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Mode of Action of a *Staphylococcus epidermidis* Bacteriocin

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Staphylococcin 1580, a bacteriocin produced by *Staphylococcus epidermidis* 1580, is bactericidal to sensitive cells of many gram-positive bacteria and stable staphylococcal L-forms. The bacteriocin inhibited simultaneously the syntheses of deoxyribonucleic acid, ribonucleic acid, and protein, and caused neither degradation of deoxyribonucleic acid nor induction of phages in lysogenic, sensitive cells. After 1 hr of treatment, extensive degradation of ribonucleic acid occurred, which was accompanied by leakage of ultraviolet-absorbing material out of the cell. The incorporation of glucose in acid-precipitable and glycogenlike material was inhibited. Furthermore, the staphylococcin inhibited the transport of glucose, glutamic acid, rubidium ions, and *o*-nitrophenyl-β-galactoside. The uptake of oxygen was only gradually affected, but the intracellular adenosine triphosphate level fell rapidly to 15% of the control value. The motility of sensitive *Bacillus subtilis* cells was markedly reduced on treatment. Staphylococcin 1580 exhibited no phospholipase activity. The phenomena are interpreted as resulting from an altered conformation and composition of the membrane, from an inhibition of transport through the membrane, or from a combination of these effects.

Adsorption of bacteriocins to specific receptor sites on the cell membrane induces striking alterations of cellular functions and loss of viability. The various bacteriocins exert their lethal action by affecting different biochemical targets such as deoxyribonucleic acid (DNA), ribosomes, and the electron transport chain, all of which have physical connection with the bacterial membrane. Thus, it is quite possible that the bacteriocins act on these targets by way of the mediation of changes in the cell membrane.

Colicin E2 causes degradation of DNA as a result of the activation of one or more nucleases (2, 33). Colicin E3 (6, 25) and cloacin DF 13 (20) affect protein synthesis. The former causes cleavage of 16*S* ribosomal ribonucleic acid (rRNA) at a specific position near the 3′-terminus, which leads to the inactivation of the 30*S* subunits (25). Recent results of Boon (5) and Bowman (7) show that colicin E3 inactivates ribosomes in vitro and suggest that the killing of bacteria by this bacteriocin involves penetration into the cell. Treatment of cells with cloacin DF 13 results in an inhibition of binding fMet-transfer RNA^{fMet} to the messenger RNA-ribosome complex (20). Colicins E1, K, A, Ia, and Ib (27, 29, 30) all inhibit energy-dependent processes such as macromolecular synthesis and active transport.

Little is known about the mode of action of staphylococcins. Dajani et al. (13) isolated a bacteriocin from phage-type 71 *Staphylococcus aureus*. This substance causes immediate cessation of protein and DNA synthesis as well as degradation of newly and previously formed RNA (14). Like colicins G, Q, and E1 (8), it causes extensive structural changes in susceptible bacteria (12).

Previously we have shown (21, 22) that the nature of staphylococcin 1580, a bacteriocin produced by *S. epidermidis* 1580, is different from that of the bacteriocin described by Dajani et al. (13). This paper deals with the mode of action of staphylococcin 1580. Its effect on macromolecular syntheses and transport phenomena will be described and compared with that of other bacteriocins.

MATERIALS AND METHODS

Microorganisms. The bacteriocin-producing strain was *S. epidermidis* 1580. *S. aureus* Oxford 209P and *S. aureus* NCTC 6571 were used as bacteriocin-sensitive and -resistant strains, respectively. *Bacillus cereus* V 5003 and *B. subtilis* NCTC 60015 were used as indicator strains in the motility experiments. *S. aureus* strains 34 and NCTC 8319 were used in the phage induction experiments. The strains were subcultured once a week on Trypticase soy broth agar (BBL). Stable L-forms of *S. aureus* 502 A were ob-

tained from B. Kagan, Cedars-Sinai Hospital, Los Angeles, Calif. The L-forms were maintained on medium composed of 1.3% agar, 3.8% brain heart infusion, and 5% NaCl, and containing 20% unheated horse serum.

Production, purification, and assay of staphylococcin 1580. The production and purification of staphylococcin 1580 and the assay of its activity have been described previously (22). The activity was expressed as arbitrary units (AU) per milliliter, as defined before (22).

Medium. Medium AJ1, containing 1% casein hydrolysate (enzymatically hydrolyzed; Difco), 0.8% glucose, 2×10^{-2} M potassium phosphate (*p*H 7.0), 10^{-3} M $MgSO_4 \cdot 7H_2O$, 1 ml of the oligodynamic solution described by Pollock and Kramer (32) per liter; 2×10^{-5} M thiamine, 2×10^{-3} M cysteine, 2×10^{-3} M histidine, and 1 μg of thymidine, uracil, adenine, and xanthine per ml, was used as the basic semisynthetic medium. The casein hydrolysate contained 0.495 μmole of glutamic acid/mg (Difco).

