Effect of sodium chloride on the respiratory function of *Staphylococcus aureus*

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Summary. Sodium chloride at concentrations below 0.5 M, enhanced the respiratory activity (O_2-consumption) of *Staphylococcus aureus* under endogenous and sugar-supported conditions, but did not overcome the inhibitory action of sodium azide. Several sugars, including the glucose analogue α-methylglucoside, and their metabolites enhanced bacterial O_2-consumption, but acetylmethylcarbinol was ineffective.

It is well known that *Staphylococcus aureus* possesses a mechanism for resistance to salt, which enables it to grow in media with relatively high salt concentrations[2-4]. However, salt resistance in staphylococci is different from that in halophiles[5], because staphylococci also grow well in low salt media, and therefore, this property is of interest physiologically.

Recent investigations in this laboratory indicated that the production of staphylococcal nuclease, a representative enzyme secreted by *S. aureus,* increased significantly after the addition of adequate amounts of sodium chloride (NaCl) into the culture medium[6], but the stimulatory effect of NaCl does not overcome the suppression of nuclease production by specific inhibitors of energy metabolism or respiratory functions (unpublished data). To clarify further the regulatory mechanism of nuclease production, effect of NaCl on the respiratory function of *S. aureus* was examined.

Materials and methods. The bacterial strain used in the present investigation was *Staphylococcus aureus* strain Wood 46 (ATCC 10832). The organisms were grown overnight (18 h) in brain-heart infusion broth (Difco, Detroit, Mich., USA) with constant shaking on a reciprocating shaker, harvested by centrifugation at 6000×g for 10 min at 4 °C, and washed 3 times with 0.05 M Tris-acetate buffer, pH 7.0. The washed cells were resuspended in the same buffer. Cell concentrations were determined at 540 nm using a Shimadzu (Kyoto, Japan) UV-200 spectrophotometer by relating the OD to dry weight.

Respiratory activity was measured manometrically in a Warburg apparatus from Ikemoto Sci. Instr. (Tokyo, Japan). For the measurement of endogenous or glucose-supported respiration, the equivalent of 4 or 8 mg dry weight of cell suspensions was placed in the main vessel with or without the desired concentrations of NaCl or sodium azide (NaN_3). The volume was then made up to 2.7 ml with Tris-acetate buffer. Unless otherwise indicated, 0.3 ml of 0.3 M glucose solution or Tris-acetate buffer was tipped into the main chamber from the side arm after temperature equilibration. The temperature was 37 °C, the atmosphere was air, and 0.2 ml of 20% KOH was in the center well. The incubation was continued for 60 or 120 min, and the results (Q_{O2}) were expressed as the mean of at least 3 experiments showing less than 7.5% variation. All the chemicals used were of analytical grade and were obtained from Wako pure Chemical Instr. Ltd. (Osaka, Japan), except for acetylmethylcarbinol which was obtained from Tokyo Kasei Instr. Co., Tokyo.

Results and discussion. Table 1 shows the effect of various sugars and their metabolites added as a substrate for respiration. With the exception of acetylmethylcarbinol, most compounds stimulated bacterial O_2-consumption remarkably. The effect was more pronounced with the sugars themselves (glucose or maltose) than with the metabolites.

The lack of stimulation by acetylmethylcarbinol may be due to the fact that, as pointed out previously[7], this is a final product (or the nearest metabolite to it) of fermentation in several bacteria including *S. aureus*[8,9]. α-Methylglucoside, which is considered to be transported into the cell but not further metabolized[10,11], also significantly enhanced the O_2-consumption. This reagent was chromatographically[12] pure and it is difficult, therefore, to explain why bacterial respiration is enhanced so significantly by the addition of α-methylglucoside.

Table 2 shows the effect of NaCl on glucose-supported or endogenous respiration. The bacterial O_2-consumption was enhanced efficiently by the addition of NaCl at the concentrations of 0.1 or 0.5 M, but was rather suppressed at the concentration of 1 M. Similar results as with NaCl were obtained with potassium chloride (data not shown).

The inhibition of glucose-supported or endogenous respirations with different concentrations of NaN_3 and its reversibility by the addition of NaCl were examined (table 3).

Table 1. Effect of various sugars and their metabolites on the respiratory rates of *Staphylococcus aureus*

Substrate (10 mM)*	Q_{O_2}**	Substrate (10 mM)*	Q_{O_2}**
None	2.2	Ethanol	18.4
Glucose	31.4	Pyruvate	15.0
Maltose	27.6	Lactate	13.5
α-Methylglucoside	9.8	Acetate	7.6
		Acetylmethylcarbinol	2.0

* Substrate was added from the side arm to a final concentration of 10 mM after the temperature equilibration (15 min). ** Q_{O_2} equals µl of O_2/h/mg dry weight of cells.

Table 2. Effect of NaCl on glucose-supported and endogenous respirations of *Staphylococcus aureus*

NaCl (M)	Q_{O_2} (µl of O_2/h/mg dry weight of cells)	
	Glucose-supported*	Endogenous
0	29.8	5.8
0.1	33.3	7.9
0.5	38.8	8.0
1.0	26.0	4.5

* Glucose was added from the side arm to a final concentration of 30 mM after temperature equilibration (15 min) in the presence or absence of NaCl at the concentration indicated.

