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NOTES

Heat Injury in *Staphylococcus aureus* 196E: Protection by Metabolizable and Non-Metabolizable Sugars and Polyols

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Polyols and sugars, which were not metabolized (O_2 uptake or fermentation was not demonstrated), protected *Staphylococcus aureus* 196E against heat injury as well as metabolized compounds. Inhibitors of glucose metabolism decreased O_2 uptake with glucose but did not affect the protective ability of glucose against heat injury.

Polyols and sugars increase the heat resistance (reduce stress-induced injury and death) of bacteria (2, 4, 6, 7, 9, 11, 13, 16), yeasts (6, 9), and fungal spores (3, 5) and reduce acid injury of staphylococci (15). When polyols or sugars are present in the heating menstruum, higher temperatures are required to effect a given killing or injury level (16). The mechanism of the solute protective effect is unknown and does not appear to be a direct function of decreased water activity (a_w) (16, 16a). The present study was undertaken to determine if protective polyols or sugars are metabolized by *Staphylococcus aureus*. The effect of inhibitors of glucose metabolism on the protective action of glucose was also investigated.

Stationary-phase *S. aureus* 196E cells were heated in 0.1 M phosphate buffer (pH 7.2), with added sugar or polyol, at 50°C for 45 min as previously described (16a). The numbers of injured and noninjured cells were determined, and the relative protective effect (RPE) was calculated (16).

For most of the compounds tested, high solute concentration produced greater protection against thermal injury, i.e., the RPE approached 1.0 (Table 1). However, maltose gave only limited protection regardless of concentration, and xylose protected only at low concentrations. Xylose was found to be bactericidal at the 40 and 50% levels. On the basis of O_2 uptake and fermentation studies (Table 1), it would appear that compounds need not be metabolized to protect against thermal injury. Oxygen uptake by washed cells was stimulated by only 6 of the 14 compounds tested, and fermentation-mediated acid production was demonstrated in 5 of those 6 compounds. Moreover, two glucose analogs, 2-deoxyglucose and α-methylglucoside, were as protective as glucose at the 50% level. These analogs are known to penetrate the cell membrane of *S. aureus* (10); both compounds are phosphorylated to the 6-phosphate level, but further metabolism does not occur.

Inhibitors of glucose metabolism reduced O_2 uptake by *S. aureus* (Table 2) but did not affect the RPE of glucose at the 50% level, further demonstrating that protection against thermal damage does not depend on cellular metabolism. Inhibitors included oxidative phosphorylation uncouplers (2,4-dinitrophenol, arsenate, carbonyl cyanide *m*-chlorophenylhydrazone), inhibitors of electron transport (NaF, KCN), and an agent reactive with sulfhydryl groups (arsenite). The inhibitors did not potentiate heat injury, and 2,4-dinitrophenol, NaF, and carbonyl cyanide *m*-chlorophenylhydrazone actually had appreciable protective effects when used alone (Table 2).

The mechanism by which sugars or polyols protect *S. aureus* against heat injury is not understood. The relationship of decreased water activity (a_w) of the heating menstruum and thermal injury was evaluated by Smith et al. (16, 16a), who tested the protective ability of salts, sugars, polyols, and amino acids at a_w values ranging from 0.80 to 0.98. No consistent relationship between protection and a_w was found (16, 16a). However, Ng (14) has suggested that NaCl plasmolyzes bacterial cells and that the resulting dehydration lowers the internal a_w, thereby stabilizing macromolecules against sublethal heat effects. A potential site of stabilization for protective solutes is the cell membrane. The addition of NaCl or sucrose to the heating menstruum is known to prevent leakage of UV-

TABLE 1. Relationship between O_2 uptake on polyols and sugars and their ability to protect *S. aureus* from heat injury

Compound	O_2 uptake (μl/h)[a]	Fermentation with acid[b]	RPE at designated sugar or polyol level[c]				
			20%	30%	40%	50%	60%
Maltose, water	225	+	NT	0.42	0.45	0.49	0.45
Sucrose	191	+	NT	0.79	0.89	0.96	0.99
Mannose	147	+	NT	NT	NT	0.91	NT
Glucose	144	+	0.32	0.58	0.86	0.99	NT
Fructose	90	+	NT	NT	0.25	0.47	0.71
Glycerol	66	−	0.89	0.92	0.94	NT	NT
Melezitose, water	0	−	NT	NT	NT	0.62	NT
2-Deoxy-D-glucose	0	−	NT	NT	NT	1.00	NT
α-Methyl-D-glucoside	0	−	NT	NT	NT	0.99	NT
Arabinose	0	−	NT	NT	NT	0.34	NT
Xylose	0	−	0.75	0.37	0.0	0.0	NT
Xylitol	0	−	0.82	0.97	0.98	0.96	0.96
Adonitol	0	−	NT	NT	NT	0.89	NT
Sorbitol	0	−	0.87	0.96	0.97	1.00	NT

[a] Fifteen-milliliter single sidearm Warburg flasks (attached to a Gilson differential respirometer) contained a total volume of 3 ml: potassium phosphate buffer (pH 7.2, 0.1 M) and 0.01 mM sugar or polyol were placed in the main compartment; 0.2 ml of 40% KOH was placed in the center well; and washed cells of *S. aureus* 196E (ca. 6.3 mg [dry weight]) were placed in the side arm. The flasks were equilibrated at 37°C, the cells were tipped in, and the uptake of O_2 was determined. Endogenous values were subtracted.

[b] Washed cells of *S. aureus* 196E were added to tubes containing 10 ml of 0.01 M potassium phosphate buffer (pH 7.2), 1% sugar or polyol, and phenol red indicator. Tubes were incubated at 37°C and observed at hourly intervals.

[c] RPE = 1 − Number of injured cells obtained when *S. aureus* was heated in buffer containing test compound/number of injured cells obtained in heated buffer alone. The RPE values range from 0.0 for no protection to 1.0 for complete protection against heat injury. NT, Not tested.

absorbing materials from cells during sublethal thermal stress (11, 16). Salts and sugars have been used to stabilize proteins in the native conformation (1, 12, 17). Since the membranes of gram-positive bacteria consist of protein and lipid in a 4:1 ratio (8), sugars and polyols may prevent denaturation of membrane proteins during thermal stress, thereby preventing leakage of essential macromolecules.

TABLE 2. Effect of metabolic inhibitors on oxygen uptake with glucose by *S. aureus* and on protection against heat injury by 50% glucose

Inhibitor (mM)	% Inhibition of O_2 uptake at 30 min[a]	RPE after 45 min at 50°C			
		− Inhibitor	+ Inhibitor	50% Glucose	Inhibitor + glucose
Na arsenate (10)	59	0.0	0.04	0.93	0.96
Na arsenite (10)	30	0.0	0.10	1.00	1.00
NaF (10)	70	0.0	0.46	1.00	1.00
KCN (10)	95	0.0	0.21	0.99	1.00
2,4-Dinitrophenol (1)	47	0.0	0.50	1.00	1.00
Carbonyl cyanide *m*-chlorophenylhydrazone (0.1)	50	0.0	0.48	0.97	0.97

[a] Determined respirometrically; procedure was similar to that described in Table 1, footnote *a*. The inhibitors, at the stated concentrations, were added to the main compartment of the Warburg flasks.

These results show that protective compounds need not be metabolic substrates. However, we did not specifically address the question of entry into the cell (permeability) as a mediator of thermal protection, and further research will be required.

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