

Enterotoxin A production in *Staphylococcus aureus*: inhibition by glucose

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Abstract. In this study, we investigated the relationship between carbohydrate metabolism and repression of staphylococcal enterotoxin A (SEA) in *Staphylococcus aureus* 196E and a pleiotrophic mutant derived from strain 196E. The mutant, designated at strain 196E-MA, lacked a functional phosphoenolpyruvate phosphotransferase system (PTS). The mutant produced acid, under aerobic conditions, from only glucose and glycerol. The parent strain contained an active PTS, and aerobically produced acid from a large number of carbohydrates. Prior growth in glucose led to repression of SEA synthesis in the parent strain; addition to the casamino acids enterotoxin production medium (CAS) led to more severe repression of toxin synthesis. The repression was not related to pH decreases produced by glucose metabolism. When *S. aureus* 196E was grown in the absence of glucose, there was inhibition of toxin production as glucose level was increased in CAS. The inhibition was related to pH decrease and was unlike the repression observed with glucose-grown strain 196E. The inhibition of SEA synthesis in mutant strain 196E-MA was approximately the same in cells grown with or without glucose and was pH related. Repression of SEA synthesis similar to that seen with glucose-grown *S. aureus* 196E could not be demonstrated in the mutant. In addition, glucose-grown *S. aureus* 196E neither synthesized β -galactosidase nor showed respiratory activity with certain tricarboxylic acid (TCA) cycle compounds. Glucose-grown strain 196E-MA, however, did not show suppressed respiration of TCA cycle compounds; β -galactosidase was not synthesized because the mutant lacked a functional PTS. Cyclic adenosine-3',5'-monophosphate did not reverse the repression by glucose of SEA or β -galactosidase synthesis in glucose-grown *S. aureus* 196E. An active PTS appears to be necessary to demonstrate glucose (catabolite) repression in *S. aureus*.

Key words: Phosphoenolpyruvate phosphotransferase system – Catabolite repression – Glucose effect – Cyclic adenosine-3',5'-monophosphate – Pleiotrophic mutant – Staphylococcal enterotoxin A – *Staphylococcus aureus*

Previous workers have shown that *Staphylococcus aureus*, under replicating as well as non-replicating conditions, produced less enterotoxin when glucose was present in the

enterotoxin production medium (Morse et al. 1969; Jarvis et al. 1975; Miller and Fung 1977; Iandolo and Shafer 1977). Most of the work has been done with staphylococcal strains that produce enterotoxin B (SEB) but Jarvis et al. (1975) have shown that SEA and SEC synthesis was repressed by glucose also. Prior growth of *S. aureus* in glucose containing media also influenced production of enterotoxin. Morse et al. (1969) demonstrated that *S. aureus*, grown in glucose containing medium, synthesized less SEB than non-glucose grown cells when the cells were subsequently placed in enterotoxin production medium with or without glucose. Similarly, Jarvis et al. (1975) found that glucose grown cells of *S. aureus*, when transferred to toxin production medium with or without glucose, produced less SEB or SEC than non-glucose grown cells. Interestingly, SEA production was not inhibited in a glucose grown strain even when glucose was present in the production medium (Jarvis et al. 1975). In the present study, we have investigated the effect of growing *S. aureus* 196E and a pleiotrophic mutant derived from strain 196E in the absence or presence of glucose on SEA synthesis.

Methods and materials

Isolation of mutants

Staphylococcus aureus 196E was inoculated into tryptic soy broth without glucose (Difco; TSB w/o glucose) containing 2-deoxyglucose (2-DOG). The glucose analogue was sterilized by filtration (0.20 micron filter) and then added to autoclaved broth to give a level of 100 mg/ml. The flasks were inoculated and subsequently incubated on a rotatory shaker (200 rpm) for 48 h at 37°C. Utilizing a spiral plater (Spiral Systems Marketing¹, Bethesda, MD, USA), dilutions of the cultures were plated on bromothymol blue agar plates (Korman and Berman 1958) containing 10 mg/ml lactose. Lactose-negative colonies were picked and purified by repeated plating. An isolate obtained in this manner was designated *S. aureus* 196E-MA and was used for further study.

