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Isolation of Mutants of *Escherichia coli* B Altered in Their Ability to Synthesize Glycogen

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Synthesis of the α-1,4 glucosidic linkages in bacterial glycogen is catalyzed by two enzymes (5, 8). The first enzyme, adenosine diphosphate (ADP)-glucose pyrophosphorylase, catalyzes the synthesis of ADP-glucose from adenosine triphosphate (ATP) and glucose-1-phosphate. The second enzyme, ADP-glucose: α-1,4 glucan, α-4-transglucosylase (transferase), catalyzes the transfer of glucose from ADP-glucose to glycogen. Previous experiments have shown that the pyrophosphorylase is an allosteric enzyme; the pyrophosphorylase from *Escherichia coli* is activated by fructose diphosphate and other glycolytic intermediates and is inhibited by 5'-adenylate (7).

This report describes the detection and isolation of mutants of *E. coli* B altered in their ability to synthesize glycogen. A preliminary description of some of these mutants is presented. Mutants with altered glycogen metabolism may be useful in investigating the physiological function of bacterial glycogen and in determining the physiological significance of the allosteric regulation of the pyrophosphorylase.

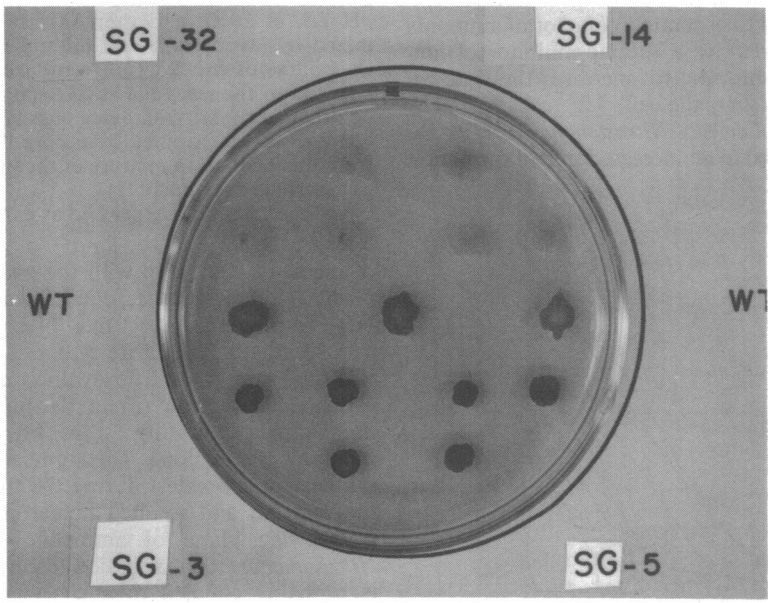

FIG. 1. *Staining of cell colonies with iodine solution. Each quadrant contains colonies of a different mutant (designated by number). Parent colonies (WT) are spotted across the center of the plate. Mutants SG32 and SG14 are "glycogen-deficient." Mutants SG3 and SG5 are "glycogen-excess" mutants.*

"Glycogen" mutants were isolated from a culture treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (100 μg/ml) in tris(hydroxymethyl)-aminomethane (Tris)-maleic buffer, *p*H 6, for 30 min (1). After mutagenesis, the cells were washed and plated immediately on an enriched medium [0.85% KH_2PO_4, 1.1% K_2HPO_4, 0.6% yeast extract (Difco), 1% glucose, 1.5% agar (Difco)]. Alterations in ability to store glycogen were detected by staining cell colonies with an

iodine solution (0.01 M I_2, 0.03 M KI, 5 ml per plate). Similar procedures were used by Chester (2, 3) to detect glycogen-deficient mutants of yeast.

A total of 800 colonies were examined by iodine staining. From these, 80 colonies were selected which had a staining intensity different from that of the parent. These colonies, when picked from replica plates, streaked, and restained, showed a consistent staining intensity with respect to the parent. From the 80 colonies, 6 mutants were isolated which show a change in their glycogen-synthetic enzymes. These mutants are able to grow on a minimal medium [basal salts solution P, *p*H 7.0, 0.12% $(NH_4)_2SO_4$, 0.6% glucose (4)] at approximately the parent growth rate, providing preliminary evidence that they have not suffered mutations in vital pathways. SG32 is an exception, having a 25% longer generation time than its parent on both enriched and minimal media. It is not known whether SG32 has suffered more than one mutation.

Figure 1 shows iodine staining of four mutants and the parent strain after a 24-hr incubation on the enriched medium at 37 C. The mutants in the top two quadrants give a negative stain and are glycogen-deficient, whereas those in the bottom two quadrants give a positive stain and are glycogen-excess mutants (*see* Table 1). The intensities of staining of both the parent strain and the mutants are dependent on the medium and on the time of incubation. However, under the same conditions, "glycogen-excess" mutants always stain darker than or the same as parent colonies, and glycogen-deficient mutants always stain lighter than or the same as parent colonies. Differences in staining are consistent with quantitative differences in glycogen accumulation (Table 1).

