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Conditional Killing Effect of Staphylococcin 1580 and Repair of Sublethal Injury in *Staphylococcus aureus*

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Treatment of sensitive cells with staphylococcin 1580, a bacteriocin of *Staphylococcus epidermidis*, rapidly induced gross changes in the permeability of the membrane. However, only a small fraction of the cells was killed when treated cells were plated on media containing low amounts of salts. Killing was greatly enhanced by increasing the amounts of small cations incorporated in the plate medium and raising the alkalinity of the medium. The effect of cations correlated inversely with the ion radius. These conditions are shown to affect the repair mechanism of the sublethally injured cells rather than the interaction of staphylococcin 1580 with the cells. A model is proposed in which the killing effect of staphylococcin 1580 is a result of the inability of cells to maintain the protonmotive force at neutral or alkaline pH as a result of the permeation of cations. Recovery of sublethal damage appears to be a complex process requiring protein and probably also ribonucleic acid synthesis and the addition of a suitable energy source.

Staphylococcin 1580 is a bacteriocin produced by *Staphylococcus epidermidis* (16, 18). In sensitive cells it rapidly affects the permeability of the cell membrane, which results in a simultaneous inhibition of several energy-dependent processes, such as active transport and macromolecular synthesis, and in a rapid decline of cellular adenosine 5'-triphosphate (17). Like colicins E1 and K (5, 6) and A (19), it exerts its action probably directly on the cell membrane, since amino acid transport in membrane vesicles is inhibited too (19). Staphylococcin 1580 also resembles a number of bacteriocins produced by a variety of other gram-positive bacteria, such as other staphylococci (3), clostridia (1, 15), streptococci (21, 30), and lactobacilli (32).

Though numerous studies have been focused on the cellular events after treatment with bacteriocins of this type, the factor(s) causing death, measured by inability to form colonies, has as yet not been fully clarified. Mutant cells lacking the Ca^{2+}, Mg^{2+}-activated adenosine triphosphatase activity (*uncA*) were killed by colicin K in spite of the absence of an effect on the adenosine 5'-triphosphate level and on macromolecular synthesis (26). Recently, Kopecky et al. (21) showed that colicin E1- or K-treated cells may be rescued by plating on media with appropriate concentrations of potassium and magnesium ions. They concluded that killing results from the failure to restore the specific intracellular concentration of ions required for proper metabolic functions.

In this paper we report on the conditions required for an effective killing by staphylococcin 1580 and the repair of sublethal injury.

MATERIALS AND METHODS

Bacterial strains. Staphylococcin 1580-producing *S. epidermidis* 1580, colicin A-producing *Citrobacter freundii* C31, and the indicator strains *Staphylococcus aureus* Oxford 209P and *Escherichia coli* K-12 were described previously (19). *S. aureus* C55, which produces staphylococcin C55, was kindly donated by L. M. Wannamaker; the indicator strain used for this bacteriocin was *S. aureus* 502A.

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

Media. Medium CY (low-salt medium) consisted of 1% casein hydrolysate (enzymatically hydrolyzed; Nutritional Biochemicals Corp.), 0.2% yeast extract L21 (Oxoid), and 1.5% agar (Difco) (final pH, 6.5). This medium contained less than 2×10^{-3} M sodium chloride.

The basal semisynthetic medium A was derived from the previously described AJ-1 medium, except that casein hydrolysate (enzymatically hydrolyzed) was obtained from Nutritional Biochemicals Corp. and glucose was substituted by various carbohydrates in a 1% concentration (17).

In the recovery experiments the medium proposed by Iandolo and Ordal (14) was used, which consisted of 0.25% glucose or sodium pyruvate; 30 mM potassium phosphate buffer (pH 7.2); 0.4 mM L-alanine, L-valine, L-leucine, L-glycine, L-proline, L-hydroxy-

proline, L-aspartic acid, and L-glutamic acid; 0.15 mM L-methionine, L-phenylalanine, L-tyrosine, L-arginine hydrochloride, L-histidine hydrochloride, and L-lysine hydrochloride.

Production and purification of bacteriocins. Colicin A-C31 was obtained by the modified method of Dandeu (4), as previously described (19). Staphylococcin C55 was produced by the method of Dajani and Wannamaker (2).