Production of acid from various carbohydrates by *S. aureus* 196E and 196E-MA

The bromothymol blue agar of Korman and Berman (1958) lacking carbohydrates was sterilized by autoclaving, concentrated carbohydrate solutions were sterilized by autoclaving

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Abbreviations. SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B; SEC, staphylococcal enterotoxin C; PTS, phosphoenolpyruvate phosphotransferase system; CAS, casamino acids salts medium; TCA, tricarboxylic acid cycle

¹ Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned

Table 1. Production of acid in carbohydrate-containing media by *Staphylococcus aureus* 196E and 196E-MA

Addition	Reaction on bromothymol blue agar plate + 10mg/ml carbohydrate		pH after 16 h grown in broth containing 10mg/ml carbohydrate	
	196E	196E-MA	196E	196E-MA
No sugar	B ^a	B	7.9	8.0
Maltose · H ₂ O	A	B	5.2	7.9
Sucrose	A	B	5.2	7.9
Lactose	A	B	5.3	7.9
Trehalose · 2H ₂ O	A	B	5.3	7.8
Mannose	A	B	5.3	7.9
Galactose	A	B	5.2	7.9
Fructose	A	B	5.1	7.7
Glucose	A	A	5.0	5.5
Glucosamine · HCl	NG	NG	5.8 ^b	7.7 ^c
Glycerol	A	A	4.9	6.1
Mannitol	A	B	5.1	8.0

^a B, basic reaction; A, acid reaction; NG, no growth

^b Tested at 5 mg/ml level; there was no growth at 10 mg/ml glucosamine · HCl

^c Tested at 2.5 mg/ml level; there was no growth at 5 mg/ml glucosamine · HCl

(glucosamine · HCl was neutralized with NaOH and sterilized by filtration) and then added aseptically to the sterile agar to give a final carbohydrate concentration of 10 mg/ml. The plates were streaked with a single line of *S. aureus* 196E and 196E-MA, incubated at 37°C, and observed 24 and 48 h for acid production.

To determine acid production in broth, TSB w/o glucose was sterilized by autoclaving, and sterile (by autoclaving) carbohydrate solutions added to the broth to give a final concentration of 10 mg/ml (except for filter-sterilized neutral glucosamine; see Table 1). Flasks were inoculated with *S. aureus* 196E and 196E-MA and incubated on a rotary shaker (200 rpm) at 37°C. The pH of the flask contents was determined at 16 h using a pH meter.

Growth of *S. aureus* 196E and 196E-MA

S. aureus 196E or 196E-MA was inoculated into TSB w/o glucose or TSB w/o glucose with 10 mg/ml glucose (autoclaved separately) added. Culture flasks were incubated on the rotary shaker (200 rpm) at 37°C for 16 h.

Assay of phosphoenolpyruvate phosphotransferase system (PTS) and hexokinase (glucokinase)

S. aureus 196E and 196E-MA were inoculated into 100 ml TSB w/o glucose broth ± 10 mg/ml glucose and incubated at 37°C for 16 h on a rotary shaker (200 rpm). Cells were harvested by centrifugation (16,000 × g for 5 min at 5°C), washed twice in 0.15 M KCl, and resuspended in 20 ml cold potassium phosphate buffer (0.1 M pH 7.2). The cells were decriptified as follows: 50 µl acetone-toluene (9:1 mixture) were added to 1 ml cells and the mixture was agitated with a vortex mixer for 5 min in 30 s bursts interspersed with 10 s of cooling in ice. The protocol outlined by Romano et al. (1979) was followed for the PTS and hexokinase assays.

Reactions with U-¹⁴C-labeled 2-deoxyglucose (2-DOG) and glucose were carried out at 37°C. Samples of 50 µl were removed at intervals and added to 450 µl of ice-cold unlabeled glucose or 2-DOG (10 mg/ml). The samples were then transferred to Whatman DE81 filter paper disks (2.5 mm) held on vacuum funnels, and allowed to soak into the paper. The disks were washed with three successive 10 ml portions of cold distilled water. The washed disks were folded and placed in 5 ml of scintillation fluid (Hydromix, Yorktown Research, Hackensack, NJ, USA) for counting with a Beckman Liquid Scintillation Spectrophotometer. Cell protein levels were determined by the Bio-Rad protein assay kit with bovine serum albumin as the standard.

