Physiological Conditions Affecting *Staphylococcus aureus* Susceptibility to Staphylococcin 1580

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Loss of salt tolerance, irreversible loss of viability, inhibition of L-glutamic acid uptake and effects on the high energy state of the membrane were used as parameters to measure the injury induced by staphylococcin 1580, a bacteriocin of *Staphylococcus epidermidis*, in susceptible cells of *Staphylococcus aureus* Oxford 209P. A small part of a growing cell population appeared to be temporarily resistant to the bacteriocin, and the cells were arrested in this stage when suspended in buffer. The proportion of susceptible cells may rapidly shift during exponential growth, apparently concomitantly with a change in cell metabolism. Glucose- and pyruvate-grown cells were equally susceptible to salts after staphylococcin treatment. Only in pyruvate-grown cells was amino acid uptake strongly inhibited, and the membrane potential was abolished after a short lag time. Also, irreversible killing was more distinct in pyruvate-grown cells. The proton gradient across the cell membrane was only slightly disturbed in both types of cells. Specific inhibitors of the energy metabolism revealed that the high energy state of the membrane was largely supported by hydrolysis of adenosine 5'-triphosphate in glucose-grown cells, whereas the oxidative input through electron transport appeared to be relatively more important in pyruvate-grown cells. Staphylococcin 1580 affected primarily the oxidative energy metabolism, although electron transport is not inhibited. Below a distinct incubation temperature cells were completely resistant to the action of the bacteriocin. Varying the growth temperature had only a slight effect on the transition temperature, but growth in the presence of Tween 80, which greatly enhanced the proportion of unsaturated fatty acids, decreased the transition temperature.

Bacteriocins are widely produced among various bacteria, and some of them, especially colicins, have been studied extensively (22). Bacteriocins from gram-positive bacteria (reviewed recently by Tagg et al. [23]) have been, with a few exceptions, investigated much less thoroughly.

Staphylococcin 1580, a bacteriocin produced by *Staphylococcus epidermidis* 1580 (11, 14), exerts its action on the cytoplasmic membrane and causes a simultaneous inhibition of active transport and macromolecular synthesis, a rapid leak of potassium ions from the cells, and a decline in the cellular adenosine 5'-triphosphate (ATP) level (10). In this it resembles colicins E₁ and K (7) and A (12) and also a variety of bacteriocins from gram-positive bacteria (23).

In a previous study it was shown that susceptible cells are not irreversibly damaged as a consequence of staphylococcin treatment, but the injury may be repaired under appropriate conditions (27). It was also noticed (13) that the staphylococcin 1580-induced killing effect is

strongly influenced by the growth temperature of the susceptible cells as well as by the temperature of incubation, which suggests a dependence on the physiological state of the susceptible cells. Similar conclusions were drawn for the action of colicins K (20) and E₁ (4) and various bacteriocins from gram-positive bacteria, such as staphylococcin C55 (5) and streptococcin A-FF22 (24). It was shown that expression of colicin K action was dependent on a proper fluidity of the membrane (21) and the supply of energy (9, 19), both probably required for the access of the colicin molecule to its target on the cytoplasmic membrane.

In this paper we report on conditions required for the action of staphylococcin 1580 and the role of energy involved in the expression of the killing effect.

Energy may either be derived directly from ATP or drawn from the high energy state of the membrane (8). According to the chemiosmotic hypothesis (17), a protonmotive force ($\Delta\mu_{H^+}$) is created by the extrusion of protons by either

oxidation of the substrate or hydrolysis of ATP (8) and is composed of an electrical potential ($\Delta\psi$) and a chemical potential ($\Delta\mu$).

MATERIALS AND METHODS

Bacterial strains. Staphylococin 1580-producing *S. epidermidis* 1580 and the indicator strain *S. aureus* Oxford 209P have been described previously (14). A staphylococin 1580-resistant mutant, strain N3, was derived from the latter by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1) and selection for staphylococin 1580 resistance.

The strains were subcultured each fortnight on tryptone soya agar (TSA) (Oxoid Ltd., London) and stored at 4°C.