Staphylococcin 1580 was produced and purified in a way different from the previously used method (16). *S. epidermidis* 1580 was grown overnight at 37°C under vigorous shaking in 1-liter batches in semisynthetic A-pyruvate medium. The cells were removed by centrifugation for 15 min at 8,000 × *g*. All subsequent treatments were carried out at 4°C. The supernatant was brought to 65% saturation by slowly adding powdered ammonium sulfate under constant stirring. After a further 30 min the precipitate was pelleted by centrifugation for 30 min at 12,000 × *g* and dissolved in about 50 ml of 0.02 M potassium phosphate buffer (pH 7.0). To remove undissolved material this solution was centrifuged for 10 min at 6,000 × *g*, and the pellet was discarded. The supernatant was layered on top of a CM-Sephadex C-25 (Pharmacia, Uppsala) column (50 by 1.5 cm) and eluted with a linear 0 to 0.8 M sodium chloride gradient in 0.02 M potassium phosphate buffer (pH 7.0). Fractions (10 ml) were collected and assayed for staphylococcin 1580 activity, and the absorption at 280 nm was monitored for detection of protein. The single peak eluted at about 0.6 M NaCl contained over 80% of the total staphylococcin activity and was pooled and dialyzed overnight against excess 0.02 M phosphate buffer, followed by another 4 h with two charges of deionized water. The dialyzed preparation was freeze-dried and stored at 4°C.

Assay of bacteriocin activity. The bacteriocins were assayed as described previously (16). The activity was expressed in arbitrary units per milliliter.

Viable count. Total viable counts after treatment with bacteriocin were determined by rapidly diluting samples 1,000-fold into ice-cold tryptone soya broth and subsequently plating suitably diluted amounts on agar plates, as indicated in the relevant experiments. The R_t value was defined as the ratio between the viable counts of treated and untreated bacteria.

Conditions of bacteriocin treatment. Cells in the midlogarithmic phase of growth were centrifuged at 4°C, washed, and suspended in incubation buffer, which contained 0.05 M potassium phosphate (pH 7.0), 0.05 M sodium chloride, and 2×10^{-3} M magnesium sulfate, and stored on ice for no longer than 2 h. Cell suspensions (1 ml) were preincubated for 5 min at 37°C, subsequently supplied with bacteriocins (10-μl volume), and further incubated for 5 min. After that, samples were rapidly diluted and plated as described.

Recovery. The time course of recovery was followed by diluting staphylococcin 1580-treated cells 1,000-fold into the recovery medium under investigation and further incubating at 37°C in static culture, unless otherwise indicated. At the times indicated, samples were withdrawn and plated as described.

Transport of glutamic acid. The assay system was the same as that used for staphylococcin 1580 treatment, with the exception that after 5 min of preincubation at 37°C L-[^{14}C]glutamic acid (final concentration, 10^{-5} M) was added. Samples (0.1 ml) were withdrawn, rapidly filtered on membrane filters (0.45-μm pore size; Millipore Benelux, Brussels, Belgium), and washed with 4 ml of incubation buffer. After drying, filters were counted in a liquid scintillation counter.

Chemicals. L-[*U*-^{14}C]glutamic acid (270 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks, England; actinomycin D and mitomycin C from Sigma, St. Louis, Mo., and 2,4-dinitrophenol was from Baker, Deventer, The Netherlands. All other chemicals were reagent grade.

RESULTS

Influence of salts. The viability of sensitive cells, treated with relatively low amounts of staphylococcin 1580 or colicin A, both of which are assumed to act in a similar way (19), was much more reduced when the cells were plated on media containing increasing amounts of sodium chloride than on low-salt medium (Fig. 1). In particular, staphylococcin 1580-treated cells were nearly completely viable when plated on low-salt medium. In contrast, the sodium chloride concentration only slightly influenced the survival ratio after treatment with staphylococcin C55.

The effect of salts on the viability after treatment with bacteriocin was investigated more in detail with staphylococcin 1580. Figure 2 shows that prolonged incubation times hardly further enhanced the amount of cells killed at each salt concentration applied.

