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Biosynthesis of the *N*-Acyl-Galactosamine in Cell Walls of *Bacillus subtilis*

F. E. YOUNG[1] AND L. ARIAS

Departments of Microbiology and Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

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Recent studies have demonstrated that glucosylated polyglycerol teichoic acid (TA) is essential for the adsorption of five serologically distinct groups of phage in *Bacillus subtilis* 168 (F. E. Young, Proc. Natl. Acad. Sci. U.S., *in press*). Cell walls of phage-resistant mutants have polyglycerol TA which is devoid of glucose and contains varying amounts of *N*-acetylgalactosamine (GalNAc). Most of the GalNAc in the cell wall is in the teichoic acid fractions (F. E. Young, J. Bacteriol. **92**:839, 1966). As shown in Fig. 1, three steps are required for the transfer of glucose-6-phosphate to polyglycerol phosphate to produce glycosylated TA in which the following enzymes are involved: phosphoglucomutase (PGM), uridine-5'-diphosphate glucose pyrophosphorylase (UDPG pyrophosphorylase), and polyglycerol glucosyl teichoic acid transferase (TAG transferase). Mutants blocked in PGM (class C) and those which physiologically appeared to be deficient in UDPG despite normal levels of the enzymes involved in glucosylation of TA (class B) had only traces of GalNAc in the cell walls, whereas the level of GalNAc in mutants blocked in TAG transferase (class A) was only slightly lower than in the wild-type strain (F. E. Young, Proc. Natl. Acad. Sci. U.S., *in press*). This surprising observation suggested that UDPG may act either as the precursor of GalNAc or as a key compound in the control of biosynthesis of GalNAc. Experiments with another strain, *B. subtilis* ATCC 9945 (L. Glaser, J. Biol. Chem. **234**:2801, 1959), demonstrated that extracts catalyze the interconversion of UDP-*N*-acetylglucosamine (UDP-GlcNAc) and UDP-*N*-acetylgalactosamine (UDP-GalNAc). The presence of enzymatic activity does not necessarily demonstrate that the pathway is physiologically significant, as evidenced by the study on deoxythymidine-5'-diphosphate *N*-acetylglucosamine pyrophosphorylase (R. Kornfield, S. Kornfield, and V. Ginsburg, Biochem. Biophys. Res. Commun. **17**:578, 1964). Therefore, *B. subtilis* 168 was grown under conditions in which the concentration of the carbon source, glucose, was approximately 1,000-fold greater than the concentration of radioactive GlcN. Exogenous GlcN is the preferential source of amino sugars for the bacterial cell wall, presumably by the simultaneous repression of L-glutamine D-fructose-6-phosphate transamidase and induction of specific kinases for GlcN or GlcNAc, as discussed by Davidson (*The Amino Sugars*, Academic Press, Inc., New York, 1966). Thus, the ratio of the specific activity of GlcN to GalN would indicate whether the GalN was derived via epimerization of UDP-GlcN or UDPG. If GlcN was the precursor of GalN, the specific activity of these compounds would be similar; however, if the GalN was derived from UDPG, the specific activity of GalN would be negligible.

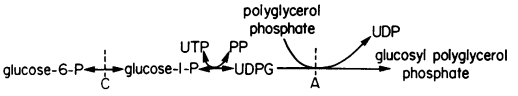

FIG. 1. *Pathway for glucosylation of polyglycerol teichoic acid. Class A and class C phage-resistant mutants have defects at A (polyglycerol glucosyl teichoic acid transferase) and C (phosphoglucomutase), respectively.*

The wild-type phage-sensitive strain *B. subtilis* 168 was grown for 3 hr in minimal growth medium at 37 C (F. E. Young, I. P. Crawford, and J. Spizizen, J. Biol. Chem. **238**:3119, 1963). The cells were harvested and resuspended in the same volume of growth medium supplemented with 0.25 μmole of nonradioactive GlcN per ml and 2.2 μmoles of GlcN-*1*-^{14}C per ml (specific activity, 11.5 mc/mmole). After 210 min of incubation at 37 C with vigorous aeration, the cells were harvested and the cell walls were isolated (F. E. Young, I. P. Crawford, and J. Spizizen, J. Biol. Chem. **238**:3119, 1963). During this incubation, 54% of the radioactive isotope was incorporated into the cells.

