutz (30) and Soskin and Levine (31) to support the statement that "... in all but the most severe states of insulin deficiency a new equilibrium with essentially normal rates of peripheral glucose assimilation ... albeit obtained at much higher blood glucose concentrations ... is reached." In untreated diabetes, the ability of the kidney proximal convoluted tubule to reabsorb sugar is exceeded, and urine glucose concentrations in the range 0.5 to 12% (500 to 12,000 mg/100 ml) are observed (32); certainly, the concentration of glucose in the distal tubule under these conditions must be the highest found in the body. In contrast with phosphotransferase activity of alkaline phosphatase (33), which has been implicated in the absorptive process (see review by Rosenberg and Willbrandt (34)), phosphotransferase activity of kidney glucose 6-phosphatase approximates phosphohydrolase activity at high glucose concentrations (see Figs. 3b and 5). It therefore seems reasonable that kidney glucose 6-phosphatase may be involved in the active reabsorption of tubular glucose by a phosphorylation-dephosphorylation mechanism such as that shown in Fig. 7. The involvement of hexokinase plus a phosphatase in a similar scheme has been suggested by Drabkin (28). However, hexokinase is not inhibited by a possible exception is the lumen of the small intestine after ingestion of a meal. Interestingly, PPi-glucose phosphotransferase activity recently has been detected in small intestine of rabbit and guinea pig (R. C. Nordlie and D. G. Lygre, unpublished observations).

**Fig. 7.** Proposed mechanism for participation of glucose 6-phosphatase-phosphotransferase (P-Tase) in transport of glucose across kidney tubular epithelial cell of the diabetic animal. PPi, PPi, nucleoside diphosphate, or nucleoside triphosphate. The mechanism involves basically the phosphorylation of glucose near the luminal membrane of the cell and hydrolysis of the sugar phosphate near the base of this same cell. Both processes are catalyzed by glucose 6-phosphatase; the synthetic reaction is favored by high glucose concentrations and acidic pH, while hydrolysis of glucose-6-P is preferentially favored by higher pH approaching that of blood. The phosphoryl donor pool is maintained in equilibrium with ATP which is continuously regenerated in mitochondria located near the base of the cell (30).
by phlorizin, which prevents tubular sugar reabsorption (see Reference 34 for additional arguments against and for this mechanism). Glucose 6-phosphatase is, however, inhibited by this compound (36, 37). While a physiologically significant role for glucose 6-phosphatase phosphotransferase has been questioned previously (15) because of (a) the high glucose requirement ($K_{\text{glucose}} = 90$ mM), and (b) the rather acidic $pH$ optimum, conditions near the luminal side of the tubular epithelial cell of the kidney of untreated diabetic animals would preferentially favor the phosphotransferase reactions. The currently accepted view (39, 40) is that kidney glomerular filtrate is acidified in the distal convoluted tubule (41); however, Ellinger and Hirt (42) have observed an acid filtrate in the proximal tubule. Pitts (43) also states that the acidifying mechanism may operate not only in the proximal as well as in distal tubular segments. The effects of acido- sis, which often accompanies the diabetic state, also is reflected in the $pH$ of the urine (44). A higher $pH$, approximating that of blood, would selectively favor hydrolysis of glucose-6-P near the base of the tubular cell. The differences in observed effects of $pH$ on kidney glucose 6-phosphatase phosphohydrolase and phosphotransferase activities (Fig. 1) are consistent with such a phosphorylation-dephosphorylation process. In untreated diabetes the kidney enzyme would, through its role in reabsorption of sugar from the kidney tubule, promote the maintenance of blood glucose at considerably higher than normal levels. This elevated glucose concentration would make possible the phosphorylation-dephosphorylation process. Thus, in untreated diabetes the kidney enzyme would, through its role in reabsorption of sugar from the kidney tubule, promote the maintenance of blood glucose at considerably higher than normal levels. This elevated glucose concentration would make possible the phosphorylation of this hexose by phosphotransferase activity of this enzyme in liver (11, 15) and, perhaps, other tissues as well.

Acknowledgments—We wish to thank Drs. E. G. Olmstead and W. E. Cornatzer for helpful discussions.

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