Growth media and preparation of cells. Semi-synthetic medium A was derived from the previously described AJ-1 medium (10), except that casein hydrolysate (enzymatically hydrolyzed) was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio), and either glucose (1%, wt/vol) or sodium pyruvate (1%, wt/vol) was used as carbon source. Unless otherwise indicated, exponentially growing cells were harvested by centrifugation, washed once, and resuspended to a final concentration of 0.25 mg (dry weight)/ml in incubation buffer, which consisted of 50 mM potassium phosphate (pH 7.0), 50 mM sodium chloride, and 2 mM magnesium sulfate. Cell suspensions were stored at 0°C and used within 1 h after preparation.

Production, purification, and assay of staphylococin 1580. The bacteriocin was prepared and assayed as previously described (27). The activity was expressed in arbitrary units per milliliter.

Viable count. Viable counts after staphylococin 1580 treatment were determined by rapidly diluting samples 1,000-fold into ice-cold tryptone soya broth and subsequently plating suitably diluted amounts on both TSA and TSA supplemented with 1 M sodium chloride (TSAS) to measure loss of salt tolerance (27). The survival ratio (R_s) was defined as the ratio between the viable counts of treated and untreated bacteria.

Transport of amino acids. Unless otherwise indicated, the incubation mixtures contained 50 mM potassium phosphate (pH 7.0), 50 mM sodium chloride, 2 mM magnesium sulfate and 0.25 mg (dry weight) of cells per ml. After 5 min of preincubation at 37°C, the radioactive label was added to a final concentration of 10^{-6} M. Samples (0.1 ml) were withdrawn at the times indicated, rapidly filtered on membrane filters (0.45- μ m pore size; Millipore Benelux, Brussels, Belgium), and washed with 4 ml of the aforementioned incubation buffer. After the filters were dried, radioactivity was counted in a liquid scintillation counter.

Metabolic inhibitors added to the incubations were dissolved in ethanol, except for KCN, which was dissolved in buffer. The final ethanol concentration never exceeded 1%, which did not affect amino acid uptake.

Determination of the intracellular pH. The intracellular pH was calculated according to Waddell and Butler (25) from the distribution of 5,5-dimethyl-2,4-[14 C]oxazolidinedione (DMO) (final concentration, 21.4 μ M; 1.0 μ Ci/ml). Extracellular water was moni-

tored by the use of [3 H]inulin (final concentration, 20.0 μ M; 1.4 μ Ci/ml). Cells were suspended in 50 mM potassium phosphate (pH 7.0) in a final concentration of 0.45 mg (dry weight)/ml and incubated at 37°C. Samples of the incubation mixture were filtered on Millipore filters (0.45- μ m pore size) without washing. Filters were dried (15 min, 105°C), and the radioactivity was determined. After the last sample had been taken, the remainder of the incubation mixture was centrifuged, and 10- μ l samples of the supernatant were counted to determine the radioactivity in the external liquid. Counts were corrected for channel efficiency and spillover. The intracellular water space was taken to be 1.55 ml/g (dry weight) of cells (18).

Fluorescence measurements. Fluorescence of 3,3'-dipropylthiodicarbocyanine (DiS-C₃) allows the continuous monitoring of the membrane potential (2, 16, 26). Washed cells were suspended in incubation buffer (pH 7.0) in a standard 1-cm-pathlength fluorescence cuvette at a final concentration of 50 μ g (dry weight)/ml. DiS-C₃ was added to a concentration of 800 nM, and fluorescence was excited at 620 nm and measured at 662 nm by use of an Aminco Bowman spectrofluorimeter with 3-mm slits in positions 3 and 4. Measurements were carried out at 37°C, and the suspensions were aerated through a 28-gauge needle. Scatter due to the bacteria was less than 2%. At the end of each experiment maximum fluorescence of the system was determined after the addition of gramicidin D (1 μ g/ml), which allows leveling of H⁺, Na⁺, and K⁺ over the membrane and abolishes the membrane potential completely. DiS-C₃ did not affect the viability of the cells and did not influence the staphylococin 1580-induced killing effect.

