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# Kinetics of Phosphoglucose Ester Hydrolysis by Rat Intestinal Mucosa (37903)

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A specific acidic glucose 6-phosphatase (D-glucose-6-phosphate hydrolase, EC 3.1.3.9) has been prepared from the small intestine of a number of animals and from human jejunum (1–3). In rat small intestine, however, it has not been possible to show directly the presence of the specific glucose 6-phosphatase (4, 5). Salomon *et al.* (6) have shown that the guinea pig intestinal glucose 6-phosphatase is inhibited by rat intestinal homogenate due to the presence of an inhibitor of this enzyme in the rat tissue. This indicates that glucose 6-phosphatase may be present in the rat intestine but not demonstrable due to endogenous inhibition. The inhibition of rabbit intestinal glucose 6-phosphatase by the rat intestinal homogenate, shown by Lygre and Nordlie (7), supports the finding of Salomon *et al.* (6). In this study we made kinetic determinations of the phosphohydrolytic activity of the homogenates of rat intestinal mucosa and delineated differences in the hydrolysis of glucose 1-phosphate (G1P) and glucose 6-phosphate (G6P).

*Methods.* The upper 15-cm segments of everted intestines from Albino rats were blotted and scraped. Homogenates (0.2 g%, w/v) of mucosal scrapings were prepared in a diluent of one part Kreb's–Ringer bicarbonate and three parts 50 m*M* tris (hydroxymethyl)aminomethane–HCl buffer (Tris), pH 8.00, using a Teflon-glass tissue grinder. The total N contents of the homogenates were determined by the microkjeldahl method (8).

The phosphatase reaction was initiated by adding 1 ml homogenate to 1 ml of disodium G6P or disodium G1P solution in 50 m*M* Tris, pH 8.00. The incubation period at 37° and initial G1P and G6P concentrations were chosen to yield linear reaction with time. The reaction vessel was transferred to a boiling water bath for 2 min to stop the reaction. The amount of free glucose and/or inorganic phosphate ($P_i$) was then analyzed to estimate the phosphatase activity. The methods of analysis of glucose and $P_i$ have been reported elsewhere (9).

The phosphoglucose isomerase activity was estimated by simultaneous measurements of amounts of $P_i$ and fructose 6-phosphate formed. The latter compound was quantitated by the method of Roe *et al.* (10). Absorbance of incubation medium with and without added sodium triphosphopyridine nucleotide (TPN) at 340 nm was monitored to measure glucose 6-phosphate dehydrogenase activity. This procedure allowed quantitation of reduced triphosphopyridine nucleotide (TPNH) and was also used for determination of phosphoglucose mutase activities in reaction mixtures containing G1P with and without purified yeast glucose 6-phosphate dehydrogenase (Calbiochem). All incubations were carried out at 37°.

*Results.* The enzyme activity with G1P and G6P was proportional to the total nitrogen contents of the homogenates over the following range: 0.010 to 0.032 mg/ml. Total nitrogen in 40 homogenate prepara-

TABLE I. Products Formed in G1P and G6P Hydrolysis.

| Expt. | G1P[a] | | G6P[b] | |
|---|---|---|---|---|
| | Glucose | $P_i$ | Glucose | $P_i$ |
| 1 | 2.23 | 2.83 | 2.98 | 2.81 |
| 2 | 2.00 | 1.76 | 4.32 | 3.33 |
| 3 | 2.23 | 2.23 | 3.42 | 3.29 |
| 4 | 2.13 | 2.04 | 3.86 | 3.76 |
| 5 | — | — | 4.20 | 4.44 |

[a] Values have units of μmoles/(mg total N × min).

[b] Values have units of μmoles/(ml homogenate × hr).

tions employed in G1P and G6P hydrolysis was 0.022 ± 0.0015 (mean ± SE) mg/ml. A proportionality was also observed between the two products, glucose and $P_i$, in G6P and G1P hydrolysis (Table I).

The phosphatase and phosphoglucose isomerase activities are given in Table II. The means of 6 determinations of glucose 6-phosphate dehydrogenase and 13 determinations of phosphatase activity under similar conditions, respectively, were: 0.08 μmole TPNH/ml homogenate and 3.40 μmoles $P_i$/ml homogenate. The presence of phosphoglucomutase activity in the tissue was shown only when exogenous TPN as well as purified glucose 6-phosphate dehydrogenase was added. In the absence of the added dehydrogenase enzyme and TPN, however, the mutase activity was not detectable.

