Regulation of Glucosamine Utilization in *Staphylococcus aureus* and *Escherichia coli*

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(Received 3 December 1976; revised 15 February 1977)

SUMMARY

Glucosamine- or *N*-acetylglucosamine-requiring mutants of *Staphylococcus aureus* 209P and *Escherichia coli* K12, which lack glucosamine-6-phosphate synthetase [2-amino-2-deoxy-D-glucose-6-phosphate ketol-isomerase (amino-transferring); EC 5.3.1.19], were isolated. Growth of these mutants on glucosamine was inhibited by glucose, but growth on *N*-acetylglucosamine was not. Addition of glucose to mutant cultures growing exponentially on glucosamine inhibited growth and caused death of bacteria, though chloramphenicol prevented death.

Uptake of glucosamine by *S. aureus* and *E. coli* mutants was severely inhibited by glucose whereas uptake of *N*-acetylglucosamine was only slightly inhibited. Uptake of glucose was not inhibited by either glucosamine or *N*-acetylglucosamine. In glucosamine auxotrophs, glucose causes glucosamine deficiency which interrupts cell wall synthesis and results in some loss of viability in the presence of continued protein synthesis.

INTRODUCTION

Glucosamine-containing macromolecules are major components of cell walls. They are synthesized *de novo* via fructose 6-phosphate (Fig. 1). Mutants lacking GlcN-6-phosphate synthetase [2-amino-2-deoxy-D-glucose-6-phosphate ketol-isomerase (amino-transferring); EC 5.3.1.19] cannot form amino sugars and lyse on removal or exhaustion of the required amino sugars (Freese *et al.*, 1970; Sarvas, 1971; Wu & Wu, 1971). We have isolated glucosamine (GlcN) or *N*-acetylglucosamine (GlcNAc) auxotrophs of *Staphylococcus aureus* and *Escherichia coli* which lack GlcN-6-phosphate synthetase. These mutants lost their viability when growing on media supplemented with GlcN and glucose, but glucose did not affect their growth on GlcNAc media. A similar phenomenon was observed with a GlcN or GlcNAc auxotroph of *E. coli* (Wu & Wu, 1971).

The inhibition by glucose of growth of the *S. aureus* and *E. coli* mutants was due to interference with the utilization of GlcN. Interruption of cell wall synthesis by limiting the supply of amino sugars was lethal for bacteria in the presence of protein synthesis.

METHODS

Organisms. *Staphylococcus aureus* 209P was provided by the late Professor S. Nakazawa, Kyoto College of Pharmacy, Japan, and *E. coli* K12 by Dr T. Iijima, Institute for Fermentation, Osaka, Japan. GlcN or GlcNAc auxotrophs, designated *S. aureus* NAG9 and *E. coli* GN4, were isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by the procedure of Adelberg, Mandel & Chen (1965); bacterial cultures treated with the mutagen

Fig. 1. Metabolism of GlcN and GlcNAc in bacteria and the postulated inhibition of GlcN transport by glucose. PTS, Phosphotransferase system.

were plated on nutrient agar containing 0.1 % (w/v) GlcNAc and colonies were replica plated on to nutrient agar without amino sugars. Bacteria which did not grow on the latter agar were selected as GlcNAc auxotrophs. All the isolated GlcNAc auxotrophs could also grow on GlcN.

Culture methods. NB medium (pH 7.0) contained (g l^{-1}): Polypepton (Daigo Nutritive Chemicals, Osaka, Japan), 10; meat extract (Wako Pure Chemicals, Osaka, Japan), 10; and NaCl, 1. Solid media contained Bacto agar (Difco) at 15 g l^{-1} . When necessary, sugars were added aseptically to a final concentration of 0.1 % (w/v) after sterilization through Sartorius membranes ($0.45 \mu\text{m}$ pore size).

Measurement of growth. Growth was routinely measured turbidimetrically in 200 ml Erlenmeyer flasks fitted with side tubes using a Spectronic 20 colorimeter (Shimadzu, Bausch & Lomb) at 600 nm. An extinction of 1.0 was equivalent to 0.44 and 0.59 mg dry wt bacteria ml^{-1} for stationary phase *S. aureus* and *E. coli* respectively. Viable cells were counted on agar plates of NB supplemented with 0.1 % GlcNAc.

