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ACTIVATION OF PHOSPHOFRUCTOKINASE FROM RAT LIVER BY 6-PHOSPHOGLUCONATE

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Summary: Low concentrations of 6-phosphogluconate activated phosphofructokinase from rat liver. 6-phosphogluconate (0.2mM) lowered the concentration of fructose 6-phosphate required to achieve one-half the maximum velocity ($S_{0.5}$) to a value within the physiological range of fructose-6-phosphate concentrations, but had no effect on the maximum velocity. 6-phosphogluconate also changed the kinetics of the enzyme from sigmoidal to hyperbolic. The effect of 6-phosphogluconate on phosphofructokinase was abolished by heating the enzyme.

Phosphofructokinase (EC 2.7.1.11) is believed to be involved in the regulation of glycolysis and, as such, is affected by a number of metabolites (see reference 1). Among the effectors of liver phosphofructokinase are the adenine nucleotides (2), although their role in the regulation of phosphofructokinase *in vivo* has been questioned (3). Several phosphorylated intermediates of carbohydrate metabolism are known to affect phosphofructokinase activity. Both fructose-6-*P* (2) and fructose-1,6-P_2 (4) are positive effectors. Recently, another phosphorylated metabolite, tentatively identified as fructose-2,6-P_2 has been shown to stimulate the enzyme (5,6).

Previous work from this laboratory demonstrated activation of rat liver pyruvate kinase activity by 6-*P*-gluconate, an intermediate of the pentose phosphate pathway (7,8). This effect is interesting in that it demonstrates coordination between two pathways which contribute to the synthesis of fatty acids in the liver. Because there is evidence that the activities of phosphofructokinase and pyruvate kinase are coordinated (for example, both enzymes are inhibited by ATP and activated by fructose-1,6-P_2), we tested the effect

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of 6-*P*-gluconate on phosphofructokinase. The results show that 6-*P*-gluconate is a positive effector of the enzyme.

Materials and Methods: A male, Sprague-Dawley rat (Hilltop Laboratory Animals, Scottsdale, PA), which weighed 320 gm and which had been starved for 2 days was killed by decapitation. The liver was removed, cooled in isotonic sucrose at 4°C and then was frozen and stored at -20°C. A piece of the frozen liver was placed in 4 volumes of a buffer which consisted of 0.25 M sucrose, 50 mM Triethanolamine, 100mM NaF and 1 mM EDTA (pH 7.5) and was then disrupted, at 4°C, using a Potter-Elvehjem tissue homogenizer. The homogenate was sedimented at 27000 x g for 30 min at 4°C. The resultant supernate was used as the source of enzyme. Phosphofructokinase was assayed at 25°C as described by Kagimoto and Uyeda (9). The reaction mixture contained, in 3 ml: 50 mM N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 5 mM $MgCl_2$, 2.5 mM Dithiothreitol, 1 mM EDTA, 0.16 mM NADH, 0.1 mM ATP, dialyzed aldolase (0.2 U), triose-*P*-isomerase (6U), α-glycero-*P*-dehydrogenase (4.6U) and various concentrations of D-fructose-6-*P* and 6-*P*-gluconate. The coupling enzymes contributed 0.47 mM NH_4SO_4. The reactions were initiated by the addition of enzyme and the disappearance of NADH was monitored at 340 nm with a Gilford spectrophotometer. The steady-state rate was used to estimate phosphofructokinase activity (10). Protein was assayed by the method of Lowry et al. (11).

All the chemicals and coupling enzymes were from Sigma. 6-*P*-gluconate was free of contamination by fructose-6-*P*, fructose-1,6-P_2 and glucose-6-*P*. (7).

Results and Discussion: The data in figure 1 and table 1 demonstrate that 6-*P*-gluconate activates phosphofructokinase from rat liver. Phosphofructokinase activity, as a function of the concentration of fructose-6-*P*, is sigmoidal and cooperative (Hill coefficient = 2.68, table 1). 6-*P*-gluconate decreased the cooperativity and lowered the apparent $S_{0.5}$ for fructose-6-*P* (table 1). In the presence of 0.2 mM 6-*P*-gluconate, phosphofructokinase displayed essentially hyperbolic kinetics (figure 1). A higher concentration of 6-*P*-gluconate (0.8 mM) did not activate the enzyme more than 0.2 mM 6-*P*-gluconate (data not shown). There was no apparent change in the maximum velocity due to 6-*P*-gluconate. The $S_{0.5}$ for fructose-6-*P* in the presence of 0.2 mM 6-*P*-gluconate is within the physiological range of fructose-6-*P* concentrations in livers from fed rats (0.05 - 0.07 mM, (12,13)). Furthermore, the effect of 6-*P*-gluconate on phosphofructokinase was most pronounced in the range of 0.05 - 0.10 mM fructose-6-*P*. At 0.075 mM fructose-6-*P*, enzyme velocity was linear with respect to 6-*P*-gluconate; 0.04 mM 6-*P*-gluconate, a concentration found in vivo (14), doubled the rate. Data similar to those in figure 1 have been obtained using fresh

