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Alternative Route for Biosynthesis of Amino Sugars in *Escherichia coli* K-12 Mutants by Means of a Catabolic Isomerase

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By inserting a λ p*lac*Mu bacteriophage into gene *glmS* encoding glucosamine 6-phosphate synthetase (GlmS), the key enzyme of amino sugar biosynthesis, a nonreverting mutant of *Escherichia coli* K-12 that was strictly dependent on exogenous *N*-acetyl-D-glucosamine or D-glucosamine was generated. Analysis of suppressor mutations rendering the mutant independent of amino sugar supply revealed that the catabolic enzyme D-glucosamine-6-phosphate isomerase (deaminase), encoded by gene *nagB* of the *nag* operon, was able to fulfill anabolic functions in amino sugar biosynthesis. The suppressor mutants invariably expressed the isomerase constitutively as a result of mutations in *nagR*, the locus for the repressor of the *nag* regulon. Suppression was also possible by transformation of *glmS* mutants with high-copy-number plasmids expressing the gene *nagB*. Efficient suppression of the *glmS* lesion, however, required mutations in a second locus, termed *glmX*, which has been localized to 26.8 min on the standard *E. coli* K-12 map. Its possible function in nitrogen or cell wall metabolism is discussed.

In *Escherichia coli* and other gram-negative bacteria, the amino sugars *N*-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN) are essential components of two major macromolecules of the cell envelope, peptidoglycan (22) and lipopolysaccharide (LPS) (26) (Fig. 1). As precursors for cell wall molecules, amino sugars derived either from intracellular biosynthesis or from exogenous sources are indispensable for cell growth. The first step in their biosynthesis is the formation of D-glucosamine 6-phosphate (GlcN-6-P) from D-fructose 6-phosphate (Fru-6-P) and L-glutamine by GlcN-6-P synthetase (amidotransferase; EC 2.6.1.16). The gene for this enzyme, *glmS*, maps at 84 min (39). It has been cloned and sequenced as part of the *oriC* region of the chromosome (35). Mutants defective for this gene are strictly dependent on the presence of exogenous GlcN or GlcNAc. Starvation for these compounds from a culture causes a rapid loss of viability and cell lysis (29, 39).

Apart from their essential function in cell wall synthesis, amino sugars can be utilized as carbon sources. The enzymes of amino sugar catabolism, GlcNAc-6-P deacetylase (EC 3.5.1.25) and GlcN-6-P isomerase (deaminase; EC 5.3.1.10) (Fig. 1), are encoded by the genes *nagA* and *nagB*, respectively, which are located at 15.6 min on the *E. coli* K-12 chromosome (12, 17, 25). Enzyme II^{Nag}, the product of the closely linked *nagE* locus, and the enzyme II^{Man}-enzyme III^{Man} complex (genes *manXYZ*; map position 40 min) are the major transport systems for amino sugars. Both take up and concomitantly phosphorylate their substrates via the phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS) (15, 17, 37).

The synthesis of GlcN-6-P from Fru-6-P and L-glutamine and the dissimilation of GlcN-6-P to Fru-6-P and NH_3 by the *glmS* and *nagB* gene products, respectively, represent a classical futile cycle, resulting in the formation of L-glutamate and NH_3 from glutamine at the expense of ATP (Fig. 1). To avoid such a cycle, a strict coordination of the expression and activity of all enzymes involved in the anabolism and catabolism of amino sugars is necessary. Earlier studies described an inverse regulation of catabolic and biosynthetic enzymes, GlcNAc inducing the isomerase and repressing the synthetase (13, 36). Both enzymes were claimed to be physiologically irreversible (39). In vitro, however, the isomerase but not the synthetase was found to react in both directions, although the equilibrium favored the catabolic reaction (5, 7, 8, 21).

In this paper, we describe the isolation of a nonreverting *glmS*-negative mutant that was used to select $GlmS^+$ suppressor mutations. As a result of two mutations, the catabolic isomerase was observed to function as a biosynthetic enzyme enabling the cells to use this alternative route for amino sugar formation. The regulation of amino sugar metabolism and its relationship to cell wall biosynthesis and ammonia assimilation were also analyzed.

MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. Descriptions of the *E. coli* K-12 strains used are given in Table 1. All strains with the designation LR, JLV, or STL are derivatives of strain LR2-162 (17) and have the following general genotype: F^- *thi-1 argG6 metB1 hisG1 manI161 manA162* (=*manXYZ*) *galT6 xyl-7 supE44 rspL104* Δ*phoA8 tonA2*. Strain JLV300 is a gal^+ Δ*lacU169* derivative of LR2-162 that lacks the D-mannose phosphotransferase system.

