A PHOSPHOGLUCOMUTASE FOR β-GLUCOSE-1-PHOSPHATE *

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A metabolic function for β-G1P[1] was first indicated when Fitting and Doudoroff (1952) demonstrated its formation from maltose in Neisseria meningitidis by the following reaction.

$$4\text{-}\alpha\text{-glucosidoglucose} + Pi \rightleftharpoons \beta\text{-G1P} + glucose$$

The present study was initiated in order to explore further transformations of β-G1P in such a system.

When maltose and Pi were incubated with a crude extract of N. meningitidis or N. perflava in the absence of divalent cations, β-G1P was the only phosphate ester which accumulated[2]. However, in presence of Mg^{++} or Mn^{++} the extracts

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1 Abbreviations: G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; Pi, inorganic orthophosphate.

2 This isolated reaction was conveniently employed for the preparation of β-G1P. After removal of Pi as magnesium ammonium phosphate, the twice precipitated barium salt analyzed as follows: $[\alpha]_D^{28} = +14$, calculated as free acid, ($[\alpha]_D^{20} = +13$, Posternak, 1957b), of the total phosphate 2.5% was Pi, < 0.3% was hexose-6-phosphate, and 102% assayed as acid labile G1P.

further converted β-G1P to hexose-6-phosphate (Table I). Under optimal conditions 1 mg. protein of N. perflava extract catalyzed the formation of 0.5 μmoles hexose-6-phosphate in 20 minutes at 37°. Whereas the known phosphoglucomutase does not react with β-G1P (Posternak, 1957a; Sutherland et al., 1949), the Neisseria preparations showed no detectable activity towards α-G1P (Table I).

Table I

Formation of Hexose-6-phosphate from β- and α-Glucose-1-phosphate

Exp.	Enzyme preparation	μmoles of hexose-6-phosphate formed	
		β-G1P as substrate	α-G1P as substrate
1	N. meningitidis extract	0.47	< 0.05
2	N. perflava extract	0.82	< 0.05
3	N. perflava purified enzyme	1.13	< 0.03

Incubation mixtures of experiments 1 and 2 in a final volume of 0.6 ml. contained: crude extract, 4.2 mg. protein; β- or α-G1P, 2.6 μmoles; manganese chloride, 2.0 μmoles and Tris buffer pH 7.4, 20 μmoles. Incubation mixture of experiment 3 in a final volume of 0.3 ml. contained: purified enzyme protein, 21 micrograms; β- or α-G1P, 1.7 μmoles; manganese chloride, 0.25 μmoles and histidine buffer pH 6.5, 8.0 μmoles. The mixtures were incubated for 40 minutes at 37° and the reaction was terminated by heating for 2 minutes at 96°.

Bacterial extracts were obtained by disrupting glucose grown cells with the aid of a Nossal shaker in 0.02 M Tris buffer pH 7.4 containing 0.5% KCl. Hexose-6-phosphates were determined as the sum of G6P and F6P with G6P dehydrogenase containing excess phosphohexoisomerase.

For further investigation, the enzyme acting on β-G1P was purified 90 fold from N. perflava. The purified enzyme had an optimum pH between 6.4 - 6.8 and was completely inactive in the absence of a divalent cation which was inhibitory when present in excess. Since the enzyme showed a net conversion of β-G1P only to G6P (Table II), it seemed feasible to test the reversibility of the reaction. On incubation of G6P with the enzyme, an acid labile product accumulated to the extent of 2.6% of the total hexose phosphate. Hydrolysis for 10 minutes in 0.1 N HCl at 96° afforded nearly equal amounts of Pi and glucose[3]. The product appeared to be β-G1P since the rate of its hydrolysis at 33° in 1 N HCl was identical with that of β-G1P and much higher than that of α-G1P. (cf. Wolfrom et al., 1942).

Table II

Stoichiometry of Glucose-6-phosphate Formation from β-Glucose-1-phosphate

β-G1P consumed	Products formed		
	G6P	F6P	Pi
μmoles	μmoles	μmoles	μmoles
9.3	10.4	< 0.3	< 0.1

The incubation mixture in a final volume of 1 ml. contained: 170 micrograms purified enzyme protein, 10.2 μmoles β-G1P, 0.8 μmoles manganese chloride and 12 μmoles histidine buffer pH 6.5. The mixture was incubated for 40 minutes at 37°.

β-G1P was determined as inorganic orthophosphate (Fiske and SubbaRow, 1925) after hydrolysis for 10 minutes at 96° in 0.1 N HCl. G6P was assayed as shown in legend to Table I, F6P was determined with the Roe reagent (Umbreit et al., 1957).

[3] Assayed with glucose oxidase (Sols and de la Fuente, 1957).

The observed interconversion of β-G1P and G6P bears a formal analogy to the known phosphoglucomutase reaction. The question whether this analogy holds also for the mechanism of the reaction remains open. The findings presented place β-G1P on the general pathway of carbohydrate metabolism in N. perflava and N. meningitidis. It also seems possible that this compound will prove to be involved in specific biosynthetic reactions.

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References

Fiske, C.H., & SubbaRow, Y., J. Biol. Chem., 66, 375 (1925).

Fitting, C., & Doudoroff, M., J. Biol. Chem., 199, 153 (1952).

Posternak, T., in "Methods in Enzymology", Vol. III, (Colowick, S.P. & Kaplan, N.O., eds.), Academic Press Inc., New York 1957. (a) p. 137; (b) p. 136.

Sols, A., & de la Fuente, G., R. Esp. Fisiol., 13, 231 (1957).

Sutherland, E.W., Cohn, M., Posternak, T., & Cori, C.F., J. Biol. Chem., 180, 1285 (1949).

Umbreit, W.W., Burris, R.H., & Stauffer, J.F., in "Manometric Methods", p. 277. Burgess Publishing Co., Minneapolis 1957.

Wolfrom, M.L., Smith, C.S., Pletcher, D.E., & Brown, A.E., J. Am. Chem. Soc., 64, 23 (1942).