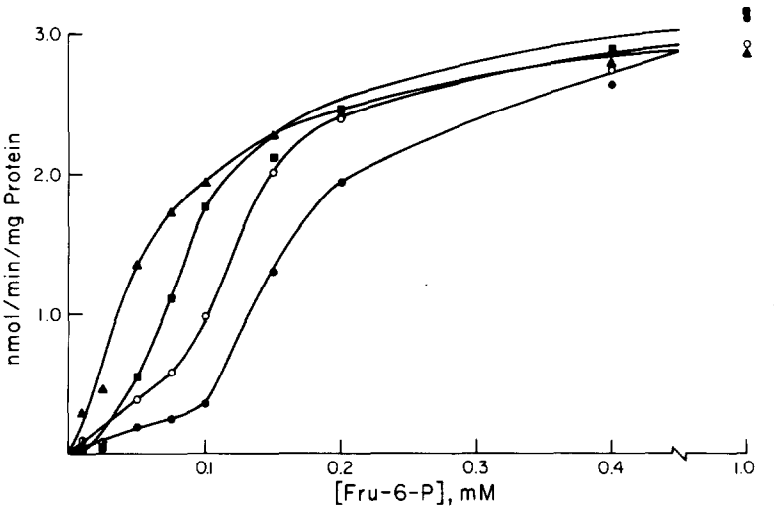

Figure 1. The effect of 6-*P*-gluconate on phosphofructokinase activity. The symbols refer to different concentrations of 6-*P*-gluconate: ●, none; ○, 0.04 mM; ■, 0.08 mM; ▲, 0.2 mM. Each point represents either one or the average of two determinations.

liver from starved rats as the source of phosphofructokinase. Because the 6-*P*-gluconate did not contain fructose-6-*P* or fructose-1,6-P_2 (7), the effect of 6-*P*-gluconate on phosphofructokinase cannot be attributed to contamination by these known effectors of the enzyme.

Attempts were made to study the effect of 6-*P*-gluconate on purified phospho fructokinase, but the first step of the purification procedure (9), heating the supernate at 55° for 3 min, rendered the enzyme insensitive to 6-*P*-gluconate. Furuya and Uyeda (15) have described an activation factor, believed to be a polysaccharide of molecular weight 3000-4000, which is dissociated from phospho-

Table 1. Effects of 6-*P*-gluconate on kinetic parameters of phosphofructokinase

	6-*P*-gluconate (mM)			
	0	0.04	0.08	0.20
Hill coefficient	2.68	2.50	1.76	1.05
$S_{0.5}$ (mM)	0.16	0.12	0.09	0.06

Hill coefficients were calculated from the data in figure 1. Values of $S_{0.5}$ for fructose-6-*P* were estimated from figure 1 using Vm = 3 nmol/min/mg supernatant protein.

fructokinase by heat (16). It may be that the effect of 6-*P*-gluconate on phosphofructokinase requires the presence of the activating factor.

Selective inactivation of the form(s) of phosphofructokinase which respond to 6-*P*-gluconate may be an alternative explanation of the apparent loss of sensitivity of the enzyme to 6-*P*-gluconate after heat treatment. Dunaway and Segal (17) described a peptide factor which stabilized the major form of rat-liver phosphofructokinase (phosphofructokinase-L_2) against thermal inactivation (18). The concentration of the factor in vivo decreased during starvation (18). Livers from starved rats were used as a source of phosphofructokinase in our studies and we observed a substantial loss (50-80%) of phosphofructokinase activity after heating the enzyme. In summary, alterations in the properties of phosphofructokinase by diet or hormones (19) may influence the activation of the enzyme by 6-*P*-gluconate as was the case with pyruvate kinase (7,8).

Studies of the effects of 6-*P*-gluconate on hepatic phosphofructokinase from starved or fed rats, in the presence of other effectors of the enzyme, are being conducted to determine the role of 6-*P*-gluconate in the regulation of hepatic carbohydrate metabolism.

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