Phage P1 *kc* was routinely used for transduction of chromosomal markers as described previously (16). Chromosomal *lacZ* fusions were introduced with λ p*lac*Mu bacteriophages, which also confer resistance to kanamycin, as described by Bremer et al. (4).

All plasmids used in this study will be described elsewhere (A. P. Vogler and J. W. Lengeler, Mol. Gen. Genet., in press). In brief, pAVL10 harbors the complete *nagBAR* operon from the closely related species *Klebsiella pneumoniae* on a 5.1-kilobase *Eco*RI fragment cloned into the multicopy vector pBR328, whereas pAVL14 expresses only the *nagA* gene. Plasmid F'nag-1 contains the complete *nag* region, including *nagE*, from *K. pneumoniae* integrated into F' lac^+. In complementation analyses, recombination of these cloned genes with the *E. coli* K-12 chromosomal *nag* genes has never been observed.

Media and growth conditions. Phosphate-buffered minimal

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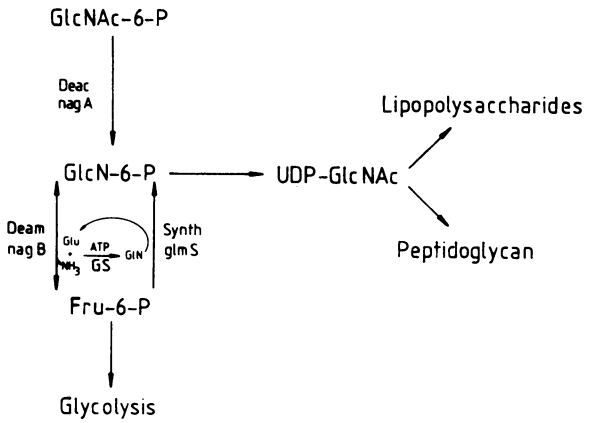

FIG. 1. Metabolism of amino sugars in enterobacteria. Abbreviations: Deac, GlcNAc-6-P deacetylase; Deam, GlcN-6-P isomerase; Synth, GlcN-6-P synthetase; GS, glutamine synthetase; Glu, L-glutamate; GlN, L-glutamine. Gene symbols are as given by Bachmann (2).

medium normally containing 10 mM $(NH_4)_2SO_4$, tryptone broth (LT), and MacConkey agar plates with 1% (wt/vol) of the carbohydrate to be tested have been described elsewhere (16). For the growth of *glmS* mutants, GlcNAc or GlcN was added to 200 mg/liter. If necessary, complex media were supplemented with 10 mM NH_4Cl. In minimal medium, 0.2% carbohydrates or 0.5% vitamin-free Casamino Acids hydrolysate (Difco Laboratories, Detroit, Mich.) was used as an energy source. The *fadR* marker was tested on minimal medium agar plates containing 0.2% proteose peptone, 5 mM Sodium octanate, and 0.0025% 2,3,5-triphenyl-2H-tetrazolium chloride (30).

Transport and enzyme assays. All enzyme assays were done with exponential cells grown in minimal medium containing 0.5% Casamino Acids or in LT medium. The enzyme activities of GlcN-6-P isomerase and GlcNAc-6-P deacetylase were determined in crude extracts of ultrasonically treated cells as described by Bates and Pasternak (3). The formation of GlcN-6-P was assayed essentially as described by Ghosh and Roseman (10). Crude extracts were incubated with 10 mM Fru-6-P and 10 mM glutamine or 15 mM NH_4Cl in 0.1 M phosphate buffer (pH 7.0). The reaction was carried out at 37°C and terminated by boiling for 3 min. The amount