Respirometric studies

To determine the utilization of carbohydrates by *S. aureus* 196E-MA, TSB w/o glucose containing 10 mg/ml carbohydrate was used. Utilization of tricarboxylic acid cycle compounds was studied by using cells of *S. aureus* 196E and 196E-MA grown in TSB w/o glucose with or without 10 mg/ml glucose. After growth was complete, cells were harvested by centrifugation (16,000 × g for 5 min at 5°C), washed 2 times with sterile distilled water and made to 1/5 of the original volume in distilled water (e. g., cells from 100 ml TSB w/o glucose were concentrated to 20 ml in distilled water). Aliquots of the washed cells were used for dry weight determinations (cell suspensions were dried at 100°C for 6 h) and for respirometry. Fifteen milliliter single sidearm flasks (attached to a Gilson Differential Respirometer) contained a total volume of 3 ml: potassium phosphate buffer (pH 7.2, 0.1 M) and washed cells (approximately 10 mg dry weight) were placed in the main compartment; 0.2 ml of 20% KOH was placed in the center well along with a folded filter paper strip; and 0.01 M (final concentration) substrate was placed in the side arm. Acidic compounds were neutralized with NaOH before use. The flasks were equilibrated at 37°C, the substrate tipped in and the O₂ uptake and CO₂ production determined. Endogenous values were subtracted.

Assay for β-galactosidase

Lactose (10 mg/ml) was used as the inducer for β-galactosidase in *S. aureus* 196E. The β-galactosidase assay was that described by Dobrogosz (1981).

Production of staphylococcal enterotoxin A (SEA)

S. aureus 196E and 196E-MA were inoculated in sterile TSB w/o glucose and incubated at 37°C for 16 h on a rotary shaker (200 rpm). Cells were harvested by centrifugation and washed twice with sterile potassium phosphate buffer (pH 7.2, 0.1 M). The production of SEA was studied using a casamino acids salts (CAS) medium which contained: Na₃ citrate · 2H₂O, 500 mg; KH₂PO₄, 200 mg; K₂HPO₄, 200 mg; MgSO₄, 180 mg; FeSO₄ · 7H₂O, 10 mg; vitamin assay casamino acids (Difco), 20 g. The ingredients were dissolved in 500 ml distilled water, the pH adjusted to 7.0, and the volume made to 1,000 ml. The medium was sterilized by autoclaving. Concentrated solutions of carbohydrates were sterilized by filtration and added to the CAS medium. Washed cells were added at a level to give approximately 5 × 10⁹ cells/ml. The flasks were then incubated at 37°C on

Table 2. Utilization of carbohydrates by *S. aureus* 196E-MA as determined by respirometry

Compound	nl/min · mg dry wt. cells
Maltose · H ₂ O	74.7
Sucrose	102.5
Lactose	0.0
Trehalose · 2H ₂ O	0.0
Mannose	0.0
Galactose	0.0
Fructose	104.3
Glucose	223.4
Glucosamine · HCl	40.9
Glycerol	157.9
Mannitol	0.0

Table 3. PTS and hexokinase activity in glucose grown cells of *S. aureus* 196E and 196E-MA

Additions to assay	Counts/min · mg cell protein	
	<i>S. aureus</i> 196E	<i>S. aureus</i> 196E-MA
2-DOG ^a	39.5	14.1
2-DOG + PEP	2,278.8	11.3
Glucose	533.2	306.3
Glucose + ATP	21,131.6	14,291.0

^a Abbreviations: 2-DOG, 2-deoxyglucose; PEP, phosphoenolpyruvate; ATP, adenosine-5'-triphosphate

a rotary shaker (200 rpm) and at intervals, samples were removed for pH and SEA determinations. No change in viable cell numbers was observed in the CAS medium over a period of 24 h.