Fatty acid composition. Extraction and methylation of fatty acids were carried out as described previously (28). Gas chromatograms were run at 170°C on a Pye series 104 gas chromatograph, equipped with 5-foot (ca. 152-cm) columns of 10% diethylene glycol succinate on 100- to 120-mesh Diatomite C.

Pyruvate concentration. The concentration of pyruvate was measured by the method of Dietz (6).

Chemicals. L-[U- 14 C]glutamic acid (270 mCi/mmol), L-[U- 14 C]glutamine (40 mCi/mmol), L-[U- 14 C]proline (290 mCi/mmol), L-[U- 14 C]leucine (330 mCi/mmol), and [3 H]inulin (695 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, England. [2- 14 C]DMO (46.6 mCi/mmol) was supplied by New England Nuclear (Boston, Mass.). Stock solutions of *N,N'*-dicyclohexylcarbodiimide (DCCD) (Aldrich-Europe, Beerse, Belgium), valinomycin (Calbiochem, Lucerne, Switzerland), gramicidin D, and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) (both from Boehringer, Mannheim, West Germany) were prepared in ethanol and stored at -18°C. DiS-C₃ was obtained from A. Waggoner, Amherst College, Amherst, Mass. Polyethylene sorbitan monooleate (Tween 80) was obtained from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were of reagent grade.

RESULTS

Composition of the incubation medium. Staphylococin 1580 appeared to be more lethal

to cells treated in growth medium than to washed cells suspended in a simple buffer medium (Fig. 1). This was evident when treated cells were plated on TSA and compared with cells plated on the same medium containing 1 M sodium chloride. The viable count on the latter medium was much lower due to the inhibiting effect of sodium chloride on the rescue of cells from the primary injury induced by the staphylococin (27). The different effect exerted by the growth medium and the buffer did not result from the different handling of the cells during the wash and resuspension procedure, since similarly treated cells, washed with and resuspended in growth medium, were killed to the same extent as cells not handled in this way. Moreover, addition of various energy sources, such as glucose, pyruvate, and L-lactate, to the buffer medium did not lower the survival ratio. Neither can the different effects be explained on basis of the presence of salts in the buffer, since salts affect the staphylococin 1580-induced killing only slightly (27). Also, pH values between 5.5 and 8.5 have only a minor influence; a distinct optimal killing effect around pH 6.5 could, however, be observed (27). The maximal number of killed cells was usually reached after about 5 min with cells treated in buffer, but cells treated in growth medium were progressively killed after that time interval. This sug-

gests that only a part of the buffer-suspended cells is susceptible, whereas in growth medium the temporarily unsusceptible cells enter the susceptible stage progressively.

Influence of growth phase. Both the survival ratio and the inhibition of glutamic acid uptake after staphylococin 1580 treatment were strongly dependent on the growth phase in batch cultures of the tested cells (Fig. 2). Cells growing in medium A with sodium pyruvate as source of energy were optimally susceptible in the mid-exponential phase. A sudden change in susceptibility occurred coincidentally with an alteration of the growth rate. At this time the pyruvate concentration in the medium still amounted to over 80% of the initial value; growth continued for at least another two generations and ceased when pyruvate was completely used up.

Effect of the energy source. The effect of the main carbon and energy source in the growth medium on the susceptibility of cells to staphylococin 1580 was investigated to elucidate the

FIG. 1. Effect of the incubation medium on the killing effect of staphylococin 1580. *S. aureus* Oxford 209P cells from an exponentially growing culture on medium A with 1% sodium pyruvate (10^8 cells/ml) were treated with staphylococin 1580, either directly in the medium (pH 7.0) or in incubation buffer (pH 7.0). Samples were withdrawn after 5 and 30 min and plated on both TSA (solid lines) and TSAS (broken lines). Viable counts are expressed as the ratio of staphylococin 1580-treated and untreated suspensions (R_t). Symbols: (●) 10 arbitrary units (AU); (○) 40 AU; (△) 100 AU; (■) 250 AU of staphylococin 1580 per ml. (A) Cells in medium A-pyruvate; (B) cells in incubation buffer.