TABLE 1. Bacterial strains, plasmids, and bacteriophages

Strain, plasmid, or phage	Genotype or phenotype[a]	Origin	Reference or source
Strains			
C600	*thi-1 thr-1 leu-6 supE44 lacY1 tonA21 rap zch*::Tn*10*		11
E110	*thi his-4 proA2 argE3 thr-1 leuB6 ara lacY1 galK2 xyl-5 mtl-1 supE44 tsx33 rpsL31 glmS*		39
GG283	*lit-6*(Con) *fadR*::Tn*10*		11
JLV3	*ilvG zid-1*::Tn*10*		33
JLV300	Δ*lacU169 gal*$^+$	LR2-162	
JLV300S	Φ(*glmS-lacZ*)	JLV300 × λ p *lac*Mu15	
JLV330	Φ(*glmS-lacZ*)	JLV300 × P1.JLV300S	
JLV330-2	GlmS$^+$	GlmS$^+$ derivative of JLV330	
JLV330-3	GlmS$^+$	GlmS$^+$ derivative of JLV330	
JLV343-2	*glmS*$^+$ *zid*::Tn*10 nag-3302*	JLV330-2 × P1.JLV3	
JLV343-3	*glmS*$^+$ *zid*::Tn*10 nag-3303*	JLV330-3 × P1.JLV3	
JLV344-2	Φ(*glmS-lacZ*) *nag-3302 kdp*::Tn*10*	JLV330 × P1.JLV345-2	
JLV344-3	Φ(*glmS-lacZ*) *nag-3303 kdp*::Tn*10*	JLV330 × P1.JLV345-3	
JLV345-2	GlmS$^+$ *kdp*::Tn*10 nag-3302*	JLV330-2 × P1.TK2309	
JLV345-3	GlmS$^+$ *kdp*::Tn*10* nag-3303	JLV330-3 × P1.TK2309	
JLV350	HfrH *nag-3303 kdp*::Tn*10*	CA8000 × P1.JLV345-3	
KL99	Hfr PO42 *thi-1 lac-42 relA1*		20
KL208	Hfr PO43 *relA1*		20
KL208-1	Hfr PO43 *nag-3303 kdp*::Tn*10*	KL208 × P1.JLV345-3	
LBG1623-1	*trp ilv purC rpsL zch*::Tn*10*		28
LR2-168	F$^-$ *ptsG168 manI161 manA162 nagE167*		18
PA340	*thi-1 thr-1 leuB6 hisG1 argH1 gdh lacY1 gal-6 malP,Q1 xyl-7 mtlA2 rpsL9 tonA2 tsx-68 supE44 gltB*		2
STL2	Φ(*glmS-lacZ*) *glmX*(pAVL10)	JLV330	
STL3	Φ(*glmS-lacZ*) *glmX*	STL2 cured for pAVL10	
STL4	Φ(*glmS-lacZ*) *glmX nag-3303 kdp*::Tn*10*	STL2 × P1.JLV345-3	
STL8	Φ(*glmS-lacZ*) *glmX nag-3303*	STL4 Tet^s	
STL13	Φ(*glmS-lacZ*) *glmX nag-3303 trp zch*::Tn*10*	STL8 × P1.LBG1623-1	
TK2309	*thi nagA kdp*::Tn*10 trpA405 trkD21 rha lacZ*	W. Epstein	
Plasmids			
pAVL10	*nagB*$^+$*A*$^+$*R*$^+$	Vogler and Lengeler, in press	
pAVL14	*nagA*$^+$	Vogler and Lengeler, in press	
F′ nag-1	*nag*$^+$ (*K. pneumoniae*)	Vogler and Lengeler, in press	
Phage			
λ p*lac*Mu15	*imm 'lacZ lacY*$^+$ *lacA' 'ara' xho*::*kan* Mu (c*Its*62 *ner*$^+$ *A*am1093′-*S*		E. Bremer
λ p*lac*Mu507	c1857 *S*am7 Mu (c*Its*62 *ner*$^+$A$^+$B$^+$)		4

[a] Phenotypic GlmS$^+$ suppressor mutants grow without hexosamines on LT but not on MacConkey agar plates. The complete genotype of JLV300, a derivative of LR2-162, is given in Materials and Methods.

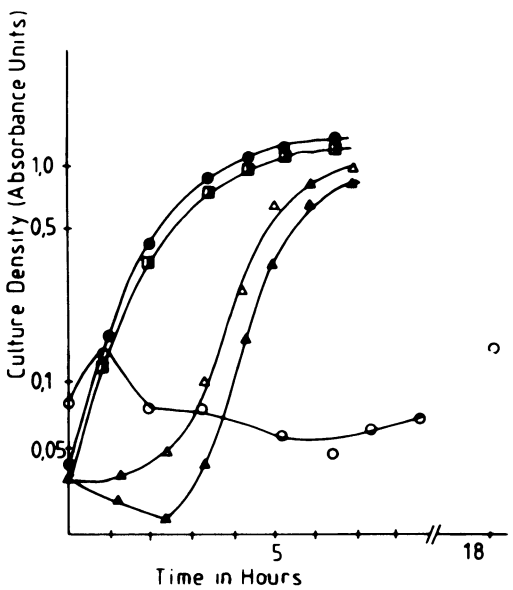

FIG. 2. Growth of *glmS* mutants in the presence and absence of GlcNAc. Cells were grown overnight in LT medium containing 200 mg of GlcNAc per liter, washed twice, and resuspended in LT medium without (open symbols) and with (closed symbols) 200 mg of GlcNAc per liter. The optical density of the culture at 650 nm was monitored. Symbols: □, ■, JLV300 *glmS*$^+$ *nag*$^+$; ○, ●, JLV330 Φ(*glmS-lacZ*) *nag*$^+$; △, ▲ JLV330-3 GlmS$^+$.