Incorporation of radioactive substrates. DNA, RNA, and protein synthesis and glucose uptake in *S. aureus* Oxford 209P cells were studied by measurement of the incorporation of the radioactive precursors ^{3}H-thymidine, ^{3}H-uracil, ^{14}C-glutamic acid, and ^{14}C-glucose, respectively. In determinations of the incorporation of ^{3}H-thymidine and ^{3}H-uracil, the cells were grown in AJ1 medium in the absence of the unlabeled compounds. In the studies on the incorporation of ^{14}C-glutamic acid or ^{14}C-glucose, cells were grown in AJ1 medium or AJ1 medium containing 1 mg of glucose per ml, respectively. The incubation media were aerated at 37 C. The incorporation of radioactivity was measured by addition of 2-ml samples to 2 ml of 10% trichloroacetic acid containing 50 μg of the unlabeled substrate per ml. After 30 min at 0 C, the acid-precipitated material was collected on membrane filters (0.45 μm pore size, Millipore Corp., Bedford, Mass.) and washed with five volumes of cold 5% trichloroacetic acid solution supplemented with 50 μg of the unlabeled substrate per ml. The filters were dried, and the radioactivity was measured in a Packard liquid scintillation counter, model 544, by use of a scintillation fluid consisting of 4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-di-2(5-phenyloxazolyl)benzene per liter of toluene.

Incorporation of ^{14}C-glucose into glycogenlike material was measured according to Abraham and Hassid (1). Total ^{14}C-glucose uptake was measured by filtering the samples through membrane filters and washing with 0.02 M phosphate buffer (*p*H 7.0) containing 20 μg of glucose per ml.

Transport of glutamic acid. The transport of glutamic acid was determined by the method of Nagel de Zwaig (29).

Measurement of $^{86}Rb^{+}$ efflux. The intra- and extracellular $^{86}Rb^{+}$ contents were determined by collecting the cells by filtration through membrane filters which were washed with 5 ml of 0.3 M sucrose solution buffered with 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (*p*H 7.0). The radioactivity on the filter and in the filtrate was measured in a liquid scintillation counter and in a Philips gamma-scintillation counter, respectively.

Respiration. Oxygen uptake was measured in a Warburg-type respirometer (Becker, Delft, The Netherlands) with standard manometric techniques described by Umbreit (36).

Measurement of ATP. Adenosine triphosphate (ATP) was assayed according to Stanley (35). Cells were grown under aeration in AJ1 medium at 37 C. In the exponential phase, samples of 2 ml were removed and added to 1 ml of 30% (w/v) $HClO_4$ at 0 C, neutralized after 15 min with 2 M KOH and 0.5 M potassium phosphate (*p*H 7.4), and centrifuged. For the assay, 0.4 ml of the supernatant fluid was added to 0.8 ml of 0.2 M glycylglycine buffer (*p*H 7.4) and 6.6 ml of water. The reaction was initiated by addition of 0.2 ml of firefly lantern extract (10 mg/ml, in 0.05 M K_3AsO_4 and 0.02 M $MgSO_4$, *p*H 7.4). The photon emission was counted after exactly 10 sec in a Packard liquid scintillation counter operating with the photomultipliers out of coincidence, at 75% amplification and at settings of 65 to 85 divisions.

β-Galactosidase assay. The hydrolysis of *o*-nitrophenyl-β-galactoside (ONPG) was determined by the method of Kennedy and Scarborough (24).

Motility. The cells were grown at 37 C in AJ2 medium containing 0.1% casein hydrolysate, 1% glucose, 2×10^{-2} M potassium phosphate (*p*H 7.0), 10^{-3} M $MgSO_4 \cdot 7H_2O$, 2×10^{-2} M $(NH_4)_2SO_4$, 2×10^{-3} M L-tryptophan, 2×10^{-3} M ammonium citrate, and 0.1% Tween SD 80. In the early exponentional phase, the motility was determined according to Shoesmith (34) and by observation of small hanging drops under a phase-contrast microscope.

Phage induction. To determine phage induction by staphylococcin 1580, cells of the sensitive and lysogenic strain *S. aureus* 34, growing in AJ1 medium, were incubated in the presence and absence of staphylococcin. The number of plaques forming units in the samples was determined against indicator strain *S. aureus* NCTC 8319.

Phospholipase activity. Phosphatidyl glycerol, diphosphatidyl glycerol, and lysylphosphatidyl glycerol were extracted from ^{32}P-labeled cells of *S. aureus* Oxford 209P by the method of Bligh and Dyer (4). The mixture of phospholipids was suspended in 0.1 M phosphate buffer (*p*H 7.5) containing 20 mM $MgCl_2$ and 0.4% Triton X-100, and an equal volume of an aqueous solution of staphylococcin 1580 or water was added. After 3 hr of incubation at 37 C, the phospholipids were extracted with a mixture of chloroform and methanol (3:1) and were separated by thin-layer chromatography on silica gel plates with a mixture of chloroform, methanol, and water (65:25:4) as solvent. An autoradiogram was made to detect the ^{32}P-labeled compounds, which were quantitatively determined in a liquid scintillation counter. A similar procedure was followed for unlabeled lecithin; after thin-layer chromatography lecithin and its hydrolysis products were detected with iodine vapor.