The radioactive cell walls were hydrolyzed and

[1] Faculty Research Associate, American Cancer Society.

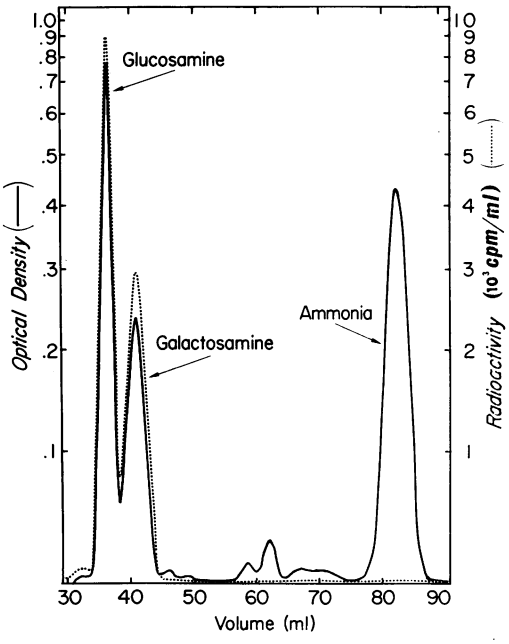

FIG. 2. *Separation of glucosamine and galactosamine on the automatic amino acid analyzer. A sample of hydrolysate of cell walls was eluted from a 12.2 × 0.9 cm column of Aminex A-5 resin (Bio-Rad Laboratories) with sodium citrate buffer (0.25 N, pH 5.28) directly into a fraction collector (0.57 ml/tube). The radioactivity of samples was determined in a Packard (model 3003) scintillation spectrophotometer.*

analyzed on an automatic amino acid analyzer (F. E. Young, J. Bacteriol. **92**:839, 1966). The radioactivity coincided with the peak of ninhydrin reactivity for GlcN and GalN in hydrolysates of cell walls (Fig. 2). The radioactivity of the amino acids in the hydrolysates was negligible. As shown in Table 1, there was only a 9% dilution of the specific activity of GlcN in the cell wall, presumably by endogenous biosynthesis of GlcN.

TABLE 1. *Specific activity of amino sugars in cell walls of Bacillus subtilis 168*

Amino sugar	Location	Specific activity (counts per min per nmole)
Glucosamine	Medium[a]	101.75
Glucosamine	Cell wall	93.17
Galactosamine	Cell wall	84.16
Muramic acid	Cell wall	77.05

[a] Radioactivity of medium at the onset of the 210-min incubation.

The high specific activity of GalN and muramic acid indicates either: (i) that GlcN is the primary precursor of both of these compounds under physiological conditions or (ii) that exogenous GlcN induces an abnormal pathway for the biosynthesis of GalN and muramic acid in cell walls. The observation that mutants defective in PGM (Fig. 1), a defect which does not influence the normal biosynthesis of GlcN in peptidoglycan or TA, are unable to utilize this hypothetical abnormal pathway for the biosynthesis of GalN, the in vitro studies of L. Glaser (J. Biol. Chem. **234**:2801, 1959), and the results of the isotopic experiments discussed by Davidson (*The Amino Sugars*, Academic Press, Inc., New York, 1966), make the second interpretation unlikely. Therefore, the extremely low level of GalN in cell walls of class B and class C mutants is probably due to a regulation of biosynthesis of GalN in TA by the intracellular levels of UDPG. The mechanism of such a hypothetical control process remains to be elucidated.

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