of amino sugar produced was determined after N acetylation under alkaline conditions by a modified Elson-Morgan procedure (19). The assay of glutamate dehydrogenase was carried out as described by Doherty (9) by measuring the reductive conversion of 2-ketoglutarate and ammonia to glutamate.

Transport of [^{3}H]GlcNAc (10 μM; Amersham Buchler, Braunschweig, Federal Republic of Germany) was assayed in exponentially growing cells as described previously (17).

RESULTS

Isolation of a nonreverting *glmS* mutation. To isolate a nonreverting *glmS* mutation, strain JLV300 (Δ*lacU169*) was infected with the hybrid phage λ p*lac*Mu15. Among 10^4 independent insertions, one mutant, named JLV300S, had the expected GlmS$^-$ phenotype. It showed an absolute requirement for GlcNAc or GlcN and thus resembled the authentic *glmS* mutant E110 characterized previously (39) (Fig. 2 and Table 2). The λ p*lac*Mu insertion of strain JLV300S was transduced to the parental strain JLV300 with the help of phage P1, and a GlmS$^-$ Km^r transductant, JLV330, was used in all further experiments (Table 2). By using the *ilvG* marker and a closely linked *zid*::Tn*10* insertion (34), it was shown by P1 transduction that these were closely linked to the GlmS$^-$ Km^r mutations of strain JLV330 (data not shown). These results allocate the λ p*lac*Mu insertion close to or into the *glmS* locus at 83.7 min of the chromosome.

Selection and characterization of suppressor mutations. Phage λ p*lac*Mu15 is very stably integrated, and no spontaneous loss from its target gene or transposition to new locations has been observed thus far. In contrast to strain E110, with its high reversion rate (39; unpublished results), strain JLV330 could therefore be used to isolate second-site mutations suppressing the *glmS* defect. Phenotypic revertants were selected on tryptone plates without amino sugars, where they appeared after 2 to 3 days of incubation in patch streaks at an apparent rate of 10^{-9} to 10^{-10}. About 50 independent mutants were isolated, purified, and tested for phenotype.

The majority resembled mutant JLV330-3 (Table 2 and Fig. 2). They grew independently of exogenous hexosamines on LT medium with a normal generation time but not on MacConkey plates. Furthermore, a characteristic growth lag in liquid LT medium could not be shortened by the addition of GlcNAc, nor could any hexosamine-independent colonies be isolated on MacConkey plates. Obviously, true *glmS*$^+$ reversions never occurred. Instead, all mutants expressed the *nag* operon and the corresponding enzymes GlcNAc deacetylase and GlcN-6-P deaminase as well as GlcNAc transport activity (which is already semiconstitutive in wild-type strains [33]) constitutively and at a level that significantly exceeded the fully induced wild-type level (Table 2). The mutations responsible for this elevated expression map in the *nag* regulon, as shown by cotransduction with the closely linked *kdp*::Tn*10* insertion of strain TK2309, and more precisely in the newly discovered repressor gene *nagR* (see below; data not shown).

A minor class (about 10%) of suppressor mutants not only grew in the absence of hexosamines on LT plates but had become sensitive to exogenous GlcNAc such that growth on MacConkey plates was possible only in the presence of GlcN. When transduced to a *glmS*$^+$ phenotype with the help of *zid-1*::Tn*10*, transductants of both classes of suppressors retained constitutive *nag* gene expression and regained the ability to grow on MacConkey plates (data not shown). Transductants of the minor class, however, had all of the