Mutant SG32 is a transferase-negative mutant, having only 5% of the parent activity (Table 1). SG32 can synthesize 3 to 14% as much glycogen as its parent. Mutant SG14 has an altered ADP-glucose pyrophosphorylase. When assayed under optimal conditions for the parent enzyme, the SG14 pyrophosphorylase shows 8 to 15% parent activity. If the activator and substrate concentrations in the reaction mixture are increased 10-fold, the mutant enzyme shows 85% of the parent activity. This suggests that the SG14 pyrophosphorylase has decreased binding affinities for its substrates and for the activator, fructose diphosphate. In the enriched medium, SG14 can synthesize 75% as much glycogen as its parent; in the minimal medium, SG14 can synthesize only 50% as much glycogen as its parent.

The glycogen-excess mutant SG3 accumulates 1.3 to 2 times as much glycogen as its parent. In the stationary phase of growth, SG3 has a two- to threefold higher level of pyrophosphorylase and a twofold higher level of transferase than the parent strain. Concomitant with these elevations, there appears to be an increase in the level of

TABLE 1. *Glycogen accumulation and enzyme activities for parent and mutant strains of E. coli B*[a]

Strain	Glycogen accumulated (mg/g)		ADP glucose pyrophosphorylase[b]		ADP glucose: α-1,4 glucan transferase[c]	
	Enriched medium[d]	Minimal medium	Enriched medium	Minimal medium	Enriched medium	Minimal medium
Parent	19.1	19.7	0.43	0.39	0.67	0.85
SG32	0.45	2.9	0.46	0.38	0.033	0.029
SG14	14.2	10.9	0.031 (0.37)[e]	0.061 (0.34)[e]	0.84	0.79
SG3	25.6	37.1	0.83	1.39	1.04	1.65
SG5	53.0	34.7	0.44	0.40	0.58	0.68

[a] Cell extracts for enzyme assays were prepared by suspending 0.5 g of cells in 10 ml of 0.05 M glycylglycine buffer (*p*H 7.0) containing 0.005 M dithiothreitol. The cell suspensions were then exposed to sonic oscillation for 2 to 3 min with a Biosonik III probe sonic oscillator. For cells grown on enriched medium, assays were performed on supernatant solutions obtained by centrifugation at 30,000 × *g*. Transferase activity in the supernatant solutions was 50 to 67% of the total transferase activity. For cells grown on minimal medium, assays were performed on uncentrifuged sonic extracts. Pyrophosphorylase activities were determined by following the synthesis of ^{32}P-ATP from ADP-glucose and ^{32}P-labeled pyrophosphate (7, assay conditions A-1). Transferase activities were determined by following the transfer of ^{14}C-glucose from ADP-^{14}C-glucose to glycogen (6, assay A). The reaction mixture was modified to contain 10 μmoles of Tris-hydrochloride (*p*H 8.5), 2 μmoles of KCl, 2 μmoles of glutathione, 0.1 μmoles of $MgCl_2$, 0.1 mg of bovine plasma albumin, 0.5 mg of rabbit liver glycogen, 0.15 μmole of ADP-^{14}C-glucose (3×10^5 to 6×10^5 counts per min per μmole) and enzyme in a total volume of 0.2 ml. For glycogen determinations, 0.5 g of cells (wet weight) was hydrolyzed in 2 N H_2SO_4 for 1 hr at 100 C. The hydrolysates were neutralized and centrifuged, and then assayed for glucose by the method of glucose-oxidase (Glucostat Technical Bulletin, Worthington Biochemical Corp., Freehold, N.J.). Glycogen is reported as milligrams of anhydrous glucose per gram (wet weight) of cells (9).

[b] Expressed as micromoles of ATP per milligram of protein in 10 min.

[c] Expressed as micromoles of glucose per milligram of protein in 10 min.

[d] Enriched medium, 1% glucose; minimal medium, 0.6% glucose.

[e] Assayed under SG14 optimal conditions.

glycogen phosphorylase. Mutant SG5 accumulates two to three times as much glycogen as its parent strain. SG5 has normal levels of both the pyrophosphorylase and the transferase. However, the SG5 pyrophosphorylase shows altered regulation by its allosteric effectors. It has an increased binding affinity for the activator, fructose diphosphate, and a decreased binding affinity for the inhibitor, adenosine monophosphate.

These results indicate that there is a correlation between the iodine staining of cell colonies and their glycogen accumulation. Further work is being done with mutants SG3 and SG5 to investigate the importance of enzyme synthesis and of the allosteric regulation of enzyme activity in controlling the rate of glycogen synthesis in *E. coli*.

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