FIG. 2. Relation between the growth phase of *S. aureus* Oxford 209P cells and their susceptibility to staphylococin 1580. At intervals samples were taken from a culture growing on medium A-pyruvate, diluted with fresh, prewarmed medium to give about 5×10^8 cells/ml, and incubated with 100 arbitrary units of staphylococin 1580 per ml and ^{14}C -labeled L-glutamic acid ($2 \mu\text{Ci/ml}$) for 15 min at 37°C . Controls received no staphylococin. Viability is given as the survival ratio (R_t), and the uptake of L-glutamic acid is given as the ratio of disintegrations per minute between bacteriocin-treated and untreated cells. Symbols: (●) viability; (○) L-glutamic acid uptake. Insert: (□) growth curve.

possible involvement of distinct catabolic pathways in the staphylococcin action. Although both glucose- and pyruvate-grown cells were killed by the bacteriocin to the same extent when plated on TSAS (log R_t values were -2.32 and -2.45 , respectively, after 5 min of staphylococcin treatment), pyruvate-grown cells were apparently damaged more severely, since the survival ratio on TSA was considerably smaller: the log R_t values were -0.22 and -1.15 for glucose- and pyruvate-grown cells, respectively. This was particularly clear when the effect of the bacteriocin on uptake of glutamic acid (Fig. 3) or on the membrane potential (Fig. 4) was studied. Uptake in pyruvate-grown cells was inhibited by staphylococcin 1580, but, in contrast, it was stimulated in glucose-grown cells. In both cases the K_m for transport, which amounted to 1.2×10^{-4} M, was not affected, which is in accordance with the previous reported results, obtained with membrane vesicles (12). Similar observations were made with the amino acids leucine, proline, and glutamine.

The effect of various inhibitors on the L-glutamic acid uptake in both types of cells is shown in Table 1. Uptake was strongly inhibited by the uncoupler FCCP and by valinomycin in the presence of external potassium ions, which confirms the previous conclusion on its coupling to a high energy state of the membrane (12). DCCD, an inhibitor of the membrane-bound Ca^{2+} , Mg^{2+} -adenosine triphosphatase (ATPase),

FIG. 3. Effect of staphylococcin 1580 on the uptake of L-glutamic acid in *S. aureus* Oxford 209P cells grown with either glucose or sodium pyruvate. Cells were preincubated for 5 min at 37°C , after which staphylococcin (100 arbitrary units/ml, final concentration) or only buffer was admixed, followed 1 min later by $\text{L-[}^3\text{C]glutamic acid}$. (A) Cells grown with glucose; (B) cells grown with sodium pyruvate. Inserts: Lineweaver-Burk plots of initial L-glutamic acid uptake rates. Symbols: (●) treated with staphylococcin 1580; (○) treated with buffer only.

exhibited a divergent effect: uptake in glucose-grown cells was severely inhibited at concentrations that did not affect uptake in pyruvate-grown cells. Experiments carried out with toluene-treated cells, grown and treated under the same conditions as used in the uptake experiments, showed that DCCD inhibits the Ca^{2+} , Mg^{2+} -ATPase activity to the same extent in both glucose- and pyruvate-grown cells. Moreover, 4×10^{-5} M DCCD did not affect L-glutamic acid uptake in pyruvate-grown cells but inhibited the Ca^{2+} , Mg^{2+} -ATPase activity 62 and 63%, respectively, in glucose- and pyruvate-grown cells. This suggests that energy used for L-glutamic acid uptake in glucose-grown cells is at least in part coupled by way of the membrane ATPase, whereas in pyruvate-grown cells the oxidative input through electron transport is relatively more important.