TABLE 2. Phenotypes of *glmS* mutants and GlmS$^+$ suppressor strains

Strain	Relevant genotype	Phenotype[a]		Activity (nmol/mg of protein per min)[b]					
				Deaminase		Deacetylase		Transport	
		LT	LT + GlcN	−	+	−	+	−	+
JLV300	*glmS*$^+$ *nag*$^+$	+	+	0.08	2.55	23	220	3.7	5.9
JLV330	Φ(*glmS-lacZ*) *nag*$^+$	−	+	0.13	1.52	74	195	3.7	6.3
JLV330-3	Φ(*glmS-lacZ*) *nag-3303*	+	+	22.60	32.00	560	740	7.5	13.3
JLV344-3	Φ(*glmS-lacZ*) *nag-3303*	−	+	25.20	28.20	420	620	10.8	12.4
JLV330-3 (F′ *nag-1*)	Φ(*glmS-lacZ*) *nag-3303* (*nag*$^+$)	−	+	0.11	2.10	<5	125	ND	ND
JLV330(pAVL10)	Φ(*glmS-lacZ*) *nag*$^+$ (*nag*$^+$)	−	+	35.60	25.00	474	504	ND	ND
E110	*glmS nag*$^+$	−	+	ND	ND	ND	ND	ND	ND

[a] Growth on LT plates with or without GlcNAc or GlcN (200 mg/liter) was scored as positive (+) or negative (−).
[b] Activity after growth of cells in LT plus 20 μg of GlcN per ml and 0.2% GlcNAc where indicated (+) for induction. ND, Not determined.

TABLE 3. Rates of GlcN-6-P formation in cell extracts[a]

Strain	Induction	GlcN-6-P synthesized (nmol/mg of protein per 30 min)		Deaminase activity (nmol/mg of protein per min)
		Glutamine	NH_4Cl	
LR2-162	−	31	≦1	0.8
	+	7	11	6.5
JLV330	−	≦1	≦1	0.4
	+	≦1	10	5.1
JLV330-3	−	≦1	65	32.4
	+	≦1	28	21.8

[a] Crude extracts of cells grown in LT medium containing 20 μg of GlcN per ml were tested for rate of GlcN-6-P formation as described in Materials and Methods, using either 10 mM glutamine or 15 mM NH_4Cl. The extracts were tested for deaminase activity in a parallel assay. If indicated (+), GlcNAc was added as an inducer to a final concentration of 0.2% 60 min before the cells were harvested.

properties of other polar *nagA* mutant strains described thus far (17, 36), i.e., sensitivity to GlcNAc but not GlcN, lack of GlcNAc-6-P deacetylase activity, a defect complemented by the $nagA^+$ plasmid pAVL14, and constitutive expression of the GlcN-6-P deaminase. This phenotype is due to the fact that polar *nagA* mutations also prevent expression of the downstream *nagR* gene and consequently synthesis of the repressor (Vogler and Lengeler, in press).

Biosynthesis of amino sugars in *glmS* suppressor mutants. Being a common feature of all *glmS* suppressor mutants, constitutive deaminase expression could be responsible for the amino sugar-independent cell growth. When the mutants were complemented with the single-copy plasmid F'nag-1, harboring the complete *nag* regulon from *K. pneumoniae*, including the *trans*-active repressor gene *nagR*, expression of the chromosomal *nag* genes returned to the inducible wild-type level (Table 2). At the same time, the mutants again became strictly dependent on the supply of exogenous hexosamine, further substantiating the correlation of high deaminase expression and amino sugar dependence.

In the suppressor mutants, the deaminase itself may act as a biosynthetic enzyme (Fig. 1). To test this possibility, we measured the formation of GlcN-6-P from Fru-6-P in crude cell extracts (Table 3), using as a cosubstrate either ammonia to test the biosynthetic function of the isomerase or deaminase or glutamine for the assay of GlcN-6-P synthetase activity. In the wild-type strains, hexosamines were synthesized exclusively by the GlmS, unless the deaminase was induced, which resulted in a significant rate of ammonia-dependent formation of GlcN-6-P (and reduced synthetase activity, as described earlier [36]). In contrast, JLV330 and the suppressor mutant JLV330-3 no longer exhibited any synthetase activity, and the latter strain was able to synthesize GlcN-6-P from ammonium at a very high rate even without previous induction of the *nag* enzymes. Obviously, the constitutively expressed catabolic isomerase can substitute for the biosynthetic GlcN-6-P synthetase in vivo and in cell extracts. Addition of neither GlcN-6-P transacetylase nor acetyl coenzyme A was necessary. Although it has not been shown directly that the velocity of the amination reaction is sufficient under physiological concentrations of ammonium, it seems clear that the overexpressed deaminase fulfills the amino sugar requirement of *glmS* mutants under the growth conditions used here.