Incorporation of labelled sugars. Bacteria were cultivated at 37°C for 16 h in 40 ml of NB containing 0.1 % GlcNAc in 200 ml Erlenmeyer flasks with shaking. The organisms were harvested, washed twice with NB and then resuspended in NB to an extinction of 1.0 at 600 nm. The incubation mixture, containing 1 ml bacterial suspension, 0.01 ml labelled sugar solution and 0.01 ml H_2O or unlabelled sugar solution in a plastic tube (11 mm i.d.), was incubated at 37°C with reciprocal shaking ($120 \text{ oscillations min}^{-1}$). After incubation the mixture was cooled immediately by immersing the tube in an ice bath and was then passed through a Sartorius membrane ($0.45 \mu\text{m}$ pore size). The membrane was washed twice

with 2 ml portions of NB and dried under an infrared lamp. Radioactivity retained on the membrane was counted using toluene-based scintillation liquid with an Aloka 502 liquid scintillation spectrometer (Nihon Musen Irigaku Kenkyusho, Tokyo, Japan). When necessary, cellular components of *S. aureus* were fractionated according to the procedure of Park & Hancock (1960) and the radioactivity in each fraction was counted using dioxan-based scintillation liquid.

Preparation of extracts and assay of GlcN-6-phosphate synthetase. Strains of *S. aureus* were cultivated at 37 °C for 16 h in 40 ml of NB containing 0.1 % GlcNAc in 200 ml Erlenmeyer flasks with shaking. Stationary phase organisms were harvested and washed twice with cold TM buffer (20 mM-Tris buffer containing 10 mM-2-mercaptoethanol, pH 7.6). The crude extracts were obtained by grinding with alumina. Strains of *E. coli* were grown similarly and 20 ml portions of the cultures were transferred to 200 ml of the fresh medium in 1 l Erlenmeyer flasks. After incubation for 3 h with rotary shaking, organisms were harvested, washed twice with TM buffer and crude extracts were obtained by sonic oscillation. GlcN-6-phosphate synthetase activity in the extracts was assayed by the method of Ghosh & Roseman (1962). Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Chemicals. Radioactive sugars were purchased from The Radiochemical Centre, Amersham. An enzyme cleaving mucopeptides of *S. aureus* was kindly provided by Mr Y. Yamasaki of our laboratories (unpublished). Other chemicals were of the highest purity available commercially.

RESULTS AND DISCUSSION

Growth tests on agar plates and GlcN-6-phosphate synthetase activity of parental and mutant strains

Parental strains grew on NB without added sugars, but the mutants *S. aureus* NAG9 and *E. coli* GN4 required addition of GlcN or GlcNAc. Fructose or glucose did not support growth of the mutants (Table 1). The mutants had greatly reduced levels of GlcN-6-phosphate synthetase; their requirements for amino sugars can be ascribed to the defect in this enzyme. Growth of these GlcN or GlcNAc auxotrophs on solid NB containing GlcN was inhibited by the simultaneous addition of glucose, but growth on GlcNAc-containing plates was not (Table 1). All the other mutants of *S. aureus* and *E. coli* isolated as GlcNAc auxotrophs showed a similar growth pattern. The selective growth inhibition by glucose of the mutants on GlcN-containing medium suggests that glucose only interferes with the process of GlcN utilization, as postulated in Fig. 1.

Growth inhibition by glucose of GlcN or GlcNAc auxotrophs in GlcN-containing liquid media

Staphylococcus aureus NAG9 and *E. coli* GN4 were cultivated in NB containing various concentrations of both GlcN and glucose. Growth of both mutants was inhibited by glucose, the concentration of glucose necessary for complete inhibition being proportional to the concentration of GlcN in the medium (data not shown).

Addition of glucose to cultures of these mutants growing exponentially in NB containing 0.1 % GlcN resulted in loss of viability and lysis (decrease in turbidity) after a lag period of about 2 h (Fig. 2).