Determination of SEA

Cells were removed from the CAS medium by centrifugation. To remove non-specific IgG binding protein A, normal rabbit serum was added to the cell-free culture supernatant to give a final concentration of 5% (Fey et al. 1984). Tubes were incubated at 5°C for 1 h and then centrifuged. Dilutions of the culture supernatant fluid freed from protein A were used for enzyme-linked immunosorbent assay (ELISA) for SEA.

The double sandwich ELISA of Berdal et al. (1981) was used to quantitate SEA production. The ELISA procedure was modified as follows: Nunc-Immuno Plates I (Vanguard International, Neptune, NJ, USA) were used as the solid support; rabbit antiserum against SEA was used as the first antibody and goat anti-SEA was used as the second antibody. The enzyme was horseradish peroxidase conjugated to rabbit anti-goat IgG (Sigma). The substrate for enzyme action was ABTS-H₂O₂ [2,2'-azinodi-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, Sigma]. Goat and rabbit anti-sera and purified SEA were generously supplied by Dr. Anna Johnson, US Army Medical Research Institute of Infectious Diseases, Frederick, MD, USA.

The absorbancy of the chromogen developed by peroxidase activity on ABTS-H₂O₂ was determined by the use of a Microelisa Minireader (Model MR 590, Dynatech Instruments, Torrance, CA, USA) at 410 nm. Absorbancies

Table 4. PTS and hexokinase activity in cells of *S. aureus* 196E and 196E-MA not grown in glucose containing medium

Additions to assay	Counts/min · mg cell protein	
	<i>S. aureus</i> 196E	<i>S. aureus</i> 196E-MA
2-DOG ^a	956.3	30.5
2-DOG + PEP	974.0	30.8
Glucose	4,701.9	537.8
Glucose + ATP	4,321.6	670.0

^a See footnote, Table 3

Table 5. Effect of the absence or presence of glucose in TSB w/o glucose on respiration of washed cells *S. aureus* 196E

Compound tested	nl gas/min · g dry weight cells; grown in			
	TSB w/o glucose		TSB w/o glucose + 10mg/ml glucose	
	O ₂	CO ₂	O ₂	CO ₂
Glucose	880	378	745	404
Fumarate	532	431	0	0
Succinate	335	240	0	0
Malate	202	206	0	0
Pyruvate	139	123	0	0
Glutamate	426	238	97	52 ^a

^a 45 min lag before CO₂ production started

of the unknowns were transformed into ng SEA/ml by comparison to a standard curve prepared with purified SEA.

Results

The mutant strain, *Staphylococcus aureus* 196E-MA, has limited ability to produce acid from carbohydrates (Table 1). Under aerobic conditions, strain 196E-MA did not produce acid from maltose, sucrose, lactose, trehalose, mannose, galactose, fructose, glucosamine, or mannitol while acid was produced only from glucose and glycerol. Addition of higher concentrations (up to 40 mg/ml) of the refractory compounds did not result in acid production. The parent strain, *S. aureus* 196E, produced acid from all of these carbohydrates (Table 1).

While acid was produced only from glucose or glycerol, respirometric studies indicated that the mutant took up oxygen in conjunction with maltose, sucrose, fructose, and glucosamine (Table 2). Analysis of these sugars using a commercial enzymatic glucose analysis technique (no. 15-UV, Sigma) indicated that none were contaminated with any significant amount of glucose (< 0.24%).