Involvement of a second locus in the suppression of *glmS* mutations. To test whether derepression of the deaminase alone is sufficient to suppress the *glmS* defect, the *nag* region from suppressor strains was transduced to the parental strain JLV330, selecting for the adjacent *kdp*::Tn*10*. All transductants (e.g., JLV344-3; Table 2) that acquired the donor *nag* regulon showed high constitutive enzyme activities. Despite its high isomerase level, the transductant JLV344-3 did not exhibit the amino sugar-independent $GlmS^+$ phenotype of its parent, JLV330-3 (Tables 2 and 4). Likewise, transformation of pAVL10, a multicopy plasmid expressing deaminase and deacetylase to very high levels (Table 2), did not confer amino sugar prototrophy to the *glmS* strain JLV330 (Table 4). Therefore, derepression of the deaminase alone could not be responsible for the *glmS* suppression.

The transformant JLV330(pAVL10), when grown in the absence of external amino sugars, gave rise at an accelerated rate to $GlmS^+$ colonies, i.e., within 24 h of incubation instead of the 2 to 3 days required for JLV330. These colonies, one of them named STL2, were shown to be not true $glmS^+$ revertants by their failure to grow in the absence of amino sugars on MacConkey plates and the lack of GlmS activity (data not shown), thus resembling strain JLV330-3. When strain STL2 was cured of plasmid pAVL10, the resulting colonies (e.g., strain STL3; Table 4) again became auxotrophic for hexosamines. Reintroduction of an overexpressed *nagB* gene either by multicopy plasmids or by transduction of the *nag-3303* allele from strain JLV330-3 (as shown for strain STL4; Table 4), however, led to reversion to a $GlmS^+$ phenotype. Therefore, we concluded that STL2 acquired a second mutation which, in combination with an overproduced deaminase, was responsible for the *glmS* suppression and that neither the mutation in *glmX* nor the

TABLE 4. Phenotypes of *glmX* mutants

Strain	Genotype[a]	Phenotype[b]					
		LT	LT + GlcNAc[c]	LT + NH_3[c]	MC + Lac	MC + Lac + GlcNAc[c]	MC + GlcNAc
JLV330	nag^+	+	+	−	−	w	r
JLV344-3	*nag-3303*	−	+	+	−	w	r
JLV330(pAVL10)	nag^+ ($nagB^+A^+R^+$)	−	+	+	−	w	w[d]
STL2(pAVL10)	*glmX* ($nagB^+A^+R^+$)	+	+	+	−	w	w[d]
STL3	*glmX*	−	+	−	−	w	r
STL4	*glmX nag-3303*	+	+	+	−	w	r

[a] All strains are derivatives of strain JLV330 and retain the Φ(*glmS-lacZ*) insertion.
[b] MC, MacConkey agar; Lac, lactose; +, growth; −, no growth; w, white colonies, no fermentation; r, red colonies.
[c] Final GlcNAc concentration, 200 μg/ml; final NH_4^+ concentration, 10 mM.
[d] Despite the high expression of deacetylase and deaminase, pAVL10 causes a GlcNAc-negative phenotype because of repression of the chromosomally encoded NagE transport system not cloned on the plasmid.

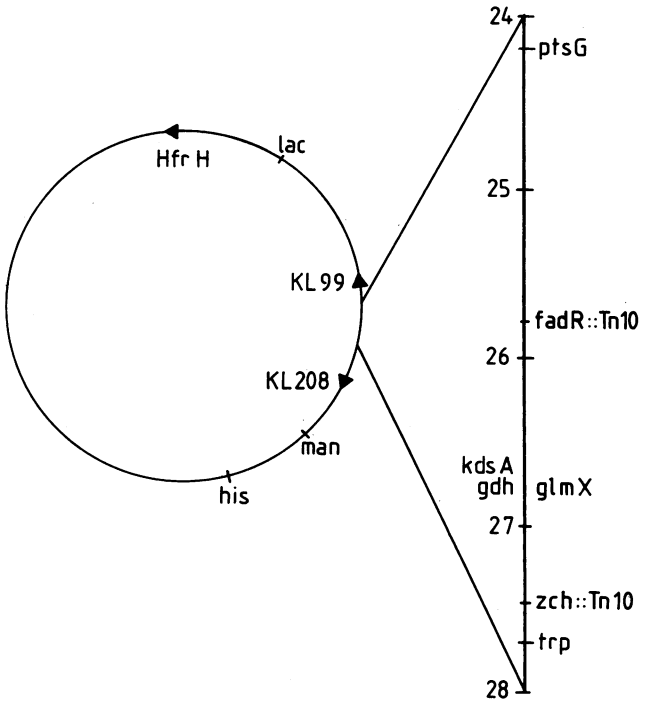

FIG. 3. Map of *E. coli* chromosome with relevant markers, origins of transfer for HfrH, Hfr PO42, and Hfr PO43, and the expanded 24 to 28-min region showing relevant markers. Notations and symbols are those of Bachmann (2), and distances were calculated from the frequencies of cotransduction according to the equation of Wu (2).

overexpression of *nagB* alone was sufficient to compensate for the defect in the GlcN-6-P synthetase gene *glmS*.