To test whether staphylococcin 1580 blocks electron transport itself, oxygen consumption of glucose- and pyruvate-grown cells was measured polarographically under the conditions used in the uptake experiments (details not shown here). Oxygen consumption was not affected in glucose-grown cells, which is in accordance with previous findings (10). However, staphylococcin

FIG. 4. Effect of staphylococcin 1580 and DCCD on DiS- C_3 fluorescence in *S. aureus* cells. The final concentration of cells was $50 \mu\text{g}$ (dry weight)/ml. Suspensions (2.5 ml) were preincubated in the cuvette for 5 min at 37°C , after which DiS- C_3 was added. Staphylococcin 1580 and DCCD were added to final concentrations of 25 arbitrary units/ml and 5×10^{-5} M, respectively. Maximal fluorescence was determined after the addition of gramicidin D ($1 \mu\text{g}/\text{ml}$). Full lines represent *S. aureus* Oxford 209P cells grown with sodium pyruvate; broken lines, *S. aureus* Oxford 209P cells grown with glucose; dotted lines, *S. aureus* N3 cells grown with sodium pyruvate. The log R_t values, determined by plating on TSAS, were -2.10 and -2.38 for *S. aureus* Oxford 209P grown on glucose and sodium pyruvate, respectively, and $+0.05$ for *S. aureus* N3. (A) Effect of staphylococcin 1580; (B) effect of DCCD.

TABLE 1. Effect of inhibitors on glutamic acid uptake in *S. aureus* Oxford 209P grown with glucose or pyruvate^a

Inhibitor	Concn (M)	Glutamic acid uptake			
		Glucose-grown cells		Pyruvate-grown cells	
		nmol/mg (dry wt) per min	% of control	nmol/mg (dry wt) per min	% of control
None		0.28	100	0.62	100
DCCD	10 ⁻⁵	0.15	54	0.60	97
	4 × 10 ⁻⁵	0.13	45	0.68	110
	10 ⁻⁴	0.08	30	0.22	36
Valinomycin	10 ⁻⁶	0.12	44	0.22	36
FCCP	10 ⁻⁶	0.03	11	0.04	7
KCN	10 ⁻²	0.05	18	0.06	9

^a Uptake was measured 30 s after addition of the label. Valinomycin and FCCP were added 1 min and DCCD and KCN were added 10 and 5 min, respectively, before addition of the label.

1580 stimulated oxygen consumption of pyruvate-grown cells 60 to 80%.

These findings are confirmed in the studies with the fluorescent probe DiS-C₃, recently used by several investigators to measure the electrical potential ($\Delta\psi$) across the membrane (2, 15, 16, 26). Preliminary experiments showed that this technique properly reflects the changes in the membrane potential expected from valinomycin treatment (1 μ g/ml) of *S. aureus* cells and membrane vesicles, suspended in either sodium phosphate buffer (hyperpolarization) or potassium phosphate buffer (depolarization) under the conditions applied in these experiments. Both glucose- and pyruvate-grown cells exhibited about identical levels of fluorescence quenching and, hence, possess a similar membrane potential (Fig. 4). DCCD did not affect the membrane potential in pyruvate-grown cells but depolarized glucose-grown cells largely (Fig. 4B), which corroborates the different way of energy coupling. Staphylococcin 1580 had a pronounced effect on the membrane potential of pyruvate-grown cells (Fig. 4A): after a 20-s lag the fluorescence quenching was partly abolished, most probably due to a partial depolarization of the membrane potential. Increasing amounts of staphylococcin 1580 did not further abolish the membrane potential, nor did it affect the length of the lag time. In contrast, the membrane potential of glucose-grown cells was affected only slightly by staphylococcin 1580 treatment. Further addition of staphylococcin 1580 did not enhance the response. The membrane potential of the staphylococcin 1580-resistant strain N3, grown on either glucose or pyruvate, was not affected by the bacteriocin.