The initial velocities ($v$) at 7 G1P concentrations, ranging from 1.41 to 9.15 m$M$,

TABLE II. Paired Comparison of Phosphoglucose Isomerase (PGI) and Phosphatase (PH) Activities.

| Expt. | PH[a] | PGI[a] | PGI/PH × 100 |
|---|---|---|---|
| 1 | 3.51 | 0.57 | 16.30 |
| 2 | 3.28 | 0.56 | 16.97 |
| 3 | 3.29 | 0.36 | 10.96 |
| 4 | 3.33 | 0.40 | 11.86 |
| 5 | 3.42 | 0.30 | 8.87 |
| 6 | 1.75 | 0.30 | 17.54 |
| 7 | 1.75 | 0.31 | 17.82 |

[a] Values have units of μmoles/(ml homogenate × hr). 20 m$M$ G6P was used as substrate.

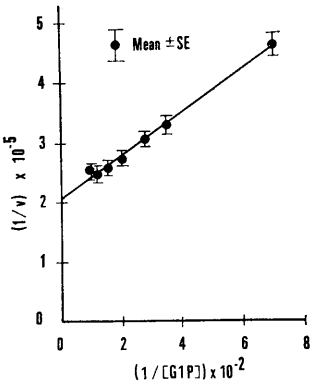


FIG. 1. Lineweaver–Burk plot of the hydrolysis of G1P. Regression line is shown in the graph; $y$-intercept is $2.10 \times 10^5$ and slope is $3.35 \times 10^2$. $K_m$ (slope/$y$-intercept) = 1.69.

were determined in homogenates from 5 different animals. The Lineweaver–Burk plot (drawn by a regression analysis) of the mean reciprocal velocities and the reciprocal substrate concentrations is shown in Fig. 1. Figure 2 shows a similar plot of the hydrolysis of G6P by homogenates from six different animals. Six initial G6P concentrations, 3.25 to 19.65 m$M$, were used. The apparent Michaelis constant ($K_m$) calculated from these plots is 1.69 m$M$ for G1P and 4.37 m$M$ for G6P. The $K_m$ and maximum velocity ($V_{max}$) values and the $K_m/V_{max}$ ratios are given in Table III. A comparison of mean $K_m$ values for G1P and G6P hydrolysis shows a significant dif-

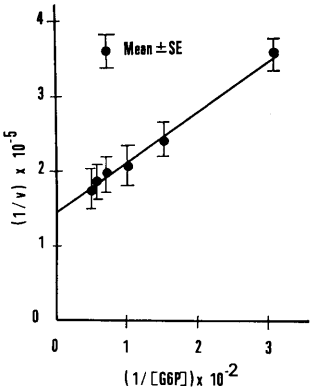


FIG. 2. Lineweaver–Burk plot of the hydrolysis of G6P. Regression line is shown in the graph; $y$-intercept is $1.45 \times 10^5$ and slope is $6.33 \times 10^2$. $K_m$ (slope/$y$-intercept) = 4.37.

TABLE III. $K_m$ and $V_{max}$ Values for the Hydrolysis of G1P and G6P.

| | $K_m$[a] | | $V_{max}$[b] | | $K_m/V_{max}$ | |
|---|---|---|---|---|---|---|
| Animal | G1P | G6P | G1P | G6P | G1P | G6P |
| 1 | 2.02 | 8.61 | 5.62 | 12.66 | 0.36 | 0.68 |
| 2 | 1.48 | 4.22 | 4.63 | 6.80 | 0.32 | 0.61 |
| 3 | 1.07 | 2.81 | 3.98 | 6.10 | 0.27 | 0.45 |
| 4 | 1.95 | 5.94 | 5.56 | 10.42 | 0.35 | 0.56 |
| 5 | 2.01 | 1.88 | 5.29 | 3.91 | 0.37 | 0.45 |
| 6 | — | 3.99 | — | 5.95 | — | 0.67 |
| Mean | 1.71 | 4.58 | 5.02 | 7.65 | 0.34 | 0.57 |
| $P$ | $<0.05$ | | $>0.05$ | | | |

[a] m$M$.

[b] $\mu$moles glucose formed/ (mg total N $\times$ min).

ference ($P < 0.05$). The $V_{max}$ values for the two substrates were not significantly different. A variability in the $K_m$ values within each substrate group and in the $V_{max}$ values of both substrates was observed and could not be accounted for by experimental error alone.