A variety of compounds had no effect on the survival ratio when present during the staphylococcin treatment only. Incorporation of salts in the plate medium, however, resulted in killing ratios characteristic of the salt applied (Table 1). Anions and compounds like leucine and sucrose exerted no special effect on either the interaction with the bacteriocin or the recovery after injury. Arranging the cations according to their ion radii strongly suggests a correlation between the radius and the effect in the plate medium in such a way that the more cells killed, the smaller the radius. The ammonium ion appears to be more lethal than would be expected from this hypothesis, possibly due to the high permeability of its unprotonated form or to effects on the pH, which will be discussed below. Potassium ions, which were nearly inert

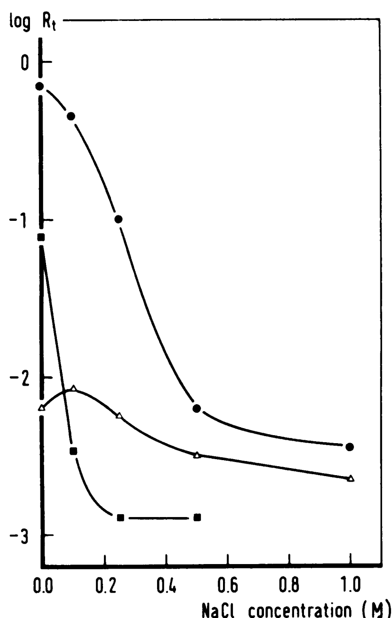

FIG. 1. *Effect of sodium chloride on the survival ratio of cells treated with staphylococcin 1580, staphylococcin C55, and colicin A. Midlogarithmic-phase cells of the indicator strain grown on tryptone soya broth were centrifuged, washed, and suspended in incubation buffer (5 × 10⁸ cells per ml). After 5 min of incubation with 100 arbitrary units of the bacteriocins per ml, the viable count on CY-agar plates (pH 6.5) with various NaCl concentrations was determined. The applied salt concentrations did not affect the viability of the cells. Symbols: ●, effect of staphylococcin 1580 on S. aureus Oxford 209P; △, effect of staphylococcin C55 on S. aureus 502A; ■, effect of colicin A on E. coli K-12 (V2005).*

in this system, partly rescued the cells from killing by a relatively low concentration of sodium chloride (Fig. 3). Possibly potassium ions compete with sodium ions for the same target, or, alternatively, their presence may facilitate the reaccumulation of potassium ions required for normal cell function. It should be noted that under conditions of sodium chloride stress, staphylococcal cells accumulate relatively high concentrations of sodium chloride, without distinct effects on the major metabolic functions (31). It is evident from the data presented that increasing concentrations of both cations killed cells progressively. To test whether the used compounds affected certain functions of the cytoplasmic membrane, their influence on glutamate uptake, which was previously shown to be energetically linked to a high energy state of the membrane (19) in *S. aureus*, was also investigated (Table 1). Although most salts increased the transport rate significantly over that of the basal incubation medium, no correlation was found with their influence on the staphylococcin 1580 killing effect.

Influence of pH. Cells treated with staphylococcin 1580 were plated on low-salt media of various pH values (Fig. 4A). At neutral pH, killing was very low under the conditions applied. Towards more acid pH values, the killing increased slightly. pH values above 7, however, drastically decreased the survival ratio. This was shown to be an effect on the viability of treated cells, rather than on the interaction of staphylococcin 1580 with the cells, since the latter has a broad range with an optimum at pH 6.5 (Fig. 4B).

Simultaneous influence of pH and salt. Cells treated with staphylococcin 1580 and subsequently plated at neutral pH were sensitive to sodium chloride (Fig. 1 and 5). At slightly alkaline pH this sensitivity of treated cells was significantly increased, whereas at pH 5.5 the survival ratio was high and almost independent of the salt concentration of the plating medium. These results point out that the effects of salts and pH are interdependent.

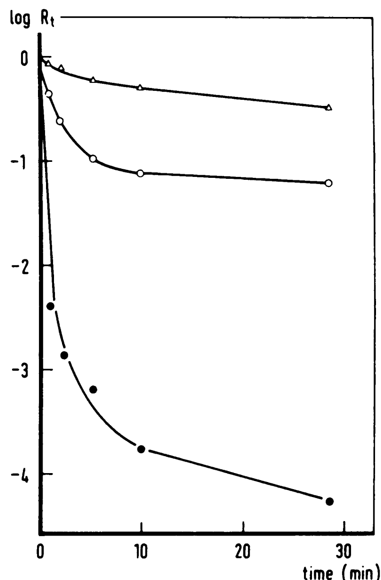

FIG. 2. *Effect of incubation time with staphylococcin 1580 on the survival ratio measured at various NaCl concentrations. Cells of S. aureus Oxford 209P, exponentially growing on tryptone soya broth were centrifuged, washed, and suspended in incubation buffer (pH 7.0) and subsequently treated with staphylococcin 1580 (100 arbitrary units/ml) for the indicated time intervals, after which the viable count was determined on CY-agar plates containing less than 2 × 10^{-3} M (△), 0.1 M (○), or 1.0 M (●) NaCl.*