Table 1. *Nutritional requirements and GlcN-6-phosphate synthetase activities of parental strains and GlcN- or GlcNAc-dependent mutants of S. aureus and E. coli*

Strain	Growth on NB agar	Growth on NB agar supplemented with 0.1 % (w/v):				GlcN plus glucose	GlcNAc plus glucose	GlcN-6-phosphate synthetase activity [units (mg protein) ⁻¹]
		Glucose	Fructose	GlcN	GlcNAc			
<i>S. aureus</i> 209P	+	+	+	+	+	+	+	0.076-0.087
<i>S. aureus</i> NAG9	-	-	-	+	+	-	+	<0.001
<i>E. coli</i> K12	+	+	+	+	+	+	+	0.254
<i>E. coli</i> GN4	-	-	-	+	+	-	+	0.037

+, Growth; -, no growth.

Fig. 2. Inhibitory effect of glucose on the growth of (a) *S. aureus* NAG9 and (b) *E. coli* GN4. Bacteria propagated on NB agar slants containing 0.1 % GlcNAc were suspended in NB and inoculated into 20 ml of NB supplemented with 0.1 % GlcN in 200 ml Erlenmeyer flasks fitted with side tubes for turbidity measurements. Glucose (final concn 0.2 %) was added at 0 (○), 1 (△), 2 (▲) and 3 (□) h after inoculation; ●, no glucose added. —, Absorbance; ---, colony-forming units (c.f.u.) ml⁻¹.

Prevention of glucose-induced loss of viability by chloramphenicol

When *S. aureus* NAG9 or *E. coli* GN4 was transferred to NB lacking amino sugars, the viable count of *E. coli* GN4 started to decrease immediately, while that of *S. aureus* NAG9 did so after a short lag period (Fig. 3). In medium containing 0.1 % GlcN and 0.2 % glucose, the viable count of both mutants decreased at approximately the same rate as in the medium lacking amino sugars though loss of viability was preceded by a lag period. After addition of chloramphenicol to the culture of *S. aureus* NAG9 in NB containing both GlcN and glucose, the viable count showed no significant change. Though the effect was less clear than with *S. aureus* NAG9, a preventative effect of chloramphenicol on glucose-induced loss of viability was also observed with *E. coli* GN4; loss of viability of this organism, which usually began 4 h after the addition of glucose, was partially prevented by chloramphenicol (Fig. 3). These results may indicate that protein synthesis is necessary for loss of viability. As described below, glucose inhibits the uptake of GlcN by *S. aureus* and *E. coli* so that GlcN or GlcNAc auxotrophs are unable to form cell wall components. Thus, continued protein synthesis

Fig. 3. Protective effect of chloramphenicol on glucose-induced loss of viability of (a) *S. aureus* NAG9 and (b) *E. coli* GN4. Bacteria were cultivated, as described in the legend to Fig. 2, in NB containing: 0.1 % GlcN (○); no sugars (●); 0.1 % GlcN plus 0.2 % glucose (□); or 0.1 % GlcN plus 0.2 % glucose plus 12.5 μ g (*S. aureus*) or 25 μ g (*E. coli*) chloramphenicol ml⁻¹ (△).

during defective cell wall synthesis appeared to promote lethal effects. Loss of viability and lysis (Figs 2 and 3) is assumed to be due to the increase of cytoplasmic volume relative to the expansion of cell wall or surface area, or to the abundance of autolytic enzymes relative to limited cell wall synthesis (see Tomasz, 1974).

Effect of glucose on uptake of GlcN and GlcNAc

Glucose interfered with the growth of GlcN or GlcNAc auxotrophs of *S. aureus* and *E. coli* on a GlcN-containing medium but not on a GlcNAc-containing medium. This discriminative effect of glucose on the two media can be ascribed to the difference in sensitivity to glucose of the uptake reactions of GlcN and GlcNAc (see Fig. 1). This assumption was substantiated by the observation that uptake of GlcN by both *S. aureus* and *E. coli* mutants was strongly inhibited by 0.1 % glucose, but uptake of GlcNAc was only slightly inhibited (Fig. 4). Cellular components of *S. aureus* which incorporated [1-¹⁴C]GlcN or [1-³H]GlcNAc were fractionated by the procedure of Park & Hancock (1960). About 40 to 50 % of the radioactivity incorporated into bacteria was recovered in the hot trichloroacetic acid-soluble fraction and 40 to 45 % in the residue after digestion with trypsin (data not shown). The radioactivity in the former fraction corresponds to teichoic acid, since neither ribonuclease nor deoxyribonuclease released substantial radioactivity from the cold trichloroacetic acid- and ethanol-washed organisms. The radioactivity in the residue fraction was solubilized by

