

## EFFECT OF GLUCOSE ANALOGS ON THE SYNTHESIS OF STAPHYLOCOCCAL ENTEROTOXIN A

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### ABSTRACT

*Glucose, 2-deoxyglucose (2-DOG), and  $\alpha$ -methylglucose ( $\alpha$ -MG) inhibited staphylococcal enterotoxin A (SEA) synthesis by Staphylococcus aureus 196E whereas  $\beta$ -methylglucose ( $\beta$ -MG) and 3-O-methylglucose (3-O-MG) did not inhibit even at high concentrations. Glucose and  $\beta$ -MG decreased the pH (< 6.0) whereas the pH with 2-DOG,  $\alpha$ -MG was above 8.0 at 24 h. Glucose (at levels not inhibitory to SEA synthesis) potentiated the inhibition of toxin synthesis by 2-DOG and  $\beta$ -MG and the glucose-analog combinations had a decreased pH. The inhibition of SEA synthesis by glucose, glucose analogs, or glucose-analog combinations does not appear to be directly related to a decline in pH. The inhibitory effects shown by glucose and its analog suggest that SEA synthesis may be regulated by a mechanism similar to catabolite repression.*

### INTRODUCTION

Glucose analogs, such as 2-deoxyglucose (2-DOG) or  $\alpha$ -methylglucose ( $\alpha$ -MG), have a variety of effects in gram-positive bacterial cells. These effects include inhibition of bacterial growth (Dietz and Heppel 1971; Iandolo and Shafer 1977; Thompson and Chassy 1982), uncoupling of energy from growth (Thompson and Chassy 1982), inhibition of enzyme synthesis or secretion (Braatz and Heath 1974; Iandolo and Shafer 1977), and inhibition of staphylococcal enterotoxin B (SEB) synthesis (Iandolo and Shafer 1977).

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The formation of staphylococcal enterotoxin A (SEA) is inhibited by glucose, with the extent of inhibition being strongly influenced by pregrowth of the bacteria in a glucose-containing medium (Smith *et al.* 1986). This study was undertaken to provide insight on how glucose analogs influence SEA production and to compare these results with those of Iandolo and Shafer (1977) for SEB production.

## METHODS AND MATERIALS

### Growth and Harvesting of Cells

*S. aureus* 196E was inoculated into tryptic soy broth w/o glucose (Difco). Culture flasks were incubated on a reciprocating shaker (200 rp.) at 37 C for 16 h. Cells were harvested by centrifugation, washed 2X with sterile 0.1M, pH 7.2 phosphate buffer, and resuspended in 10 mL of buffer.

### Enterotoxin Production and Assay

The enterotoxin production medium for SEA was a casamino acids-salts (CAS) medium (Smith *et al.* 1986). CAS medium was sterilized by autoclaving. Glucose and glucose analogs were sterilized by filtration and added to CAS medium aseptically. Washed cells, suspended in buffer, were added to CAS medium to give approximately  $5 \times 10^9$  cells/mL. Inoculated CAS medium was incubated on a reciprocating shaker (200 rpm) at 37 °C. Samples were removed from incubation at 24 h and tested for pH and SEA. Cells were removed by centrifugation. Protein A was removed from the culture supernatant fluids by adding normal rabbit serum (final concentration was 5% serum) with incubation at 5 °C for one hour followed by centrifugation (Fey *et al.* 1984). Dilution of Protein A free supernatant fluids were assayed for SEA by ELISA as described by Smith and Bencivengo (1985).

### Glucose Analogs

The analogs, 2-deoxy-D-glucose (2-DOG), 3-O-methyl-glucopyranose (3-O-MG), 1-O-methyl- $\beta$ -D-glucopyranoside ( $\beta$ -MG), and 1-O-methyl- $\alpha$ -D-glucopyranoside ( $\alpha$ -MG) were obtained from Sigma Chemical Company (St. Louis, MO). Assay of the analogs with Sigma's #15-UV glucose kit indicated that detectable amounts of glucose were not present.

## RESULTS AND DISCUSSION

Addition of glucose of CAS medium inhibited SEA production by *S. aureus* (Table 1) in a dose-dependent manner after the concentration was raised above a

threshold value. A concentration between 10.0 and 12.5 mM was needed to inhibit SEA production by approximately 50%. Lower concentrations of glucose (2.5 to 7.5 mM) stimulated toxin production to a small degree (Fig. 1A).