When *S. aureus* 196E was grown in a glucose containing medium, it demonstrated an active phosphoenolpyruvate-dependent phosphorylation of 2-DOG, whereas strain 196E-MA displayed little, if any, PTS phosphorylating activity (Table 3). Both the wild type and mutant strains were capable of ATP-dependent phosphorylation of glucose which is indicative of an active hexokinase (or glucokinase). A distinct difference in transport patterns was observed when strains 196E and 196E-MA were grown in medium lacking glucose (Table 4). Neither the wild type nor the

Table 6. Effect of the absence or presence of glucose in TSB w/o glucose on respiration of washed cells *S. aureus* 196E-MA

Compound tested	nl gas/min · mg dry weight cells; grown in			
	TSB w/o glucose		TSB w/o glucose + 10mg/ml glucose	
	O ₂	CO ₂	O ₂	CO ₂
Glucose	703	358	451	274
Fumarate	113	110	93	78
Succinate	175	118	186	139
Malate	134	130	138	166
Pyruvate	150	152	199	244
Glutamate	322	198	170	143 ^a

^a 30 min lag before CO₂ production started

mutant displayed PEP- or ATP-dependent phosphorylation. However, both strains showed increased levels of basal phosphorylation, suggesting that a third mechanism of phosphorylation may be active in non-glucose grown cells.

When cells of *S. aureus* 196E were grown in TSB w/o glucose, washed and then used in respirometric studies, O₂ uptake and CO₂ production were observed with the following substrates: glucose, fumarate, succinate, malate, pyruvate, and glutamate (Table 5). However, when strain 196E was grown in the presence of glucose at the level of 10 mg/ml, there was no demonstrable respirometric activity with fumarate, succinate, malate, or pyruvate. Glucose grown cells also showed decreased activity when glutamate was the substrate (Table 5). However, *S. aureus* 196E grown in either the absence or presence of glucose showed approximately the same respirometric activity when glucose was the substrate. The data presented in Table 6 indicated that washed cells of strain 196E-MA consumed O₂ and produced CO₂ from all the substrates equally well regardless of whether or not the cells were grown in glucose.

The data presented in Table 7 indicated that glucose-grown *S. aureus* 196E consistently produced less SEA than cells grown in the absence of glucose. Increasing the level of glucose in the CAS medium led to decreased SEA production with both glucose and non-glucose grown cells. The mutant strain, *S. aureus* 196E-MA, produced similar amounts of toxin whether or not it was grown in glucose containing medium (Table 7). Increasing the level of glucose in the CAS medium led to decreased toxin production with both glucose and non-glucose grown strain 196E-MA.

As the glucose level of CAS medium increased, the pH decrease was greater with glucose grown *S. aureus* 196E than with non-glucose grown cells (Table 7). However, such an effect was not apparent in the mutant strain. When SEA production was plotted against pH (Fig. 1), decreased production of SEA with glucose-grown *S. aureus* 196E did not appear related to pH, whereas an apparent correlation between decreased SEA synthesis and pH was observed with non-glucose grown strain 196E. Decreased synthesis of SEA by *S. aureus* 196E-MA with either glucose-grown or non-glucose grown cells correlated with decreased pH.

Attempts to reverse glucose repression of SEA synthesis in *S. aureus* 196E with cyclic adenosine-3',5'-monophosphate (c-AMP) proved futile. Addition of 1 mM c-AMP did not reverse repression of SEA synthesis. Since it was possible that *S. aureus* might contain an active c-AMP

Table 7. Effect of growth with or without glucose on pH and SEA synthesis by *S. aureus* 196E and 196E-MA in CAS medium containing varying amounts of glucose

10 mg/ml glucose in TSB w/o glucose	Concentration of glucose in CAS medium (mM)	<i>S. aureus</i> 196E		<i>S. aureus</i> 196E-MA	
		ng SEA/ml	pH	ng SEA/ml	pH
—	0.0	87.7	8.8	103.1	8.7
+	0.0	70.2	8.4	112.5	8.6
—	2.5	105.3	8.7	109.4	8.6
+	2.5	31.6	8.1	121.8	8.5
—	5.0	121.0	8.5	125.0	8.4
+	5.0	42.1	7.8	123.4	8.3
—	7.5	128.1	8.4	120.3	8.3
+	7.5	33.3	6.6	125.0	8.2
—	10.0	63.2	7.3	118.8	8.2
+	10.0	21.1	5.7	118.8	7.9
—	12.5	42.1	6.7	118.8	7.9
+	12.5	19.3	5.3	93.7	6.9
—	15.0	26.3	6.2	75.0	6.3
+	15.0	10.5	5.0	43.7	6.0
—	17.5	17.5	5.8	48.4	5.8
+	17.5	8.8	5.0	28.1	5.0
—	20.0	10.5	5.7	53.1	5.4
+	20.0	8.8	5.0	28.1	4.9

