SHORT COMMUNICATIONS

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Rabbit intestinal microsomal inorganic pyrophosphate–glucose phosphotransferase: Sugar inhibitor specificity

Classical microsomal glucose-6-phosphate phosphohydrolase (EC 3.1.3.9) of liver, kidney (see refs. 1 and 2), and intestine[3] has been demonstrated to catalyze the synthesis of glucose 6-phosphate *via* potent phosphotransferase activities. These liver and kidney PP_i– and nucleotide–glucose phosphotransferase activities have been the subjects of intensive study during the past 5 years, while some catalytic properties of phosphotransferase activies of the enzyme from small intestine of the rabbit have been described in a recent report from this laboratory[3]. Studies on the inhibition of PP_i–glucose phosphotransferase activity from this latter source by a variety of sugars, sugar derivatives, and polyols are described in the present report, and experimental results obtained are interpreted in terms of structural and configurational features requisite for effective binding of inhibitor molecule to the enzyme.

Analytical methods and sources of most chemicals were as described previously[3,4]. The various sugars, sugar derivatives, and polyols were obtained from Sigma Chemical Co., St. Louis, Mo. Microsomes were prepared from mucosa of rabbit small intestine as described previously[3], and were stored frozen at −20° as a suspension in 0.25 M sucrose solution.

K_i values were assessed as follows: In the presence and absence of other sugar, sugar derivative, or polyol, glucose concentrations were varied in the range 30–180 mM, PP_i levels were maintained constant at 10 mM, and glucose 6-phosphate formation was measured following a 10-min incubation at 30 ± 0.1° by the method described by NORDLIE AND ARION[4]. Data from such studies were plotted in conventional double-reciprocal fashion[5] and K_i values were calculated as described by DIXON AND WEBB[6] for competitive inhibitors. Results of a representative study, involving competitive attached to C-2 (2-amino-D-glucose and 2-deoxy-D-glucose were both effective inhibitors (Table I) and substrates[7,8]). A lack of specificity with regard to the nature of

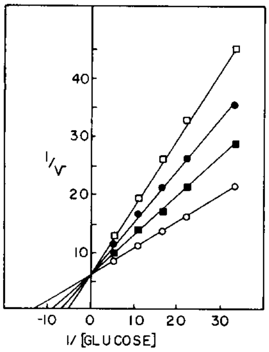

Fig. 1. Kinetics of inhibition of intestinal PP_i–glucose phosphotransferase activity by 3-*O*-methyl-D-glucose. Assay mixtures (pH 6.0) contained, in 1.5 ml, 40 mM sodium cacodylate buffer, 10 mM sodium PP_i, indicated varied concentrations of D-glucose, and no (○), 0.08 M (■), 0.17 M (●), or 0.25 M (□) 3-*O*-methyl-D-glucose. Velocity, *v*, is expressed in terms of µmoles of glucose 6-phosphate formed per 10-min incubation. K_i was calculated as 0.17 M with all three levels of inhibitor.

TABLE I

K_i VALUES FOR VARIOUS SUGARS, SUGAR DERIVATIVES, AND POLYOLS

K_m for glucose = 0.07 M.

Compound	K_i *(M)*	*Compound*	K_i *(M)*
Hexoses and derivatives		*Pentoses*	
2-Amino-D-glucose	0.12	D-Xylose	0.75
2-Deoxy-D-glucose	0.38	L-Xylose	0.90
α-Methylglucoside	0.17	D-Ribose	1.1
β-Methylglucoside	1.4	D-Arabinose	1.8
3-*O*-Methyl-D-glucose	0.17	D-Lyxose	1.9
D-Mannose	0.16	*Tetrose*	
D-Galactose	0.95	D-Erythrose	5.0
D-Fructose	1.7		
		Polyols	
Heptose		*i*-Erythritol	1.7
D-Glucoheptose	1.5	D-Mannitol	2.2

sugar derivatives, and polyols listed in Table I. With the exception of inhibitor concentrations (which ranged from 0.13 to 0.75 M, depending on effectiveness of inhibition, solubility, and availability of inhibitor), experimental details were as described in Fig. 1. In all instances, inhibitions observed were competitive with respect to glucose.

In all studies, a K_m value of 0.07 M for D-glucose was obtained. Of the variety of inhibitors tested, only D-amino-D-glucose, α-methylglucoside, 3-*O*-methyl-D-glucose, D-mannose, and 2-deoxy-2-glucose were found to be reasonably effective inhibitors. For all other tested compounds, K_i values were more than 10 times greater than the K_m (glucose) value (*i.e.*, they exceeded 0.70 M). With the possible exception of D-galactose, these patterns of relative effectiveness of inhibitors correlate well with phosphoryl acceptor specificity for the liver enzyme as reported by Stetten[7]. And while Stetten[7] reported 0.4 M D-galactose to be a reasonably active substrate, Nordlie and Arion[8] carried out kinetic studies and found this compound a relatively poor substrate in the PP_i–glucose phosphotransferase reaction and an ineffective inhibitor of liver microsomal CTP–glucose phosphotransferase activity.

Although earlier studies on the sugar specificity of phosphotransferase activities of glucose-6-phosphatase have been described[7,8], no attempt previously has been made to correlate structural and configurational features of such molecules with their relative effectiveness as inhibitors or phosphoryl group acceptors. It is apparent from a consideration of the compounds listed in Table I that those inhibiting effectively (that is, those with $K_i < 0.40$ M) all have certain structural features in common, as follows: (a) All are aldohexoses or derivatives of aldohexoses capable of forming pyranose rings with an additional $-CH_2OH$ group attached to C-5; and (b) the structure and configuration about C-4 and C-5 are identical with that in D-glucose. In addition, the fact that α-methylglucoside, but not β-methylglucoside, was an effective inhibitor suggests that the α-modification of the pyranose compounds may be the generally-active molecular species. The configuration about C-2 appears unimportant (compare results with D-glucose and D-mannose, Table I), as does the nature of the groups inhibition by 3-*O*-methyl-D-glucose, are presented in Fig. 1, along with additional experimental details. Studies of this sort were repeated with the variety of sugars, the groups attached and configuration about C-3 also is apparent from the observations that (a) 3-*O*-methyl-D-glucose inhibits effectively (Table I) and (b) D-altrose and D-allose, which differ from D-glucose with respect to the configuration about C-3, have been found effective substrates[7]. It thus appears that, while an absolute structural specificity is not exhibited by this enzyme, those sugar molecules with the structural and configuration features given in Fig. 2 bind much more effectively to the enzyme than do other compounds tested.

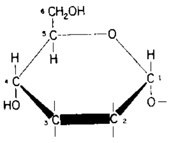

Fig. 2. Haworth formula indicating structural and configurational features essential for efficient binding of aldohexose with enzyme. Details are given in the text.

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Guy and Bertha Ireland Research Laboratory,
Department of Biochemistry,
University of North Dakota Medical School,
Grand Forks, N.D. (U.S.A.)

DAVID G. LYGRE
ROBERT C. NORDLIE

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