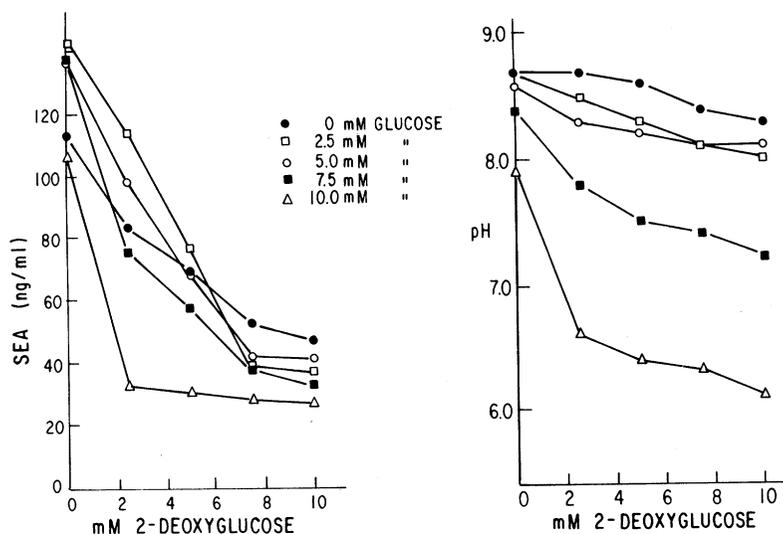


FIG. 1.  
EFFECT OF 2-DEOXYGLUCOSE IN COMBINATION WITH GLUCOSE IN CAS MEDIUM  
ON SEA SYNTHESIS (A) and PH (B) IN *S. AUREUS* 196E

The analogs, 2-DOG and  $\alpha$ -MG, were more inhibitory than glucose. Both compounds produced a 50% inhibition of SEA production at concentrations between 5.0 and 7.5 mM (Table 1). The analogs,  $\beta$ -MG and 3-O-MG were much less inhibitory than glucose. Inhibition of SEA production by 50% was not observed with concentrations up to 50 mM. Iandolo and Shafer (1977) reported that 2-DOG inhibited SEB production in *S. aureus*, but conversely found that  $\alpha$ -MG stimulated SEB formation.

After 24 h of incubation, the pH of CAS medium containing 2-DOG,  $\alpha$ -MG, or 3-O-MG was above 8.0 (Table 1), indicating that the compounds were either not being metabolized or not entering the cells. Iandolo and Shafer (1977) demonstrated that 2-DOG and  $\alpha$ -MG entered *S. aureus* cells and were metabolized only to the 6-phosphate level. The cultures containing  $\beta$ -MG showed a reduction in pH which indicated that the microorganisms were capable of catabolizing the compound to an acidic product (Table 1). There did not appear to be a relationship between final pH and inhibition of SEA production, since 2-DOG and  $\alpha$ -MG were strongly inhibitory without causing a pH decline, while

Table 1. Effect of glucose and glucose analogues on pH and synthesis of SEA in CAS medium by *S. aureus* 196E

Concentration (mM)	Effect on synthesis of SEA by														
	Glucose			2-DOG			$\alpha$ -MG			$\beta$ -MG			3-O-MG		
	% Inhibition	pH	% Inhibition	pH	% Inhibition	pH	% Inhibition	pH	% Inhibition	pH	% Inhibition	pH	% Inhibition	pH	
0.0	0.0	8.7	0.0	8.7	0.0	8.7	0.0	8.8	0.0	8.7	0.0	8.7	0.0	8.7	
2.5	0.0	8.7	24.5	8.6	37.5	8.7	3.4	8.6	3.4	8.6	1.7	8.7	1.7	8.7	
5.0	0.0	8.5	36.9	8.5	48.2	8.6	7.5	8.6	7.5	8.6	8.2	8.7	8.2	8.7	
7.5	0.0	8.4	52.0	8.4	56.5	8.6	6.3	8.6	6.3	8.5	7.5	8.8	7.5	8.8	
10.0	27.9	7.3	58.5	8.3	63.4	8.6	1.1	8.6	1.1	8.5	5.7	8.7	5.7	8.7	
12.5	52.0	6.7	<sup>a</sup>	-	-	-	-	-	-	-	-	-	-	-	
15.0	70.0	6.2	65.3	8.2	72.1	8.5	-	-	-	-	-	-	-	-	
17.5	80.0	5.8	-	-	-	-	-	-	-	-	-	-	-	-	
20.0	88.0	5.7	71.2	8.2	77.1	8.5	3.0	8.5	3.0	7.6	1.0	8.6	1.0	8.6	
30.0	-	-	-	-	-	-	18.1	-	18.1	5.5	4.6	8.6	4.6	8.6	
40.0	-	-	-	-	-	-	25.1	-	25.1	5.1	4.6	8.6	4.6	8.6	
50.0	-	-	-	-	-	-	19.4	-	19.4	4.9	7.4	8.6	7.4	8.6	

<sup>a</sup> Not determined.

