α-D-Glucopyranosyl Fluoride: A Substrate of Sucrose Phosphorylase

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α-D-Glucopyranosyl fluoride (glucosyl fluoride) was found to be a substrate for sucrose phosphorylase from Pseudomonas saccharophila. At saturating P_i (0.030 M) the apparent K_m is the same as that of sucrose, but the apparent V_{max} is 2.7 times as great; therefore, glucosyl fluoride is the best glucose donor presently known for sucrose phosphorylase. The enzyme catalyzes hydrolysis of glucosyl fluoride at the same rate as sucrose and glucose-1-P.

Sucrose phosphorylase from Pseudomonas saccharophila has been shown to operate via a covalent glucosyl enzyme intermediate by means of isotope-exchange experiments (1,2), kinetic studies, and direct observation of the glucosyl enzyme (3). In addition to

$$\text{Sucrose} + E \rightleftharpoons \text{Fructose} + \text{Glucosyl } E$$

$$\text{Glucosyl } E + P_i \rightleftharpoons \text{Glucose-1-P} + E$$

P_i and D-fructose, a variety of other substances are capable of accepting the glucosyl group from the glucosyl enzyme. These are analogs of P_i, such as arsenate (4), or of fructose, such as L-sorbose, D-xylulose, L-arabinose, and L-arabinulose (1,5). Water also accepts the glucosyl group, resulting in slow hydrolysis of the glucosyl donor (6). We have observed that α-D-glucopyranosyl fluoride (glucosyl fluoride) is capable of efficiently donating the glucosyl moiety to the enzyme.

EXPERIMENTAL PROCEDURES

Sucrose phosphorylase was assayed by the method of Silverstein et al. (3) in which the production of glucose-1-P from sucrose and P_i is coupled to the reduction of NADP in the presence of phosphoglucomutase and glucose-6-P dehydrogenase. Reaction with glucosyl fluoride as substrate was followed by the same procedure, substituting glucosyl fluoride for sucrose in the presence of 0.030 M P_i. All rate measurements were done at 25°C, pH 7.0. Inorganic phosphate was determined by the Fiske-SubbaRow method (7), fluoride ion by the method of Belcher et al. (8) , and fructose by the ferricyanide method (9). A sample of glucosyl fluoride was kindly provided by Dr. J.E.G. Barnett and sucrose phosphorylase, with specific activity of 3 units/mg and 67 units/mg, was a gift from Dr. R.H. Abeles. The more active enzyme appeared to behave similarly to the less active preparation in kinetic experiments.

RESULTS AND DISCUSSION

When glucosyl fluoride, at a concentration of 5.0 mM, is substituted for sucrose in the standard assay the appearance of NADPH is observed, indicating synthesis of glucose-1-P. The dependence of reaction rate upon enzyme concentration is illustrated in Figure 1. In order to determine the relative maximum velocities and Michaelis constants for glucosyl fluoride and sucrose the rate of glucose-1-P synthesis was determined as a function of concentration of each of the substrates at a high fixed concentration of P_i (0.030 M). Data for glucosyl fluoride and sucrose obtained on the same day using the same dilute enzyme

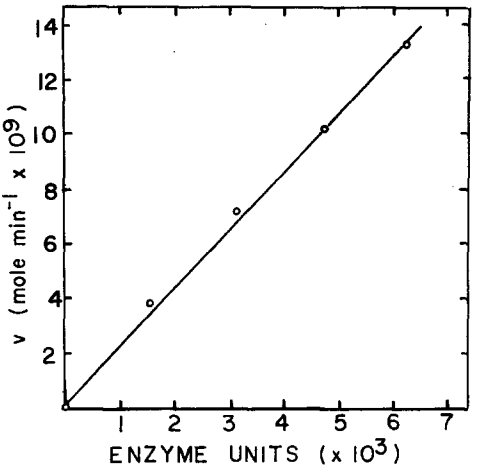

Figure 1. Dependence of Rate of Phosphorolysis of Glucosyl Fluoride Upon Enzyme Concentration. Concentration of P_i is 0.030 M.