TABLE 1. *Effect of various salts on staphylococcin 1580 action, the recovery of treated cells, and glutamate uptake*

Compound added (0.25 M)	Effect on viability (log R_t): Salt present during staphylococcin treatment[a], Plated on CY agar	Salt present during staphylococcin treatment[a], Plated on CY agar + 1 M NaCl	Salt present in plate medium only[b]	Glutamate uptake (nmol/mg [dry wt]/min)[c]
None	−0.2	−2.8	−0.2	1.10
$MgCl_2$	−0.2	−1.9	−3.1	2.16
LiCl	−0.3	−2.2	−2.8	1.80
NaCl	−0.2	−2.1	−2.1	2.42
$CaCl_2$	NT[d]	NT	−0.7	NT
KCl	−0.2	−2.6	−0.5	1.48
NH_4Cl	−0.2	−2.2	−1.9	1.54
RbCl	−0.6	−2.9	−0.2	1.18
KCl	−0.2	−2.6	−0.6	1.48
KBr	−0.2	−2.0	−0.5	1.68
KNO_3	−0.2	−2.1	−0.6	1.80
K_2SO_4	−0.2	−2.5	−0.5	1.08
K-acetate	NT	NT	−0.6	NT
Leucine	NT	NT	−0.3	NT
Sucrose	−0.2	−2.7	−0.2	1.21

[a] The basal incubation medium contained 0.05 M potassium phosphate (pH 7.0), 0.05 M NaCl, and 2×10^{-3} M $MgSO_4$, to which the indicated salts were added. Washed cells of *S. aureus* 209P (6×10^8 cells per ml) were preincubated for 5 min at 37°C, after which staphylococcin 1580 was added (100 arbitrary units/ml) and incubation was continued for 5 min. Subsequently, samples were rapidly diluted more than 1,000-fold and plated on the media indicated.

[b] Viable counts after staphylococcin treatment in basal medium were determined on CY-agar with incorporated salts. Viable counts are expressed as the logarithm of the survival ratio (R_t).

[c] Uptake after 1 min by cells suspended in basal incubation medium with the indicated salts was measured.

[d] NT, Not tested.

Mechanism of recovery. Loss of salt tolerance on staphylococcin 1580 treatment appears concomitantly with a collapse of energy-dependent functions of the cell (17, 19). Since treated cells are able to resume growth under favorable conditions, injuries must be repairable. It is shown in Fig. 6 that after a 60-min incubation treated cells start to recover from the injury, and the process is completed within 120 min under optimal conditions. Table 2 gives the results obtained under various incubation conditions and the effect of specific metabolic inhibitors on the recovery process. Optimal recovery requires a rather rich growth medium. The choice of the main carbon and energy source seems to be crucial.

Pyruvate, which supports growth of untreated cells very well, was most beneficial in the recovery process. Succinate, a less preferable substrate for growth, was also less effective in the recovery process, and the omission of an energy source results in a slow recovery. However, glucose, which acts as well as pyruvate in supporting growth of untreated cells, was not suited at all in sustaining the recovery. Incubation in a glucose medium even improved the killing observed on tryptone soya agar. Although staphylococcin 1580-treated cells are only slightly impaired with respect to the rate of glucose uptake (Weerkamp, unpublished observation), they might be blocked in the metabolism of glucose or the energy derived from glycolysis might not be suited for the recovery process. An effect of pH is probably not involved since the internal pH, as determined from the distribution of the weak acid ^{14}C-labeled 5,5-dimethyl-2,4-oxazolidinedion, was found to be 7.65 in both pyruvate- and glucose-grown cells (unpublished observation). Media shown to be suitable for recovery of heat-induced loss of salt tolerance in *S. aureus* (14) did not allow the recovery of cells from staphylococcin 1580-induced injury; this holds for media