In a previous paper it was shown by use of the acid-pulse technique that the permeability

TABLE 2. Effect of staphylococcin 1580 on the intracellular pH of cells grown with either glucose or sodium pyruvate^a

Time after addition of staphylococcin 1580 (min)	Internal pH	
	Glucose-grown cells	Pyruvate-grown cells
1	7.78	7.53
5	7.74	7.58
10	7.65	7.55
15	7.62	7.61
No staphylococcin 1580	7.65 ± 0.03	7.65 ± 0.02

^a Staphylococcin 1580 was admixed immediately after the radioactive labels in a final concentration of 100 arbitrary units/ml.

of the membrane to protons was not altered by treatment with staphylococcin 1580 (12). Table 2 shows the intracellular pH values for glucose- and pyruvate-grown cells in the presence or absence of staphylococcin 1580, as calculated from the distribution of the weak acid [¹⁴C]DMO. The intracellular pH, and, hence, the pH gradient (Δ pH) over the cytoplasmic membrane at constant external pH were identical in glucose- and pyruvate-grown cells and remained constant throughout the incubation period. Incubation with staphylococcin 1580 had only a slight effect: a small and transient rise of the internal pH could be observed in glucose-grown cells, whereas the internal pH of pyruvate-grown cells was temporarily lowered.

Effect of temperature. The extent of staphylococcin 1580-induced injury, as determined by culturing treated cells on high-salt (TSAS) medium, was strongly dependent on the incubation temperature applied during the treatment with the bacteriocin (Fig. 5). A transition from resis-

FIG. 5. Effect of the incubation temperature on staphylococcin 1580-induced killing of *S. aureus* Oxford 209P. Cells were grown at 37°C into mid-exponential phase on tryptone soya broth, and prepared as described in the text. After 5 min of preincubation at various temperatures, staphylococcin 1580 (100 arbitrary units/ml, final concentration) or only buffer was added, and the mixtures were incubated further for 5 min. Samples were plated on both TSA (○) and TSAS (●). The viability is plotted against the temperature of incubation.

tance to full susceptibility took place over a relatively small temperature range, which suggests that a conformational change occurs in either the staphylococcin molecule or the indicator cells. Attempts to demonstrate a conformational change in the staphylococcin molecule by use of the fluorescent probe 1-anilino-8-naphthalene sulfonate were unsuccessful.

A variation of the growth temperature over a range of 22 to 42°C mainly affected the proportion of the cells susceptible to the bacteriocin and only slightly affected the temperature profile observed in the susceptibility test (Table 3). Cells cultured at either 37 or 22°C differed only slightly with respect to their fatty acid composition (Table 4). However, the transition temperature range was decreased in cells cultured in the presence of Tween 80, which greatly enhanced the proportion of unsaturated fatty acids (Table 4). Therefore, the transition temperature may reflect changes in the fluidity of the membrane lipids.

DISCUSSION

The effects of staphylococcin 1580 on susceptible cells, such as killing, inhibition of amino

acid transport, and abolition of the membrane potential, require distinct physiological conditions of the cells for full expression. It must be noted here that energy is required to induce any physiological damage, since energy-depleted cells are entirely resistant to the bacteriocin (Weerkamp, manuscript in preparation). Cells pregrown with either glucose or sodium pyru-

TABLE 3. Effect of culture conditions on the temperature profile of staphylococcin 1580-induced loss of salt tolerance^a

Growth temp (°C)	Transition temp (°C)	log R _t
22	14	-3.5
29	12	-2.8
37	12	-2.2
42	14	-2.4
37 (10% Tween 80)	6	-3.0

^a The experiment was carried out as shown in Fig. 5, except that the indicated growth temperatures were applied. In one experiment the growth medium contained 10% (vol/vol) Tween 80. Viable counts were determined on TSAS and expressed as the survival ratio. The transition temperature is defined arbitrarily as the temperature at which the survival ratio was 10% and was calculated from curves similar to that shown in Fig. 5.