Fig. 4. Inhibitory effect of glucose on uptake of GlcN and GlcNAc by *S. aureus* NAG9 and *E. coli* GN4. Sugar uptake was examined as described in Methods: 0.01 ml of 1 mM-[1-¹⁴C]GlcN (5.9 mCi mmol⁻¹) or 1 mM-[1-¹⁴C]GlcNAc (5.3 mCi mmol⁻¹) was added to 1 ml of bacterial suspension. (a) GlcN uptake by *S. aureus*, (b) GlcN uptake by *E. coli*, (c) GlcNAc uptake by *S. aureus*, and (d) GlcNAc uptake by *E. coli*. ●, No glucose; ○, with 5 mM-glucose.

an enzyme which cleaves mucopeptides of *S. aureus*. About 10% and 25% of the radioactivity incorporated into *E. coli* GN4 from labelled GlcN and GlcNAc, respectively, was solubilized by lysozyme (data not shown). Thus GlcN and GlcNAc were used for the synthesis of cell wall materials and limitation of GlcN utilization by glucose leads to the defective synthesis of cell walls in the GlcN or GlcNAc auxotrophs.

Unilateral inhibitory effect of glucose on GlcN uptake reaction

In *S. aureus* all sugars are transported by phosphoenolpyruvate:sugar phosphotransferase systems (Roseman, 1969). *Escherichia coli* also transports most sugars, including GlcN and GlcNAc, by phosphotransferase systems (Roseman, 1969; White & Kent, 1970; White, 1970). The inhibition of GlcN uptake by glucose is therefore presumed to be due to the inhibition of the phosphotransferase system operating for GlcN uptake. One possibility is that a single phosphotransferase system promotes uptake of both GlcN and glucose and that the inhibition is the result of competition for that common system. To examine this possibility, the effects of glucose on GlcN uptake and of GlcN on glucose uptake were compared. The uptake of 0.01 mM-GlcN by *S. aureus* NAG9 was slightly inhibited by 0.1 mM-glucose and strongly inhibited by 1 mM-glucose (Table 2). The uptake of 0.01 mM-GlcN by *E. coli* GN4 was strongly inhibited by 0.1 mM-glucose. Conversely, 1 mM-GlcN did not inhibit the uptake of 8.9 μ M-glucose in either mutant. These results indicate that glucose and GlcN do not compete for the same transport system. Curtis & Epstein (1975) demonstrated that in *E. coli*, GlcN is transported by the mannose phosphotransferase system, whereas glucose is transported mainly by the glucose phosphotransferase system and subsidiarily by the mannose phosphotransferase system. Saier, Feucht & Hofstadter (1976) isolated a 2-deoxyglucose-resistant mutant of *E. coli* in which the utilization of GlcN as well as mannose was impaired but that of glucose was unimpaired. Thus in *E. coli*, and possibly in *S. aureus*, GlcN and glucose are likely to be transported by different phosphotransferase systems.

GlcNAc uptake was relatively insensitive to glucose (Table 2). Neither GlcNAc nor GlcN inhibited the uptake of glucose by either organism. The uptake of GlcN in *E. coli* GN4 was strongly inhibited by GlcNAc, but that in *S. aureus* NAG9 was not. Since the transport

Table 2. *Inhibition of uptake of GlcN and GlcNAc by S. aureus* NAG9 *and E. coli* GN4

Organisms were cultivated for 16 h at 37 °C in NB containing 0.1 % GlcNAc and bacterial suspensions were prepared as described in Methods. To 1 ml of the suspensions were added (i) 0.01 ml of 1 mM-[1-¹⁴C]GlcN (5.9 mCi mmol⁻¹), 1 mM-[1-¹⁴C]GlcNAc (5.3 mCi mmol⁻¹) or 0.89 mM-[U-¹⁴C]glucose (17.9 mCi mmol⁻¹) and (ii) 0.01 ml of H₂O or unlabelled sugar solution. The mixture was then incubated for 10 min at 37 °C.

