METABOLISM AND CELL WALL STRUCTURE OF A MUTANT OF SALMONELLA TYPHIMURIUM DEFICIENT IN PHOSPHOGLUCOSE ISOMERASE [1]

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Received May 6, 1963

Mutations affecting glycolytic enzymes would be of considerable biochemical interest; to our knowledge none has previously been reported. A mutant of S. typhimurium recently isolated by Smith (1963) has now been shown to be deficient in phosphoglucose isomerase activity. The organism is characterized by defective utilization of glucose, galactose, and maltose, but normal utilization of fructose and of gluconate. Because of the defect in isomerase, G-6-P[4] must be metabolized by pathways other than glycolysis, and the mutant strain therefore provides a system for investigation of the contribution of alternate pathways (e.g. the phosphogluconate pathway) to glucose metabolism.

In the absence of exogenous glucose (or galactose) phosphoglucose isomerase is required for the biosynthesis of glucose, which

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1/ Supported in part by grants-in-aid from the National Institutes of Health and the National Science Foundation.

2/ Postdoctoral Fellow, National Foundation.

3/ Research Career Development Awardee, National Institutes of Health.

4/ The following abbreviations are used: glucose 6-phosphate, G-6-P; fructose 6-phosphate, F-6-P; tris (hydroxymethyl) aminomethane, tris; lipopolysaccharide, LPS; 2-keto-3-deoxyoctonate, KDO; 6-phosphogluconate, 6-PG.

is a constituent of the normal cell wall polysaccharide. We have found that the mutant strain forms a lipopolysaccharide lacking glucose and all other normal carbohydrate constituents except hexosamine, heptose, and the recently described component, 2-keto-3-deoxyoctonate (Heath and Ghalambor, 1963).

Genetics — The mutant, SL797, was detected as a galactose-non-fermenting colony after ethyl methane sulphonate treatment of SL698 (S. typhimurium LT2 metA-22 tryB-2 azi-r H1-b H2-e, n, x (colE1)). Mapping by colicine-factor mediated recombination (Smith and Stocker, 1962) shows that the site of mutation is remote from the gal region and very close to metA, though not co-transducible with it. The mutant character is reasonably stable although revertants with wild-type fermentation character are easily obtained.

Hexose metabolism — At 37° the mutant does not ferment glucose, maltose, or galactose, as tested by growth either in sugar peptone water or on indicator agar plates; fructose is fermented, producing acid and gas. Glucose utilization is not completely blocked, for the mutant grows slowly in liquid minimal medium with glucose as the sole carbon source; the time of doubling is 240 min, in contrast with 40 min for the parent. In fructose-minimal medium the doubling times of the two strains are identical (50 min).

The growth pattern of the mutant strain is consistent with a block in the conversion of G-6-P to F-6-P. Phosphoglucose isomerase levels in sonic extracts were therefore determined. Barely detectable levels of isom ase were found in strain SL797 while normal levels of glucokinase and the dehydrogenases of the oxidative pathway were present (Table I).

Metabolism of glucose in the mutant occurs primarily by the oxidative shunt. During growth on glucose-1-C^{14}, 0.7 mole of $C^{14}O_2$ was produced per mole of glucose consumed; the corresponding value for the parent strain was 0.2 (the growth yields were the same for the two strains: 5 μmoles glucose per ml allowed a Δ O.D.$_{580}$ of 1.0). The fact that less than 1 mole of CO_2 is produced from C-1 in the mutant may result from the incomplete block in isomerase or may be due to an additional pathway not involving obligatory oxidation of C-1 to CO_2.

TABLE I

Activity of Enzymes of Glucose Metabolism

Enzyme	Specific Activity (mμmoles/min/mg protein)	
	Parent Strain (SL698)	Mutant Strain (SL797)
Glucokinase	84	88
Phosphoglucose isomerase	535	< 15
Glucose-6-phosphate dehydrogenase	193	160
6-Phosphogluconate dehydrogenase	91	67

Extracts of cells grown on fructose at 37° were prepared by sonication in Tris-Mg^{++} buffer for 10 min at 10 KC. Enzyme activities were determined on the supernatant solution after centrifugation for 30 min at 15,000 xg. Spectrophotometric assays were carried out as follows; all incubation mixtures contained 0.05 M tris, pH 7.65, 0.01 M $MgCl_2$, 0.2 μmole of TPN, and the indicated additions in a volume of 1.0 ml. For assay of glucokinase, 0.5 μmole of glucose, 4 μmoles of ATP, 4 μmoles of $MgCl_2$, and 0.4 unit of G-6-P dehydrogenase (Boehringer) were added; for the isomerase assay, 0.1 μmole of F-6-P and 0.4 unit of G-6-P dehydrogenase; for G-6-P dehydrogenase, 0.14 μmole of G-6-P; for 6-PG dehydrogenase, 0.4 μmole of 6-phosphogluconate. TPNH formation was measured at 340 mμ in the Beckman DU Spectrophotometer.