TABLE 4. Effect of growth temperature and of Tween 80 on the fatty acid composition of *S. aureus* Oxford 209^a

Fatty acid ^a	% of total fatty acids		
	37°C	22°C	37°C + Tween 80
br-C _{13:0}	0.7	0.5	0.5
n-C _{13:0}	0.5		1.1
br-C _{14:0}	2.6	2.4	6.7
br-C _{15:0}	38.0	42.9	33.5
n-C _{16:0}	8.6	8.2	6.3
br-C _{17:0}	6.0	3.8	3.1
n-C _{18:0}	17.6	15.5	10.7
n-C _{18:1}	1.4	4.3	17.3
br-C _{19:0}	0.7	1.0	1.0
n-C _{19:0}	8.1	4.7	4.9
n-C _{20:0}	8.2	9.8	6.1
n-C _{20:1}	0.5	1.2	7.4
Total branched	51.6	53.6	45.4
Total unsaturated	1.9	5.5	24.7
Mean chain length (no. of carbon atoms)	15.6	15.7	16.7

^a Cells were grown in tryptone soya broth or tryptone soya broth supplemented with 10% (vol/vol) Tween 80 at the temperature indicated into the mid-exponential phase and washed twice with 50 mM potassium phosphate buffer (pH 7.0). Fatty acids were extracted as described in the text. Minor fractions, containing less than 0.5% of the total fatty acids, are not shown. br, Branched-chain fatty acids; n, straight chains.

vate as carbon and energy source differed as to the extent of primary injury induced by staphylococcin, but they were equally susceptible to killing by high concentrations of salt after treatment with the bacteriocin. The primary injury was much more severe in pyruvate-grown cells, as was obvious from its expression in the extent of irreversible killing, the inhibition of active transport, and the depolarization of the membrane potential. The effects of DCCD indicate that the membrane energization results from ATP hydrolysis by the Ca^{2+} , Mg^{2+} -ATPase in glucose-grown cells, whereas an electron transport-coupled membrane potential is predominant in pyruvate-grown cells. These results suggest that staphylococcin 1580 acts primarily on a target specifically involved in the maintenance of the membrane potential coupled to electron transport. However, electron transport is not inhibited but is even stimulated by staphylococcin 1580 in pyruvate-grown cells, which may be a reflection of the uncoupling of electron transport in these cells.

Staphylococcin 1580 abolished the membrane potential only partly. Brewer (3) suggested recently that only part of the sources of the membrane potential are blocked by colicin K in *Escherichia coli*. A similar conclusion may be valid for the action of staphylococcin 1580.

An exponentially growing cell population was heterogeneous with respect to staphylococcin susceptibility. The rapid shift in the proportion of susceptible cells during exponential growth may be due to a change in energy metabolism of the cells.

Both the growth temperature of the indicator cells and the incubation temperature during staphylococcin treatment affected the susceptibility of the cells to the bacteriocin. Cells were unsusceptible to staphylococcin when incubated with it at a temperature below a rather sharply defined limit. The access of staphylococcin to its target may be prevented at low temperature or the expression of its effect may be blocked. The action of colicin K also depends strongly on the temperature. The effect was attributed to changes in the membrane lipids affecting the fluid properties of the membrane (19, 20). This may be valid also for the effect of temperature on cells treated with staphylococcin 1580, since the temperature at which cells become susceptible was lower in cells containing increased levels of unsaturated fatty acids.

Cells grown at suboptimal temperatures were killed to a greater extent when cultured in the presence of high concentrations of salt after the staphylococcin treatment than cells grown at the optimal temperature. This result seems to contradict previous ones, since cells grown at

suboptimal temperatures were killed to a less extent than those grown at optimal temperatures, when cultured on normal media after treatment with staphylococcin (13). This discrepancy may be explained by assuming effects of the growth temperature on the ability of the cells to repair injury after staphylococcin treatment (27), as well as on the extent and expression of the primary injury caused by the bacteriocin.

Various degrees of injury may be observed in cells treated with staphylococcin 1580, but they reflect distinct physiological conditions of the cell. Our data do not justify the distinction of the action of staphylococcin 1580 into successive stages, as was possible for the action of various colicins (21). Staphylococcin-treated cells were not rescuable by trypsin (13), and physiological injury occurred in the very early stages of treatment. Effects exerted by the energy metabolism of the cell and by the temperature are similar to those found with colicins E1 and K during the transition step from stage I to stage II (9, 19, 20).

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