Table 3. Inhibition of respiratory rates of *Staphylococcus aureus* by NaN_3, and its reversibility with NaCl

Concentration of		Q_{O_2} (µl of O_2/h/mg dry weight of cells)	
NaCl (M)	NaN_3(mM)*	Glucose-supported	Endogenous
	0	11.5	3.0
	1	7.9	3.5
	2	6.3	2.9
	5	5.9	2.3
	10	8.3	1.5
0.5	10	8.4	1.6

* NaN_3 was added after temperature equilibration (15 min) from the side arm to a final concentration indicated in the presence (glucose-supported) or absence (endogenous) of glucose (30 mM) and NaCl (0.5 M).

Endogenous O_2-consumption was slightly stimulated at a concentration of 1 mM NaN_3 and the inhibitory effect of NaN_3 was slightly reduced on glucose-supported respiration at the concentration of 10 mM as compared to lower NaN_3 levels. These results are of interest for analyzing the principle of the uncoupling effect of this inhibitor[13,14].

Although NaCl enhanced the consumption of oxygen by *S. aureus* as shown in table 2, the salt did not overcome the inhibitory action of NaN_3. This observation seems to exclude a mechanism for the observed enhancement of bacterial O_2-uptake by salt that is based on a direct activation of enzymes constituting the electron transport system. As a working hypothesis, we assume that the activity of oxidative phosphorylation, depending on the membrane-associated K^+- or Na^+-stimulated ATPase[15], may control indirectly the respiratory function of *S. aureus*.

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The turnover of F-actin-bound ADP in vivo[1]

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Summary. A procedure for estimating the rate of turnover of F-actin-bound ADP in vivo is described. A turnover rate of 0.88 h^{-1} was determined for mouse muscle F-actin. The validity of the method when used to estimate the turnover rate of F-actin per se is discussed in relation to the possible exchange of F-actin-bound ADP.

The polymerization of actin has been extensively studied in vitro. However, relatively little is known about the polymerization process in vivo[2]. Actin exists in a monomeric state, G-actin, to which is bound one molecule of ATP and in a polymeric form, F-actin. The polymerization process involves the sequential addition of monomers with the concomitant dephosphorylation of the ATP to ADP which remains tightly bound to each actin subunit[2]. In earlier work[3] ADP bound to washed muscle homogenates has been used as a marker to estimate the actin concentration in muscle. The present report describes a procedure for estimating the rate of actin polymerization in vivo by determining the rate of turnover of [^{3}H]-ADP bound to F-actin. F-actin-bound ADP may be conveniently radioactively-labelled by administration of [2-^{3}H]-adenosine.

Materials and methods. Male adult mice, Quackenbush strain weighing 35–45 g, were injected s.c. with 200 µCi [2-^{3}H]-adenosine (Radiochemical Centre, Amersham) in 0.2 ml-saline. In order to avoid leakage of the solution from the puncture site, injections were performed by passing the needle s.c. from the puncture site in the pelvic region to the mid-point on the dorsal surface of the thigh. In this manner each hind-leg received 0.1 ml of the isotope solution.

At various times up to 1 h after injection mice were anaesthetized with ether, the hind-limbs skinned and the muscle removed and placed in liquid nitrogen. This procedure was carried out as rapidly as possible. The frozen tissue was powdered using a percussion mortar. A sample of this frozen tissue powder was homogenized in 4.0 ml of ice-cold 0.5 M-perchloric acid in order to extract the tissue nucleotides. The perchloric acid extract was neutralized with 5.0 M-KOH and the concentration of tissue nucleotides determined in the extract by ion-exchange chromatography[4]. The eluate fractions containing individual nucleotides were collected and the radioactivity in aliquots of these fractions determined by liquid scintillation spectrometry.

A 2nd sample of the tissue powder was homogenized in 20 mM-Tris buffer containing 100 mM-KCl and the precipitate washed repeatedly with further aliquots of buffer until no radioactivity above background was detectable in aliquots of the supernatants. The final washed precipitate was extracted twice with 2.0 ml 0.5 M-perchloric acid. The concentration of ADP in neutralized samples of this extract was determined enzymatically[5]. Further aliquots were used for liquid scintillation spectrometry. The presence of radioactivity in ADP only was confirmed by preparative electrophoresis[6].

The precipitates remaining after extraction were dried and the concentration of protein in the dry residue determined according to the method of Lowry et al.[7].

Turnover rate of F-actin-bound ADP

Time t (h)	ATP:ADP sp. act. ratio (^{S}ADP/^{S}ATP)	Fractional turnover rate k (h^{-1})
0.25	0.11 ± 0.04 (3)	0.89 ± 0.36
0.50	0.23 ± 0.06 (3)	1.10 ± 0.34
0.75	0.22 ± 0.09 (2)*	0.75 ± 0.35
1.0	0.30 ± 0.08 (3)	0.76 ± 0.28

Mean ± SEM (number). * Duplicates only, mean ± range given.