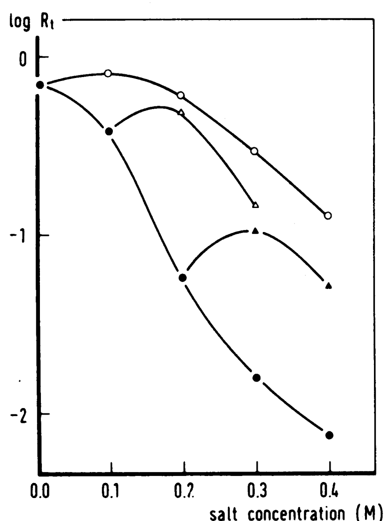

FIG. 3. *Effects of mixtures of sodium chloride and potassium chloride on the killing effect of staphylococcin 1580. Conditions were identical to those in Fig. 1. Cells were plated on CY-agar (pH 6.5) with various amounts of NaCl (●) or KCl (○) or mixtures of 0.1 M NaCl plus additional KCl (△) and 0.2 M NaCl plus additional KCl (▲). The indicated salt concentrations refer to the final sum of concentrations of both salts.*

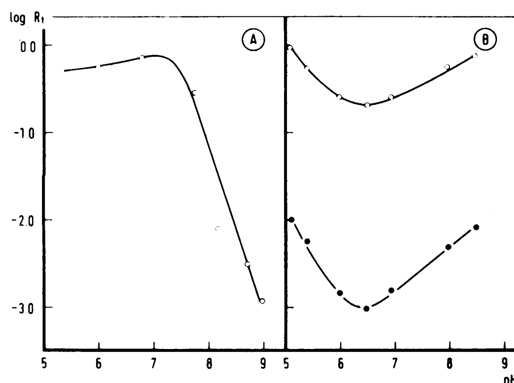

FIG. 4. *Effect of pH on staphylococcin 1580-induced killing. (A) Washed cells of S. aureus Oxford 209P (5 × 10^8 cells per ml) were incubated with staphylococcin 1580 (100 arbitrary units/ml) for 5 min and subsequently plated on CY-agar, of which the pH was adjusted to the indicated values by the addition of potassium phosphate buffers to a final concentration of 0.05 M. The extreme pH values required the addition of small amounts of HCl or KOH. The potassium ion concentration of the medium, however, never exceeded 0.1 M. (B) Cells were suspended in incubation buffers of various pH values (5 × 10^8 cells per ml). After 5 min of incubation with staphylococcin 1580 (100 arbitrary units/ml), the viable count was determined by plating on CY-agar (pH 6.5) containing either 0.1 (○) or 1.0 (●) M sodium chloride. Cells not treated with the bacteriocin were completely viable at the pH values applied.*

with various energy sources and buffered by either tris(hydroxymethyl)aminomethane or phosphate. Incubation in phosphate buffer alone was extremely harmful to staphylococcin-treated cells.

The experiments performed with various metabolic inhibitors show a complete inhibition of recovery by the inhibitors of ribonucleic acid synthesis (actinomycin D) and protein synthesis (chloramphenicol) and by the uncoupler 2,4-dinitrophenol. Also, dicyclohexylcarbodiimide, an inhibitor of membrane adenosine triphosphatase, appeared to prevent recovery, but the results were obscured by the lethal effect of this agent on cells not treated with staphylococcin 1580. The results obtained with actinomycin D should also be taken with care, since it also might inhibit amino acid transport (11).

Mitomycin C, an inhibitor of deoxyribonucleic acid synthesis, delayed but did not prevent the recovery. Therefore, de novo synthesis of ribonucleic acid and protein, together with a suitable energy supply, is required to restore the injury induced by staphylococcin 1580.

Preliminary studies (not shown here) on the incorporation of radioactive precursors into macromolecules suggest a specific incorporation of amino acids into membrane proteins during the early stages of the recovery process.

DISCUSSION

Staphylococcin 1580, like colicins E1, K, Ia and Ib, and A (5, 6, 8, 19, 23), simultaneously inhibits active transport of various substrates and synthesis of macromolecules, induces leak of potassium or preloaded rubidium ions from the cells, and decreases adenosine 5′-triphosphate level (17). However, as recently shown by Kopecky et al. (21), these events do not implicate cell death, since plating of colicin E1- or K-treated cells on a medium containing particular concentrations of potassium and magnesium ions allows survival of the cells. Similarly, treatment of sensitive cells with staphylococcin 1580 is not simply bactericidal, but cells may survive if (i) the concentration of cations is rather low or (ii) the medium is slightly acidic. Since the presence of salts or alkalinity in the medium during the short incubation of cells with staphylococcin 1580 does not bring about the observed effect on the killing ratio, it is likely that under the described conditions the restoration of the induced injuries is prevented.