Mapping of the *glmX* locus. Since the new locus, for which we propose the preliminary designation *glmX*, was not linked to the *nag* locus, strain STL4 was crossed with the HfrH derivative JLV350, carrying like STL4 the *nag-3303* constitutivity allele. Thus, *glmX*$^+$ exconjugants could be detected on LT plates as cells requiring externally supplied amino sugars for growth. Linkage to the *his* locus (47% linkage), the *manI* locus (75% linkage), and the *lac* marker (21% linkage), as well as the subsequent use of Hfr KL208-1 and KL99, located *glmX* between their origins of gene transfer close to 25 min on the gene map (Fig. 3).

P1 transductions showed the *glmX* mutation to be linked to the *fadR* gene (22% linkage) and the *trp* locus (19% linkage) (Table 5), placing it at 26.8 min on the standard *E. coli* K-12 map. According to the most recent map (2), *glmX* should thus be highly linked if not coincident with the *gdh* locus encoding glutamate dehydrogenase. Mutations in *glmX* allowed strains with high levels of GlcN-6-P isomerase to grow on tryptone plates in the absence of added amino sugars (Table 4). Addition of high concentrations of ammonium (e.g., 10 mM NH_4Cl) allowed the growth of *glmX*$^+$ strains like JLV344-3 [Φ(*glmS-lacZ*) *nag-3303*] and JLV330(pAVL10) in tryptone medium and thus seemed to mimic the effect of *glmX* mutations. This finding could indicate that the *glmX* mutation increases the availability of intracellular ammonium as a cosubstrate for GlcN-6-P synthesis by the catabolic isomerase. However, when glutamate dehydrogenase activity was measured under several growth conditions of either nitrogen or carbon limitation, we were unable to detect any difference in the activity of *glmX* mutants as compared with the wild-type activity (data not shown). Moreover, the *glmX* marker was not cotransducible with the *gdh* marker of strain PA340. This *gdh* marker was linked in P1 transductions to neither the *fadR*::Tn*10* nor the *zch*::Tn*10* insertion (Table 5). It therefore clearly does not map at 26.8 min on the gene map, and the precise location of *gdh* remains to be shown.

TABLE 5. Cotransduction frequencies between *glmX*, *trp*, *gdh*, *zch*::Tn*10*, and *fadR*::Tn*10*[a]

Strain		Marker		% Cotransduction (unselected/selected)
Donor	Recipient	Selected	Unselected	
STL8	GG283	*fadR*::Tn*10*	*glmX*$^+$	22 (22/100)
STL13	PA340	*trp*$^+$	*zch*::Tn*10*	79 (38/48)
			glmX$^+$	19 (9/48)
			zch::Tn*10 glmX*$^+$	31 (15/48)
			gdh	2 (6/48)
PA340	LBG1623-1	*zch*::Tn*10*	*gdh*$^+$	0 (0/30)
LR2-168	GG283	*fadR*::Tn*10*	*ptsG*	1.5 (1/69)

[a] The *glmX*$^+$ wild-type allele was scored by the failure to grow on LT plates in the absence of amino sugars. The glutamate dehydrogenase (*gdh*) lesion was scored by the enzyme assay described in Materials and Methods. The Tn*10* insertion into the *fadR* locus caused red coloration of colonies grown on 2,3,5-triphenyl-2H-tetrazolium chloride plates supplied with sodium octanate (see Materials and Methods).

All strains carrying a *glmX* mutation were unable to grow on any medium at temperatures above 40°C. This phenotype is linked to *glmX*, since transduction of this mutation, by means of the closely linked *zch*::Tn*10* insertion into otherwise wild-type strains, rendered these cells temperature sensitive too. The mutation is recessive to the *glmX*$^+$ allele whether present on an F plasmid or in the chromosome of *recA* strains, and the diplogenetic strains resemble in all properties, including temperature resistance, the corresponding haploid *glmX*$^+$ strains (S. Trentmann, Diplomarbeit, University of Osnabrück, Osnabrück, Federal Republic of Germany, 1989).