*Discussion.* The phosphoglucose isomerase and the glucose 6-phosphate dehydrogenase activities, respectively, were approximately 14 and 3% of the phosphatase activity in the mucosal homogenates. The utilization of G1P or G6P by the mucosal phosphoglucomutase was negligible. Studies of Villar-Palasi and Larner (11) have shown that rat intestinal mucosa contains extremely low quantities of the enzyme phosphorylase. These authors have also reported that the phosphorylase activity was equal to less than 1% of the phosphoglucomutase activity. It appears that in rat intestinal mucosa phosphatase activity is more prevalent than other phosphoglucose-utilizing enzyme systems. Thus, influence of enzymes other than phosphatase on the "apparent $K_m$" measurements of G1P and G6P hydrolysis might not be significant.

Since the Lineweaver–Burk plots of G1P and G6P hydrolysis were reasonably linear, we assumed that the reactions conformed to the following Michaelis–Menten scheme:

$$\begin{matrix}\text{Enzyme} \\ + \\ \text{Substrate}\end{matrix} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} \text{Enzyme-Sub. Complex(s)} \xrightarrow{K_3} \begin{matrix}\text{Enzyme} \\ + \\ \text{Product}\end{matrix}$$

According to this scheme, $V_{max} = K_3 \times e$ (where $e$ = enzyme concentration), and $K_m = K_2/K_1$, $(K_2 + K_3)/K_1$, or $K_3/K_1$ (12). Should the $V_{max}$ variation observed in homogenates of different animals be due to enzyme concentration differences and be independent of changes in $K_3$, one would not observe variability in $K_m$ determinations. Since $K_m$ variation within each substrate group was observed, the $V_{max}$ variation in all probability was the result of changes in $K_3$ as well. This would mean that $K_m$ cannot be equal to $K_2/K_1$ in the hydrolysis of G1P or G6P. Lumrey *et al.* (13) interpreted $K_m$ as equal to $K_3/K_1$ because of the occurrence of proportional changes in $K_m$ and $V_{max}$ values. Dixon and Webb (14) state that if $K_2$ is negligible for an enzyme, any factor that would produce a change in $V_{max}$ would change $K_m$ proportionately. These generalizations seem to apply to the data presented in this report, as the $K_m/V_{max}$ ratio was constant for G1P or G6P (see Table III). This would mean that $K_m$ values in all likelihood were equal to $K_3/K_1$. As $K_3$ is equal to $V_{max}/e$, $K_m$ would be equal to $V_{max}/K_1 \times e$ and the ratio $K_m/V_{max}$ equal to $1/K_1 \times e$. It becomes possible then to approximate the value of the quantity $K_1 \times e$ from the $K_m/V_{max}$ ratio. Assuming that roughly equal quantities of enzymes were employed in G1P and G6P reactions, a ratio of $K_{1(G1P)}/K_{1(G6P)}$ can be calculated. This ratio has a value of 1.68 and indicates that the constant $K_1$, which describes the formation of the enzyme–substrate complex, for G1P hydrolysis is significantly

higher (68%) than that for G6P hydrolysis. Although statistically significant differences were present in the $K_m$ values for G1P and G6P, the variability of this parameter in intestinal homogenates of different animals was too large to substantiate the difference. The estimated difference between the $K_1$ values for the two phosphoglucose esters was clearly substantial because of much smaller variance in the $K_m/V_{max}$ ratios.

Our observations indicate that the ratio of $K_1$ for G1P and G6P hydrolysis calculated from $K_m$ and $V_{max}$ determinations is a better criterion for differentiating the two hydrolytic reactions than the kinetic parameter of $K_m$ itself. As pointed out by Slater (15), knowledge of $K_1$ is valuable in evaluating the functional capacity of enzymes *in vivo*. This constant describes the rate of formation of the enzyme–substrate complex at concentrations of substrate that may exist physiologically and not near the level of concentration required to saturate the enzyme.

*Summary.* The enzymatic hydrolysis of glucose 1-phosphate (G1P) and glucose 6-phosphate (G6P) by homogenates of rat intestinal mucosa was quantitated. These substrates were not utilized by enzymes other than phosphatases to any appreciable extent. The mean $K_m$ value for the hydrolysis of G6P was higher (4.3 m$M$) than that for G1P (1.69 m$M$). A variability was observed in $K_m$ values in each substrate group. However, the $K_m/V_{max}$ ratio was constant for each of the two substrates. Such proportional changes in $K_m$ and $V_{max}$ values allow the approximation of the rate constant ($K_1$) related to the formation of the enzyme–substrate complex. The $K_1$ value thus estimated for G1P was approximately 1.68 times that for G6P.

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