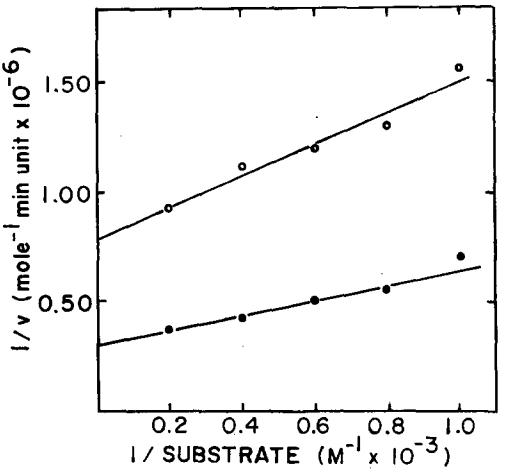

Figure 2. Dependence of Reaction Rate Upon Substrate Concentration. Sucrose, open circles; glucosyl fluoride, closed circles.

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solution are presented in reciprocal form in Figure 2. Kinetic parameters are compared in Table I. It appears that the two glucose donors have the same apparent K_m but that glucosyl fluoride has an apparent V_{max} 2.7 times as great as that of sucrose.

TABLE I

Kinetic Constants for Sucrose Phosphorylase

Substrate	V_{max}(apparent)	K_m(apparent)
	moles min^{-1} $unit^{-1}$ x 10^6	M x 10^3
Sucrose	1.28 ± 0.06	0.92 ± 0.14
α-Glucosyl Fluoride	3.4 ± 0.2	1.17 ± 0.23

P_i is fixed at 0.030 M. Conditions are pH 7.0, 25°. Constants were evaluated by use of a computer program described by Cleland (10) for a hyperbolic function. Limits are standard error.

Since water is known to accept the glucosyl group from the enzyme, the hydrolysis reaction was studied with glucosyl fluoride as substrate. When the reaction was carried out with 5.0 mM glucosyl fluoride in the presence of 0.020 M potassium maleate, liberation of fluoride ion was found to be linear with time. Figure 3 illustrates the rate of hydrolysis as a function of enzyme concentration. The hydrolytic activity is 23 x 10^{-9} moles min^{-1} $unit^{-1}$, in close agreement with the hydrolysis rates of sucrose and glucose-1-P (3). Reduction of the concentration of glucosyl fluoride to 0.5 mM did not significantly reduce the hydrolysis rate; therefore, K_m is probably less than 0.1 mM.

Attempts were made to demonstrate the occurrence of a back-reaction by treating 10 mM glucose-1-P with 0.10 M NaF in the presence of 0.1 unit of enzyme. The small amount of P_i that appeared could be accounted for by hydrolysis. Similar experiments were carried out with sucrose and NaF, but the results were also ambiguous. Although no evidence was obtained for a back-

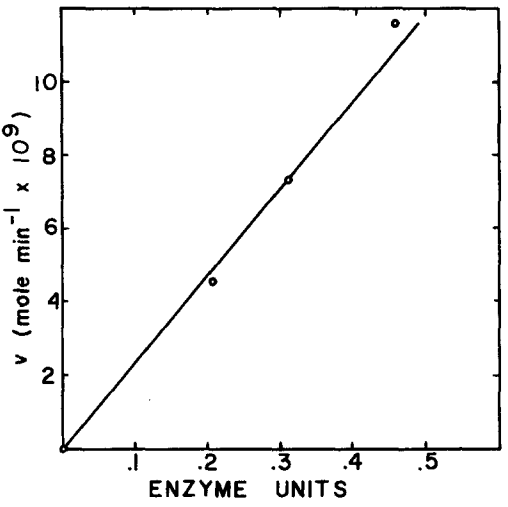

Figure 3. Dependence of Rate of Hydrolysis of Glucosyl Fluoride Upon Enzyme Concentration.

reaction, the possibility cannot be ruled out on the evidence available.

Glycosyl fluorides have been shown by Barnett (11,12,13) to be excellent substrates for certain glycosidases, but this is the first case of a glycosyl fluoride acting as substrate for a glycose-transferring enzyme. Sucrose phosphorylase does not have great specificity for the leaving group of the glucose donor, so it is not altogether surprising that it will accept glucosyl fluoride as a substrate. Glucosyl fluoride is the best donor presently known for this enzyme. Phosphorylase b from rabbit muscle, which has much more stringent specificity requirements for donors and acceptors, fails to utilize glucosyl fluoride as a substrate.

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