DISCUSSION

Biosynthesis of amino sugars, the precursors for cell wall macromolecules by GlcN-6-P synthetase (gene *glmS*), which catalyzes the formation of GlcN-6-P from Fru-6-P and L-glutamine in an irreversible reaction, and degradation of amino sugars by the gene products of the *nag* regulon must be highly coordinated in order to avoid a futile cycle (Fig. 1). The enzyme GlcN-6-P deaminase (NagB) catalyzes the conversion of GlcN-6-P to Fru-6-P and ammonium. This reaction has been claimed to be reversible in vitro but not in vivo (5, 8, 39). In this report, we have shown that the reverse reaction is also possible in vivo and that the rate of GlcN-6-P formation via this enzyme is sufficient to allow cell growth in most of the standard media.

Derepression of the *nagB* gene is a first condition necessary to allow amino sugar synthesis in a tight *glmS* mutant completely lacking GlcN-6-P synthetase activity. Two classes of mutants exhibiting high uninduced levels of *nagB* activity were obtained in a selection for *glmS* suppressors. Most of the mutants expressed all enzymes of the *nag* regulon constitutively, whereas the remainder also were negative for deacetylase (NagA). Members of the former class, e.g., strains carrying allele *nag-3303*, were shown (Vogler and Lengeler, in press; Table 2) to carry a defect in the repressor gene *nagR*; members of the latter class carry a

polar *nagA* mutation preventing at the same time expression of the gene *nagR* located downstream of *nagA*. It should be noted that constitutive expression of the catabolic deacetylase and deaminase activities is not detrimental for cell growth of *glmS*$^+$ strains, perhaps indicating that these enzymes do not degrade internal amino sugars and cell wall precursors at any appreciable rate under normal growth conditions.

This alternative route for amino sugar biosynthesis, bypassing the synthetase reaction, requires a second mutation, termed *glmX*, that maps at 26.8 min on the *E. coli* chromosome. We do not know its precise function, but obviously a mutation in this locus is necessary for sufficient deaminase reverse reaction to proceed under physiological conditions. The mutation, as stated before, does not seem to affect glutamate dehydrogenase or ammonium transport activity, although its effect can be mimicked in certain media by high external ammonium concentrations. A specific high-affinity ammonium transport system, Amt (14), strongly repressed during ammonium excess, showed no significant difference when tested in different *glmX* mutants compared with *glmX*$^+$ strains regardless of whether the cells were pregrown in high or low ammonium and whether they were tested at high (1 mM) or low (10 μM) [^{14}C]methylammonium concentrations (data not shown).

None of the suppressor mutants was able to grow on MacConkey agar plates unless exogenous amino sugars were provided. Mutants with a similar phenotype have been isolated as a minor class of *glmS* "suppressors" in a previous study, but no further analysis has been carried out (39). MacConkey sensitivity was also reported for mutants with an altered LPS, which, because of increased permeability of the outer membrane, are sensitive to bile salts and basic dyes (23). These findings suggest that the alternative amino sugar pathway does not allow the synthesis of an intact outer membrane. Since bile salt sensitivity is observed only in mutants with highly abbreviated LPS in which the innermost heptose or 2-keto-3-deoxyoctonate (KDO) residues are missing from the core region, the lack of GlcNAc alone from the oligosaccharide side chains cannot account for the cell wall defect. Rather, the synthesis of the lipid A moiety of the LPS, which is a disaccharide of GlcN (derived from UDP-GlcNAc) linked to fatty acids of the outer membrane (1, 31), might be affected. In accordance with this suggestion, all of our *glmX* mutants exhibited significant temperature sensitivity in all growth media, a phenomenon that has been described for mutants defective in the biosynthesis of either KDO (6, 27) or lipid A (24). While this manuscript was in preparation, the precise mapping of *kdsA* mutants with a defect in KDO synthesis at 26.8 min of the chromosome, the location of *glmX*, was reported (38). Introduction of a cloned copy of the gene *kdsA* (kindly provided by G. Högenauer [38]) did not complement the *glmX* mutation, indicating perhaps the existence of a series of genes near min 28.6 involved in KDO synthesis and its regulation. Besides an increased permeability of the outer membrane, defects in KDO synthesis conceivably decrease GlcNAc incorporation into cell wall components and thus the need of a very high biosynthesis rate for amino sugars.

Further analysis of the suppressor mutants should give new insight into the genetics and regulation of cell wall biosynthesis and, hopefully, the general control of cell growth and cell division. The recent isolation of mutants that are strictly dependent either on GlcNAc or on cyclic AMP for cell division (32) may be first evidence for a predominant role of amino sugars in regulation of the cell cycle.

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