JOURNAL OF BACTERIOLOGY, July 1972, p. 290-291
Vol. 111, No. 1

Printed in U.S.A.

Mutations Affecting Amino Sugar Metabolism in *Escherichia coli* K-12

R. P. HOLMES AND R. R. B. RUSSELL

CSIRO Division of Food Research, Meat Research Laboratory, Cannon Hill, Queensland, Australia 4170

Received for publication 14 March 1972

The genetic loci determining *N*-acetylglucosamine-6-phosphate deacetylase and glucosamine-6-phosphate deaminase have been located at min 16 on the *Escherichia coli* K-12 genetic map.

Amino sugars in *Escherichia coli* are found in at least two major macromolecules of the cell envelope, peptidoglycan and lipopolysaccharide. The use of mutant strains lacking the enzymes concerned in amino sugar metabolism (Fig. 1) thus should be of value in cell envelope studies, and the construction of strains lacking glucosamine-6-phosphate deaminase should prove a useful way of increasing the specificity of incorporation of radioactively labeled glucosamine into the envelope. In wild-type cells exogenous glucosamine is largely routed into dissimilatory pathways via glycolysis (2, 5).

Recently both Sarvas (3) and Wu and Wu (6) described the isolation of mutants lacking glucosamine-6-phosphate synthetase, the responsible mutation being mapped in the *glmS* gene at min 74 on the genetic map by Wu and Wu (6). Mutants lacking either *N*-acetylglucosamine-6-phosphate deacetylase or glucosamine-6-phosphate deaminase have been isolated by White (5), and both he and Dobrogosz have investigated some aspects of the physiology of the mutants (1, 2, 5). We report here the mapping of the mutations affecting these enzymes and, because the most readily observable phenotype of these strains is an inability to grow on *N*-acetylglucosamine as sole carbon source, we propose to name the gene for the deacetylase *nagA* and that for the deaminase *nagB*.

To map the *nagA* and *nagB* genes, spontaneous streptomycin-resistant derivatives of White's deacetylaseless and deaminaseless strains were prepared. These were then used as recipients in conjugation experiments with a range of nine donor strains with different origins of chromosome transfer. We found that strain KA6 (carrying the F8 episome) transferred the ability to grow on 0.2% *N*-acetylglucosamine as carbon source (Nag^+ phenotype) within a few minutes of mixing with either recipient, but that an HfrC donor did not transfer Nag^+ early. Both *nagA* and *nagB* therefore must lie between the origin of chromosome transfer of HfrC at min 13 and that of F8 at min 17 (Fig. 2).

Transduction experiments, following the methods of Young et al. (7), were performed in which bacteriophage P1kc was propagated on $nagA^-$ or $nagB^-$ strains and used to introduce wild-type alleles into strains with mutations in a number of the genes in the 13- to 17-min region (Fig. 2). It was found that both *nagA* and *nagB* were cotransducible with *ubiF* at frequencies of 69 and 72% and with *gltA* at frequencies of 15 and 17%, respectively. Because *ubiF* and *gltA* are only 9% cotransducible, the *nag* markers must lie between them, close to min 16 on the map (Fig. 2).

These results suggested that the deacetylase and deaminase genes were very close to each other, and their linkage was confirmed by using P1 grown on a $nagA^-$ strain to introduce into a $nagB^-$ strain the ability to grow on glucosamine as carbon source. The transductants were tested for their ability to grow on *N*-acetylglucosamine, and it was found that 240 out of 246 transductants (97.6%) had received the $nagA^-$ character along with $nagB^+$. Because the absence of deaminase precludes growth on *N*-acetylglucosamine, it is not possible to perform the precise reciprocal transduction (investigation of the inheritance of $nagB^-$ as an unselected marker among $nagA^+$ transductants). However, when a recipient, which carries both $nagA^-$ and an unlinked met^- mutation, was used in a transduction with a $nagB^-$ donor, in one experiment 5,180 transductants were obtained when met^+ was selected, but

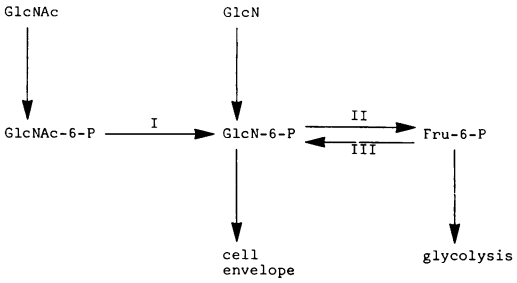

FIG. 1. *Pathways for amino sugar metabolism in E. coli. Abbreviations: GlcNAc, N-acetylglucosamine; GlcN, glucosamine; P, phosphate; Fru, fructose. Enzymes: I, N-acetylglucosamine-6-phosphate deacetylase; II, glucosamine-6-phosphate deaminase (EC 5.3.1.10); III, glucosamine-6-phosphate synthetase (EC 2.6.1.16).*

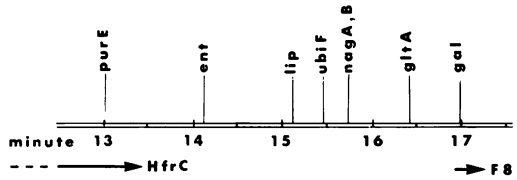

FIG. 2. *Segment of the genetic linkage map of E. coli; based on data presented in this report and in references 4 and 7.*

only 2% of that number (88 transductants) when the selection was for ability to grow on *N*-acetylglucosamine as sole carbon source, indicating that 98% of the *nagA*$^+$ recombinants had also received *nagB*$^-$.

The data presented above place the *nagA* and *nagB* genes at min 16 on the *E. coli* K-12 linkage map. Deacetylase and deaminase are inducible enzymes, both being induced by *N*-acetylglucosamine, but only the deaminase being induced by glucosamine (5). The extremely close linkage of the two genes seems likely to have some significance with regard to the regulation of their expression.

We would like to thank R. J. White and I. G. Young for providing us with *E. coli* strains.

LITERATURE CITED

1. Bernheim, Naomi J., and W. J. Dobrogosz. 1970. Amino sugar sensitivity in *Escherichia coli* mutants unable to grow on *N*-acetylglucosamine. J. Bacteriol. **101**:384-391.
2. Dobrogosz, W. J. 1968. *N*-acetylglucosamine assimilation in *Escherichia coli* and its relation to catabolite repression. J. Bacteriol. **95**:585-591.
3. Sarvas, M. 1971. Mutant of *Escherichia coli* K-12 defective in D-glucosamine biosynthesis. J. Bacteriol. **105**: 467-471.
4. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155-175.
5. White, R. J. 1968. Control of amino sugar metabolism in *Escherichia coli* and isolation of mutants unable to degrade amino sugars. Biochem. J. **106**:847-858.
6. Wu, H. C., and T. C. Wu. 1971. Isolation and characterization of a glucosamine-requiring mutant of *Escherichia coli* K-12 defective in glucosamine-6-phosphate synthetase. J. Bacteriol. **105**:455-466.
7. Young, I. G., L. M. McCann, P. Stroobant, and F. Gibson. 1971. Characterization and genetic analysis of mutant strains of *Escherichia coli* K-12 accumulating the ubiquinone precursors 2-octaprenyl-6-methoxy-1,4-benzoquinone and 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone. J. Bacteriol. **105**:769-778.