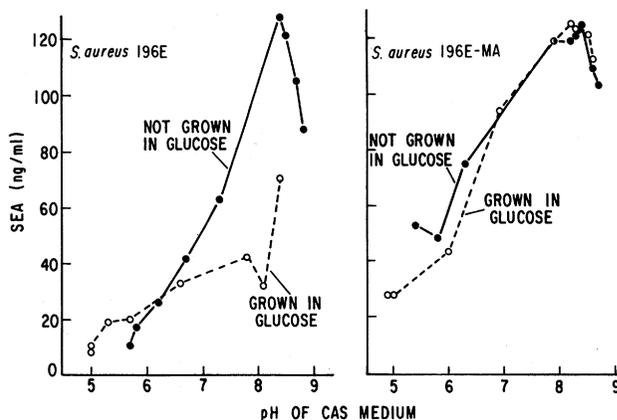


Fig. 1. The relationship between metabolically produced pH and SEA synthesis in *Staphylococcus aureus* 196E and 196E-MA

phosphodiesterase, the diesterase inhibitors caffeine, theophylline, or 3-isobutyl-methylxanthine (IBMX) were added in concentrations ranging from 0.1 to 1.0 mM. However, the repression exerted against SEA synthesis by glucose grown cells was not reversed by c-AMP plus phosphodiesterase inhibitors. *S. aureus* 196E has lactose inducible β -galactosidase which is repressed if glucose is added with the inducer. Addition of (1 mM c-AMP with or without IBMX) did not reverse glucose repression of β -galactosidase induction by lactose.

Discussion

The current study was undertaken to determine if mutant strains of *Staphylococcus aureus* deficient in carbohydrate utilization could be effectively employed as tools to elucidate

the mechanism underlying carbohydrate repression of staphylococcal enterotoxin synthesis. The specific mutant strain (196E-MA) appears to be of the class of mutants that Korman (1962) termed pleiotrophic, and is most probably an example of the *car*⁻ genotype described by Egan and Morse (1965a, b). Specifically, the genetic lesion in strain 196E-MA appears to be a non-functional PTS. The resultant phenotype is unable to produce acids from carbohydrates other than glucose and glycerol.

The alteration in phenotype appears to be specifically associated with transport mechanisms or related regulatory processes since respirometric analysis indicated that the mutant strain was capable of utilizing maltose, fructose, glucosamine, or sucrose even though these carbohydrates no longer supported acid production. This suggests that either the rate or mechanism of transport of these sugars has been altered in such a way that they no longer are effective at initiating a carbon catabolite repression of tricarboxylic acid cycle enzymes.

While the mutant strain retains the ability to form acid from glucose and glycerol, the rate of acid formation appears depressed in comparison to that of the parent strain 196E. Egan and Morse (1965b) had previously noted that the rate of acid production was slower in the *car*⁻ mutants of *S. aureus*. Glucose-grown strain 196E-MA clearly lacks the PTS phosphorylation and transport mechanism for glucose and relies on hexokinase (or glucokinase) to phosphorylate the sugar. However, the mutant must transport glucose into the cell by some mechanism other than PTS. The differences in rates of acid production between the parent and mutant strains is probably a function of their relative rate of glucose transport.

The specific glucose transport system functioning in *S. aureus* appears to be dependent on the presence or absence of glucose during growth. While glucose-grown cells of the parent strain had significant PTS transport and hexokinase activity, neither system was evident in cells grown in the absence of glucose. However, significant transport activity was still present in these cells, suggesting that *S. aureus* may have a third system for the transport and/or phosphorylation of glucose when grown in the absence of the sugar.