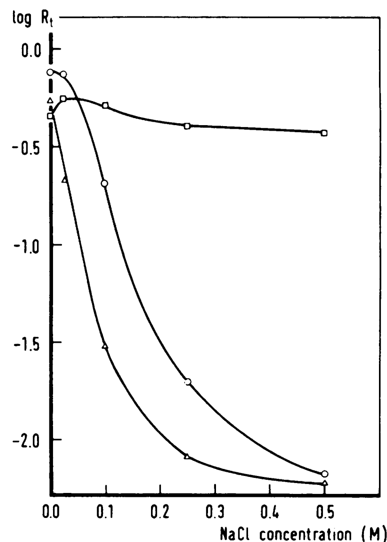

FIG. 5. *Influence of the combination of salt and various pH values on the killing effect of staphylococcin 1580. Washed cell suspensions of S. aureus Oxford 209P (4 × 10^8 cells per ml) were prepared as described in Fig. 2 and treated with staphylococcin 1580 (100 arbitrary units/ml) for 5 min. Viable counts were determined by plating on CY-agars of various pH values and NaCl concentrations. The viability of cells not treated with the bacteriocin was not affected by any of the conditions applied. Symbols: □, pH 5.5; ○, pH 6.9; △, pH 7.6.*

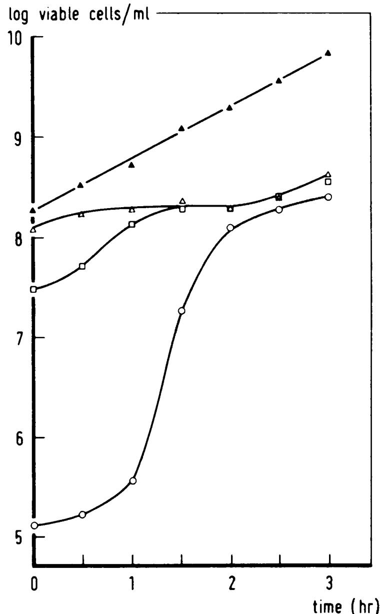

FIG. 6. *Recovery of staphylococcin 1580-induced injury. Washed cell suspensions of S. aureus Oxford 209P (2 × 10*[8] *cells per ml) were prepared as described in Fig. 2 and treated with staphylococcin 1580 (100 arbitrary units/ml) for 5 min. Cells in control experiments received only buffer. Treated (open symbols) and control cells (▲) were subsequently diluted 1,000-fold into medium A with 1% sodium pyruvate. At various time intervals samples were plated on CY-agar containing various amounts of NaCl. Symbols: △, less than 2 × 10*$^{-3}$ *M NaCl; □, 0.1 M NaCl; ○, 1.0 M NaCl. The viable count was plotted against the incubation time.*

The observed results may be interpreted on the basis of the chemiosmotic hypothesis (10, 24).

Extrusion of protons generates a protonmotive force, composed of a pH gradient (ΔpH), inside alkaline, and a membrane potential ($\Delta\psi$), inside negative. At relatively low external pH values, ΔpH is large, whereas at neutral and alkaline pH values of the medium $\Delta\psi$ is the most important or sole component. $\Delta\psi$ remains essentially constant at a pH range of 5 to 9 (25, 27). Since staphylococcin 1580 does not affect the proton permeability of the membrane (19), it does not abolish ΔpH and, hence, affects the protonmotive force less drastically at low external pH. However, $\Delta\psi$ may be abolished by the movement of permeable cations inverse to the proton extrusion, and hence the generation of the protonmotive force will be prevented at neutral and alkaline pH values. Similar mechanisms have been proposed for the action of the potassium ionophore valinomycin on both growth of and glutamate uptake by *S. aureus* in the presence of relatively high external potassium concentrations (7, 29). The action of staphylococcin 1580 may induce rather nonspecific membrane permeations, accessible to small cations except protons; the resulting effect on the protonmotive force may be the important factor causing death of the cells. Moreover, the maintenance of a limited internal concentration of potassium ions appears to be of additional importance, since the external pres-