Although metabolism of glucose by the oxidative pathway is inadequate for rapid growth of the mutant, gluconate is an excellent carbon source (doubling time of 50 min for both mutant and parent). This suggests that gluconate may not be metabolized exclusively via the oxidative shunt. Experiments to test this possibility are in progress.

Temperature effect — Certain characteristics of the isomerase-less mutant are subject to a temperature effect. For example, at 30° mutant and parent gave similar fermentation reactions (all positive); at 30° the growth obtained on fructose-minimal agar was of the "smooth" type and antigen 04 was produced, in contrast to the growth at 37°, which was of the "rough" type. However, even at 30° the growth rate of the mutant on glucose was less than that of the parent, and isomerase activity remained at the low, barely detectable level. In the experiments reported here, cells were grown at 37° unless otherwise specified.

Absence of glucose effect — In strain SL797 glucose did not prevent induction of histidase by histidine, in contrast to the parent strain in which histidase formation was completely inhibited by glucose. Similarly, glucose did not inhibit the rapid growth on fructose. Since the rate of glucose utilization was one-sixth that of the parent, these results are in accord with the catabolite repression theory of the glucose effect (Magasanik, 1961).

Glucose content — If the isomerase reaction provides the only endogenous source of glucose, it would be expected that isomerase-deficient cells would be deficient in glucose derivatives. Acid hydrolysates of mutant cells grown with glucose-free gluconate as sole carbon source contained only 0.13% (dry weight) of glucose; this is one-eighth the glucose content of the parent (1.04%). Glucose was determined with glucose oxidase, and identification of the reactive material confirmed chromatographically.

Cell-wall lipopolysaccharide — The mutant grown at 37° on peptone or on fructose minimal agar gave "rough" type growth and the cells were auto-agglutinable in saline. On glucose-supplemented media growth was of the "smooth" type, the cells were stable in saline and normally agglutinable by anti-04 serum. Cell-wall lipopolysaccharide was isolated and characterized as previously described (Osborn et al., 1962) (Table II). The LPS of the mutant grown with glucose was indistinguishable from that of the parent, but growth on fructose or gluconate resulted in a defective LPS containing only hexosamine, heptose, and 2-keto-3-deoxyoctonate (KDO) together with a trace of glucose. This deficient LPS is similar to that of UDPG-pyrophosphory-

laseless mutants of E. coli (Fukasawa et al., 1962; Sundararajan et al., 1962); it is also closely related to the LPS of Salmonella mutants lacking UDP-galactose-4-epimerase (Nikaido, 1962; Osborn et al., 1962). The latter mutants form LPS which contains glucose and heptose (1:2), whereas the LPS of the isomeraseless mutant contains only traces of glucose (glucose:heptose≅ 1:23). The polysaccharides of both strains contain phosphate in an amount equivalent to that of the heptose.

TABLE II

Carbohydrate Composition of Lipopolysaccharide

Strain	Growth Substrate	Component sugar							
		Hexosamine	KDO	Heptose	Glucose	Galactose	Mannose	Rhamnose	Abequose
Wild type	Fructose	+	+	+	+	+	+	+	+
Isomeraseless	Fructose	+	+	+	tr	-	-	-	-
Isomeraseless	Fructose + glucose	+	+	+	+	+	+	+	+
UDP-Galactose-4-Epimeraseless	Fructose	+	+	+	+	-	-	-	-

Wild type Salmonella LPS is believed to consist of a highly branched complex polysaccharide covalently linked to a glucosamine-containing lipid (Westphal and Luderitz, 1954). On the basis of the present evidence it may be concluded that the innermost core of the polysaccharide moiety consists of a polyheptose phosphate backbone to which glucose and galactose are linked in sequence. The complex outer side chains

may be linked to the core structure through these galactose residues (Nikaido, 1962). Further studies of the structure and biosynthesis of the core polysaccharide are in progress.

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