Prior growth of *S. aureus* 196E in glucose containing medium profoundly affected the manner in which it synthesized enterotoxin and β -galactosidase, and utilized TCA cycle intermediates. The synthesis of SEA was repressed in strain 196E grown in glucose medium, washed, and then resuspended in CAS medium with or without glucose. The repression was increasingly more severe as the glucose level in the CAS medium was increased. The repression with glucose-grown cells did not appear to be pH related. The mutant strain, *S. aureus* 196E-MA, did not show such a repressive mechanism. Other workers have shown that enterotoxin biosynthesis was more repressed when *S. aureus* strains were grown in glucose containing media than when the cells were grown in the absence of glucose. Morse et al. (1969) found that staphylococci grown in glucose and then resuspended in an equal volume of fresh medium, formed SEB at a reduced rate. The repressive effect was greater if the fresh resuspending medium contained glucose.

Jarvis et al. (1975) showed that SEB and SEC synthesis were not repressed when the appropriate *S. aureus* strains were grown in the absence of glucose even though the amino acids resuspending medium contained glucose. The resuspending medium did not permit replication of the cells

and there was little or no change in pH. Under similar conditions, the synthesis of β -galactosidase by *S. aureus* was severely repressed when the resuspending medium contained glucose in contrast with enterotoxin biosynthesis (Jarvis et al. 1975). However, if *S. aureus* was grown in the presence of glucose, SEB and SEC synthesis was repressed even if the resuspending medium did not contain glucose. Under these conditions β -galactosidase was not synthesized by glucose-grown cells (Jarvis et al. 1975). Thus, it would appear that repression of an inducible enzyme like β -galactosidase is not similar to repression in enterotoxin biosynthesis.

Interestingly, Jarvis et al. (1975) did not get repression of SEA synthesis under any of the conditions in which repression of SEB or SEC synthesis took place. However, the SEA producing strain did contain a glucose repressible β -galactosidase.

Glucose-grown *S. aureus* 196E, in contrast to cells not grown in glucose, lacked the ability to take up O₂ and produce CO₂ with fumarate, succinate, malate, and pyruvate as the substrates. There was also reduced activity with glutamate. The mutant strain showed respiratory activity on all these substrates regardless of the growth conditions. Other workers have shown that growth in glucose depressed TCA cycle activity. Collins and Lascelles (1962) and Strasters and Winkler (1963) demonstrated that glucose-grown *S. aureus* strains did not show oxygen uptake with a number of TCA cycle intermediates. The level of TCA cycle enzymes in cell-free preparations of *Escherichia coli* and *Bacillus subtilis* were reduced when the cells were grown in glucose containing media (Gray et al. 1966; Ohne 1975). Thus, growing microorganisms in the presence of glucose led to reduced TCA cycle enzymatic activity.

We were not successful in reversing the glucose repression of SEA or β -galactosidase synthesis in *S. aureus* by adding c-AMP with or without c-AMP phosphodiesterase inhibitors. It is well known that several glucose repressible systems present in *E. coli* and other Gram-negative bacteria are derepressed by the addition of c-AMP (Pastan and Perlman 1970; Pastan and Adhya 1976). In *E. coli*, glucose, in conjunction with Enzyme II of the PTS, inhibits adenylate cyclase activity, thereby limiting the level of c-AMP (Harwood et al. 1976; Saier et al. 1976).

Other workers have shown that c-AMP does not reverse glucose repression in *S. aureus*. The repression of SEB synthesis by glucose was not reversed by c-AMP (Morse and Baldwin 1973; Iandolo and Shafer 1977) nor was alpha toxin synthesis regained in glucose repressed cultures when c-AMP was added (Duncan and Cho 1972).

In this study, we have shown that *S. aureus* 196E contains an active PTS with SEA and β -galactosidase synthesis as well as TCA cycle activity being repressed by glucose in glucose-grown cells. However, the mutant strain, 196E-MA, does not contain an active PTS and glucose repression was not demonstrated. This suggests that the PTS of *S. aureus* is involved in regulation and repression. It is possible that a specific sugar component of the PTS in combination with glucose (or other carbohydrate) inhibits the synthesis of a key chemical messenger (similar to c-AMP) that is involved in glucose repression.