$\beta$ -MG and glucose led to decreased pH values but only glucose was inhibitory. This is in agreement with the report of Smith *et al.* (1986) which indicated that the inhibition of SEA production by glucose could not be directly attributed to a decline in pH of the *S. aureus* cultures.

SEA production in CAS medium containing various combinations of glucose and 2-DOG is shown in Fig. 1A. An upper glucose concentration of 10 mM was selected because of its limited effect on SEA production. Non-inhibitory glucose concentrations and 2-DOG combinations resulted in an inhibition of SEA production which was slightly greater than that which could be attributed to 2-DOG alone. The production of acid, presented as a decline of pH in Fig. 1B, was related to the increasing concentrations of glucose and may be involved in derepression of the acid forming metabolism although 2-DOG itself is not metabolizable. Similar results are presented in Fig. 2A and Fig. 2B for glucose interacted with  $\alpha$ -MG.

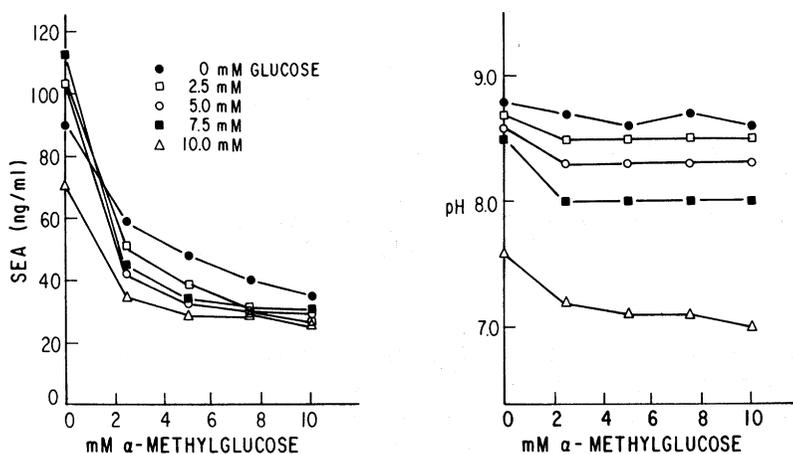


FIG. 2.  
EFFECT OF  $\alpha$ -METHYLGLUCOSE IN COMBINATION WITH GLUCOSE IN CAS MEDIUM  
ON SEA SYNTHESIS (A) AND pH (B) IN *S. AUREUS* 196E

The synthesis of SEA was not affected by  $\beta$ -MG alone, but in combination with non-inhibitory concentrations of glucose there was inhibition of toxin production (Fig. 3A). Fig. 3B demonstrates pH decreases of large magnitude resulting from the glucose and  $\beta$ -MG combination. Since *S. aureus* can metabolize  $\beta$ -MG (Table 1), the results suggest that glucose and  $\beta$ -MG were combining to raise the total concentration of carbohydrate above a minimum concentration value needed to inhibit SEA production and stimulate acid production. It is also possible that the presence of glucose may enhance the transport of  $\beta$ -MG.