		Incorporation of labelled sugar (pmol) in the presence of:							
Strain	Labelled sugar	No unlabelled sugar	Unlabelled GlcN (mM)		Unlabelled GlcNAc (mM)		Unlabelled glucose (mM)		
			0.1	1.0	0.1	1.0	0.1	1.0	
<i>S. aureus</i> NAG9	GlcN	1332	—	—	1127	1003	912	51	
	GlcNAc	4203	2906	1288	—	—	4020	2832	
	Glucose	616	—	798	—	599	—	—	
<i>E. coli</i> GN4	GlcN	497	—	—	84	74	37	16	
	GlcNAc	2193	2028	1306	—	—	1423	1357	
	Glucose	2093	—	2372	—	2285	—	—	

—, Not examined.

Table 3. *Uptake of GlcN and GlcNAc by S. aureus* 209P *and E. coli* K12 cultivated on various sugars

Bacteria were cultivated for 16 h in NB and 4 ml of the culture was inoculated into 40 ml NB supplemented with 0.1 % (w/v) of the indicated sugars and shaken for 2 h. Bacterial suspensions were prepared and the uptake of GlcN or GlcNAc was measured as described in Methods. The labelled sugars were 0.85 mM-[1-¹⁴C]GlcN (9.35 mCi mmol⁻¹) and 0.90 mM-[1-³H]GlcNAc (7.5 mCi mmol⁻¹).

Strain	Supplement	Uptake of GlcN (pmol)		Uptake of GlcNAc (pmol)	
		10 min	20 min	10 min	20 min
<i>S. aureus</i> 209P	None	201	410	379	485
	Glucose	1439	2581	474	615
	GlcN	492	827	311	304
	GlcNAc	921	1679	433	453
	Glucose plus GlcN	1533	2518	495	528
	Glucose plus GlcNAc	1596	2568	489	523
<i>E. coli</i> K12	None	1155	1868	359	422
	Glucose	2070	3117	394	557
	GlcN	1079	2026	393	504
	GlcNAc	1578	2410	427	333
	Glucose plus GlcN	2013	2436	378	361
	Glucose plus GlcNAc	1798	2568	386	368

systems for GlcN and GlcNAc in these bacterial species seem to be different (see Table 3), the strong inhibition of GlcN uptake by GlcNAc in *E. coli* may not be a result of competition between these sugars for a common component of a transport system. GlcNAc may act analogously to glucose in inhibiting GlcN uptake in *E. coli*.

In *S. aureus* 209P, the ability to transport GlcN was greatly increased by growth on glucose, while transport of GlcNAc was not significantly increased. In *E. coli* K12, a similar pattern was observed although the increase in ability to transport GlcN was not so great (Table 3). *Staphylococcus aureus*, and possibly *E. coli*, appear to transport GlcN by inducible systems, and GlcNAc by constitutive systems. GlcN uptake by *S. aureus* 209P and *E. coli* K12 grown

under any of the culture conditions listed in Table 3 was strongly inhibited by glucose (data not shown) as it was in the mutants. Thus the sensitivity of GlcN uptake to glucose is unrelated to GlcN auxotrophy.

McGinnis & Paigen (1969) observed that the utilization of various sugars in *E. coli* was generally inhibited by glucose and named the phenomenon 'catabolite inhibition'. Kornberg (1973) and Amaral & Kornberg (1975) demonstrated that fructose utilization was prevented by glucose and suggested two possible mechanisms: (i) a pleiotropic inhibition exerted by the glucose phosphotransferase system; and (ii) a reaction of glucose or glucose 6-phosphate with the inducible mechanism effecting the uptake of fructose. Clark & Holms (1976) also reported that an interaction exists between the phosphotransferase systems for glucose and fructose in *E. coli* and that the access of fructose to its own system was denied by the glucose transferase system. Saier *et al.* (1976) indicated that several reactions occurring on the membrane of *E. coli* and *Salmonella typhimurium*, such as those catalysed by adenylate cyclase and carbohydrate transport systems, are subject to regulation by a mechanism which depends on the catalytic activities of protein components of the phosphotransferase system. The regulation of GlcN uptake by glucose in *S. aureus* and *E. coli* described here seems to be analogous to the above phenomena. It is probable that the regulation due to interaction between GlcN and glucose phosphotransferase systems explains the inhibition by glucose of GlcN-supported growth of GlcN or GlcNAc auxotrophs. Furthermore, *S. aureus* and *E. coli* resembled each other with respect to the regulation of amino sugar transport.