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References

- Berdal BP, Olsvik Ø, Omland T (1981) A sandwich ELISA method for detection of *Staphylococcus aureus* enterotoxins. *Acta Path Microbiol Scand, Sect B* 89:411–415
- Collins FM, Lascelles J (1962) The effect of growth conditions on oxidative and dehydrogenase activity in *Staphylococcus aureus*. *J Gen Microbiol* 29:581–585
- Dobrogosz WJ (1981) Enzymatic activity. In: Gerhardt P (ed) *Manual of methods for general microbiology*. Am Soc Microbiol, Washington, DC
- Duncan JL, Cho GJ (1972) Production of staphylococcal alpha toxin. II. Glucose repression of toxin formation. *Infect Immun* 6:689–694
- Egan JB, Morse ML (1985a) Carbohydrate transport in *Staphylococcus aureus*. I. Genetic and biochemical analysis of a pleiotrophic transport mutant. *Biochim Biophys Acta* 97:310–319
- Egan JB, Morse ML (1985b) Carbohydrate transport in *Staphylococcus aureus*. II. Characterization of a pleiotrophic transport mutant. *Biochim Biophys Acta* 109:172–183
- Fey H, Pfister H, Rüegg O (1984) Comparative evaluation of different enzyme-linked immunosorbent assay systems for the detection of staphylococcal enterotoxins A, B, C, and D. *J Clin Microbiol* 19:34–38
- Gray CT, Wimpenny JWT, Mossman MR (1966) Regulation of metabolism in facultative bacteria. II. Effects of aerobiosis, anaerobiosis and nutrition on the formation of Krebs cycle enzymes in *Escherichia coli*. *Biochim Biophys Acta* 117:33–41
- Harwood JB, Gazdar C, Prasad C, Peterkofsky A, Curtis SJ, Epstein W (1976) Involvement of the glucose enzymes II of the sugar phosphotransferase system in the regulation of adenylate cyclase in *Escherichia coli*. *J Biol Chem* 251:2462–2468
- Iandolo JJ, Shafer WM (1977) Regulation of staphylococcal enterotoxin B. *Infect Immun* 16:610–616
- Jarvis AV, Lawrence RC, Pritchard GG (1975) Glucose repression of enterotoxins A, B, and C and other extracellular proteins in staphylococci in batch and continuous culture. *J Gen Microbiol* 86:75–87
- Korman RZ (1962) Transduction of a pleiotrophic carbohydrate locus in *Staphylococcus aureus*. *J Bacteriol* 84:1338–1339
- Korman RZ, Berman DT (1958) Medium for the differentiation of acid producing colonies of staphylococci. *J Bacteriol* 76:454–455
- Miller RD, Fung DYC (1977) The excretion of enterotoxin B from nongrowing cells of *Staphylococcus aureus* 4916. *Can J Microbiol* 23:369–377
- Morse SA, Baldwin JN (1973) Factors affecting regulation of staphylococcal enterotoxin B. *Infect Immun* 7:839–846
- Morse SA, Mah RA, Dobrogosz WJ (1969) Regulation of staphylococcal enterotoxin B. *J Bacteriol* 98:4–9
- Ohne M (1975) Regulation of the dicarboxylic acid part of the citric acid cycle in *Bacillus subtilis*. *J Bacteriol* 122:224–234
- Pastan I, Adhya A (1976) Cyclic adenosine 5'-monophosphate in *Escherichia coli*. *Bacteriol Rev* 40:527–551
- Pastan I, Perlman R (1970) Cyclic adenosine monophosphate in bacteria. *Science* 169:339–344
- Romano AH, Trifone JD, Brustolon M (1979) Distribution of the phosphoenolpyruvate:glucose phosphotransferase system in fermentative bacteria. *J Bacteriol* 139:93–97
- Saier MH, Feucht BU, Hefstadter LH (1976) Regulation of carbohydrate uptake and adenylate cyclase activity mediated by Enzymes II of the phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli*. *J Biol Chem* 251:883–892
- Strasters KC, Winkler KC (1963) Carbohydrate metabolism of *Staphylococcus aureus*. *J Gen Microbiol* 33:213–229