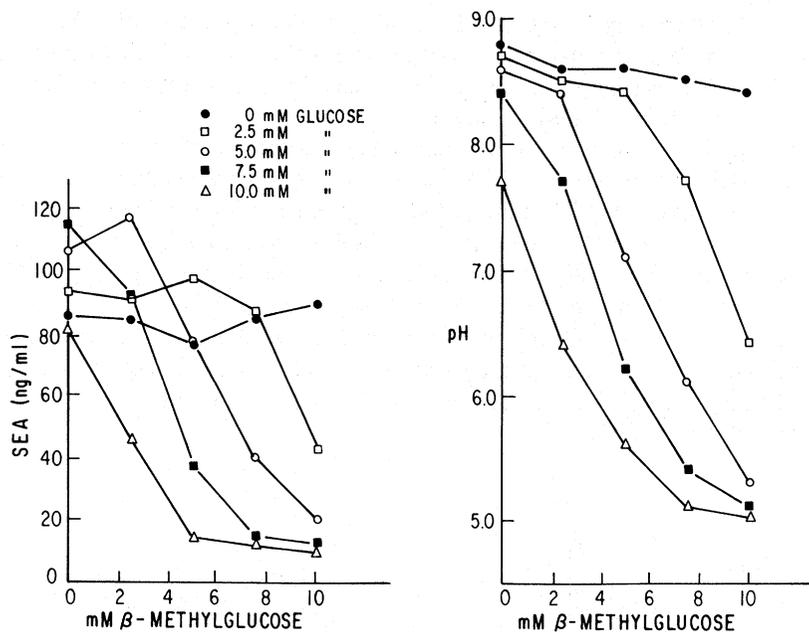


FIG. 3.  
EFFECT OF  $\beta$ -METHYLGLUCOSE IN COMBINATION WITH GLUCOSE IN CAS MEDIUM  
ON SEA SYNTHESIS (A) and pH (B) IN *S. AUREUS* 196E

The presence of 3-O-MG did not greatly affect SEA production (Fig. 4A) or pH (Fig. 4B) regardless of the glucose concentration. Preliminary investigations indicate that this analog may not be transported into *S. aureus*. Further studies are needed to confirm these observations.

The results presented demonstrate that glucose inhibits the formation of SEA when it is at a concentration above a threshold level. When  $\beta$ -MG, at non-inhibitory concentrations, and glucose, at non-inhibitory concentrations, were combined, the inhibition of SEA was greatly increased and the pH decreases observed appeared to be the result of both carbohydrates being metabolized. The possibility that SEA synthesis is catabolite repressible is suggested by these data.

Glucose, 2-DOG, and  $\alpha$ -MG are transported and phosphorylated via a phosphoenolpyruvate-mediated phosphotransferase system (PTS) (Friedman and Hays 1977). Since 2-DOG and  $\alpha$ -MG inhibit SEA production, it appears that the repressive action on SEA synthesis may involve the PTS. Previous work in our laboratory with a PTS-less mutant indicates that an intact PTS is necessary to show repression of SEA production by fermentable carbohydrates (Smith *et al.* 1986; J.L. Smith, M.M. Bencivengo, and C.A. Kunsch, Abstr. Annu. Meet.

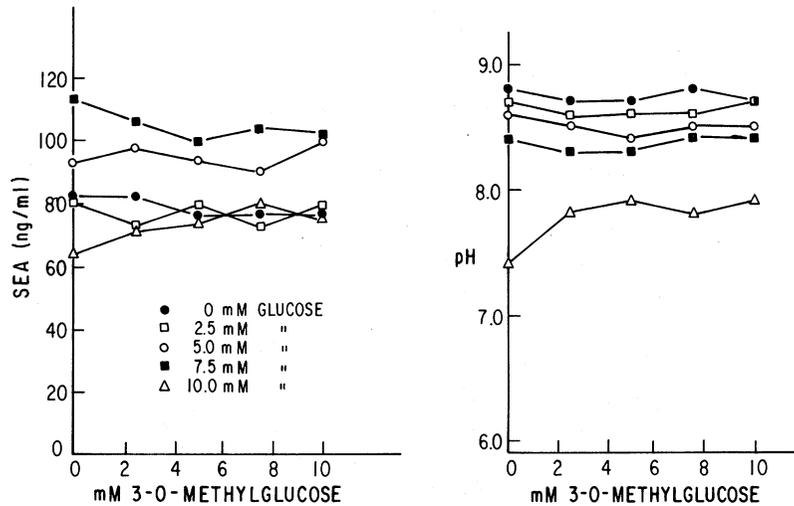


FIG. 4.

EFFECT OF 3-O-METHYLGLUCOSE IN COMBINATION WITH GLUCOSE IN CAS MEDIUM SEA SYNTHESIS (A) and pH (B) IN *S. AUREUS* 196E

Am. Soc. Microbiol. 1986, K-177, p. 223). That work and the current study suggest that SEA synthesis is regulated by a catabolite repression mechanism similar to that observed with gram-negative bacteria (Perlman *et al.* 1969; Pastan and Perlman 1970). The regulatory mechanisms appear to be different since the inhibition of SEA synthesis could not be reversed by exogenous addition of cyclic AMP (Smith *et al.* 1986). A number of studies have suggested that cyclic AMP is not involved in mediating glucose or catabolite repression in gram-positive species (Duncan and Cho 1972; Morse and Baldwin 1973; Ohne 1975; Iandolo and Shafer 1977; Price and Gallant 1983). Work is in progress to identify the chemical signal that mediates catabolite repression in *S. aureus*.

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