We are grateful to Dr E. Omura and Dr M. Isono for their encouragement, to the late Dr S. Nakazawa and to Dr T. Iijima for the gift of strains, to Mr Y. Yamasaki for his gift of a staphylolytic enzyme, and to Miss H. Sotoma for capable and conscientious technical assistance.

REFERENCES

- ADELBERG, E. A., MANDEL, M. & CHEN, G. C. C. (1965). Optimal conditions for mutagenesis by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine in *Escherichia coli* K-12. *Biochemical and Biophysical Research Communications* **18**, 788-795.
- AMARAL, D. & KORNBERG, H. L. (1975). Regulation of fructose uptake by glucose in *Escherichia coli*. *Journal of General Microbiology* **90**, 157-168.
- CLARK, B. & HOLMS, W. H. (1976). Control of the sequential utilization of glucose and fructose by *Escherichia coli*. *Journal of General Microbiology* **95**, 191-201.
- CURTIS, S. J. & EPSTEIN, W. (1975). Phosphorylation of D-glucose in *Escherichia coli* mutants defective in glucosephosphotransferase, mannosephosphotransferase, and glucokinase. *Journal of Bacteriology* **122**, 1189-1199.
- FRESE, E. B., COLE, R. M., KLOFAT, W. & FRESE, E. (1970). Growth, sporulation, and enzyme defects of glucosamine mutants of *Bacillus subtilis*. *Journal of Bacteriology* **101**, 1046-1062.
- GHOSH, S. & ROSEMAN, S. (1962). L-Glutamine-D-fructose 6-phosphate transamidase from *Escherichia coli*. *Methods in Enzymology* **5**, 414-417.
- KORNBERG, H. L. (1973). Carbohydrate transport by micro-organisms. *Proceedings of the Royal Society B* **283**, 105-123.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.
- MCGINNIS, J. F. & PAIGEN, K. (1969). Catabolite inhibition: a general phenomenon in the control of carbohydrate utilization. *Journal of Bacteriology* **100**, 902-913.
- PARK, J. T. & HANCOCK, R. (1960). A fractionation procedure for studies of the synthesis of cell-wall mucopeptide and of other polymers in cells of *Staphylococcus aureus*. *Journal of General Microbiology* **22**, 249-258.
- ROSEMAN, S. (1969). The transport of carbohydrates by a bacterial phosphotransferase system. *Journal of General Physiology* **54**, 138s-184s.
- SAIER, M. H., JR, FEUCHT, B. U. & HOFSTADTER, L. J. (1976). Regulation of carbohydrate uptake and adenylate cyclase activity mediated by the enzyme II of the phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli*. *Journal of Biological Chemistry* **251**, 883-892.

- SARVAS, M. (1971). Mutant of *Escherichia coli* K-12 defective in D-glucosamine biosynthesis. *Journal of Bacteriology* **105**, 467-471.
- TOMASZ, A. (1974). The role of autolysins in cell death. *Annals of the New York Academy of Sciences* **235**, 439-447.
- WHITE, R. J. (1970). The role of the phosphoenolpyruvate phosphotransferase system in the transport of N-acetyl-D-glucosamine by *Escherichia coli*. *Biochemical Journal* **118**, 89-92.
- WHITE, R. J. & KENT, P. W. (1970). An examination of the inhibitory effects of N-iodoacetylglucosamine on *Escherichia coli* and isolation of resistant mutants. *Biochemical Journal* **118**, 81-87.
- WU, H. C. & WU, T. C. (1971). Isolation and characterization of a glucosamine-requiring mutant of *Escherichia coli* K-12 defective in glucosamine-6-phosphate synthetase. *Journal of Bacteriology* **105**, 455-466.