TABLE 2. *Effect of the composition of the medium and of various metabolic inhibitors on the recovery of cells injured by staphylococcin 1580 treatment*[a]

Recovery medium	Log R_{rec}	
	Plated on TSA	Plated on TSAS
Medium A-pyruvate	0.82	3.67
Medium A-succinate	0.10	2.94
Medium A-glucose	−0.80	0.40
Medium A	−0.15	1.90
Medium A-pyruvate (anaerobic)	0.70	3.40
Phosphate-buffered amino acids + pyruvate[b]	−1.61	1.05
Phosphate-buffered amino acids + glucose[b]	−1.54	0.96
Phosphate buffer, 30 mM (pH 7.2)	−4.57	<−1
Medium A-pyruvate[c]		
+ chloramphenicol	−0.20	0.35
+ actinomycin D	−1.20	0
+ mitomycin C	−0.65	1.54
+ 2,4-dinitrophenol	−1.50	0.05

[a] Washed cells of *S. aureus* Oxford 209P from an exponentially growing culture on tryptone soya broth were suspended in potassium phosphate buffer (pH 7.0) in a final concentration of 5 × 10^{8} cells per ml and treated for 5 min at 37°C with 100 arbitrary units of staphylococcin 1580 per ml. Subsequently, samples were diluted 1,000-fold into the prewarmed recovery medium under investigation and incubated at 37°C for 2 h, after which samples were plated on broth tryptone soya agar (TSA) and TSA supplemented with sodium chloride to a final concentration of 1 M (TSAS) for viability testing. The logarithms of the survival ratios (R_t) prior to the incubation in the recovery media were −0.80 and −3.90 on TSA and TSAS, respectively. Log R_{rec} is defined as the ratio of the viable count after 2 h to that immediately after staphylococcin 1580 treatment. The latter was corrected for changes in the viability of cells not treated with staphylococcin 1580, induced by the presence of inhibitors or the incubation conditions.

[b] The medium consisted of 30 mM potassium phosphate buffer (pH 7.2), the amino acids listed in Materials and Methods, and 0.25% of either glucose or sodium pyruvate.

[c] Final inhibitor concentrations used were: chloramphenicol, 100 μg/ml; actinomycin D, 2 μg/ml; mitomycin C, 0.5 μg.ml; 2,4-dinitrophenol, 0.6 mM.

ence of potassium ions in addition to sodium ions partly rescues the cells from killing. Alternatively, the cations might block the recovery at the level of substrate transport. Ring et al. (28) reported that an almost identical range of cations inhibited amino acid transport in *Streptomyces hydrogenans* by competition with the transport carrier. However, L-glutamate uptake in *S. aureus* was not inhibited by the cations tested, which may argue against, although not completely exclude, this possibility.

Rescue of cells treated with colicin E1 or K in media with potassium and magnesium concentrations resembling the intracellular level was reported before (21), and we obtained similar results in studies with colicin A (unpublished data). Cells treated with colicins E1 and K are permeable to potassium ions and behave analogously to valinomycin-treated cells (9). Therefore, a similar mechanism may be involved in the rescue of cells from the injury induced by these various bacteriocins.

Recovery from injury by staphylococcin 1580 appears to be a complex process, requiring at least ribonucleic acid and protein syntheses, and, in addition, metabolic energy derived from a suitable substrate. In many respects the recovery process differs from the well-studied recovery from salt tolerance after heat injury in *S. aureus* (12, 14). The latter requires the synthesis of ribonucleic acid in particular (14) and is accompanied by the restoration of the cellular magnesium content (12) and resynthesis of teichoic acid-bound D-alanine (13). Treatment with staphylococcin 1580, however, does not induce a gross magnesium leak from the cells (Weerkamp, unpublished data). Preliminary experiments suggest that restoration of the cell damage requires the resynthesis of membrane proteins (unpublished observations). Recently, Knepper and Lusk (20) showed that specific membrane proteins disappeared after treatment of *E. coli* cells with colicin K or E1.

Although it might be concluded that the action of staphylococcin 1580 is bacteriostatic rather than bactericidal, it should be noted that in most natural environments for staphylococci (e.g., the human skin) salt concentrations are sufficiently high to induce a strong bactericidal effect. Furthermore, recovery requires rather optimal conditions even after very short incubation with the bacteriocin, and circumstances disadvantageous in this respect enhance the killing ratio strongly.

ACKNOWLEDGMENTS

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