Chemicals. The firefly lantern extract, ATP, and ONPG were purchased from Sigma Chemical Co. ^{14}C-L-glutamic acid (260 mCi/mmole), ^{3}H-uracil (1 Ci/mmole), ^{3}H-thymidine (5 Ci/mmole), ^{14}C-glucose, uniformly labeled (3 mCi/mmole), ^{86}RbCl (2 to 10 mCi/mg), and sodium dihydrogen ^{32}P-orthophosphate (10 mCi/mmole) were purchased from the

Radiochemical Centre, Amersham, England. Tween SD 80 was purchased from Atlas-Goldschmidt GMBH, Essen, Germany.

RESULTS

Macromolecular syntheses. Treatment of cells of *S. aureus* Oxford 209P with staphylococcin 1580 resulted in a rapid inhibition of the incorporation of labeled thymidine, uracil, and glutamic acid into acid-insoluble material (Fig. 1). The synthesis of macromolecules was not affected when trypsin-degraded staphylococcin was used or when the staphylococcin (1,000 AU/ml) was added to resistant *S. aureus* (NCTC 6571) cells. The inhibition occurred immediately, as was observed for colicins K and E1 (30). In contrast, the effects of colicins E2 and E3 manifest themselves slowly and at high bacteriocin concentrations (30). Staphylococcin affected the synthesis of DNA, RNA, and protein simultaneously and independently. No extensive degradation of DNA occurred because no decrease in trichloroacetic acid-insoluble thymidine-labeled material was noticed even after 2 hr of incubation with staphylococcin. Moreover, the staphylococcin did not induce vegetative replication of phages in lysogenic sensitive strains, an effect observed with colicin E2 and due to the degradation of DNA (30). After prolonged incubation of cells with staphylococcin, the RNA was extensively degraded (Fig. 2). Furthermore, after a lag period of 1 hr, material optimally absorbing at 260 nm leaked from staphylococcal cells (10^9 cells per ml) treated with 4,000 AU of staphylococcin per ml. However, the action of staphylococcin 1580 is bactericidal rather than bacteriolytic (21). Stable L-forms of *S. aureus* 502 A were also sensitive to staphylococcin and did not lyse on treatment.

No incorporation of labeled glutamic acid, uracil, and thymidine into trichloroacetic acid-insoluble material of staphylococcin 1580-pretreated cells was observed.

Glucose incorporation. Staphylococcin 1580 inhibited immediately the incorporation of ^{14}C-glucose into acid-insoluble material (Fig. 3). Moreover, the total uptake of ^{14}C-glucose and its incorporation into glycogenlike material were 90% inhibited in cells pretreated for 5 min with staphylococcin (2,000 AU/ml) and incubated for 20 min in the presence of ^{14}C-glucose (0.5 μCi/ml).

Glutamic acid transport. The uptake of glutamic acid was blocked in cells which were preincubated with staphylococcin and chloramphenicol prior to the addition of labeled glutamic acid (Fig. 4A). The addition of bacteriocin to chloramphenicol-treated cells resulted in a rapid release of preaccumulated ^{14}C-glutamic acid (Fig. 4B).

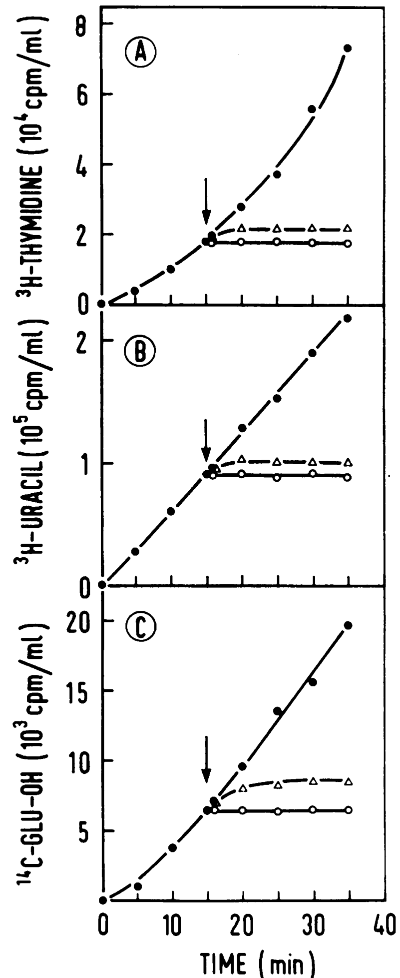

FIG. 1. *Effect of staphylococcin 1580 on macromolecular syntheses. Cells of Staphylococcus aureus Oxford 209P were grown in AJ1 medium at 37 C to a concentration of 10^8 cells per ml. (A) Effect of staphylococcin on ^3H-thymidine incorporation. The experiment was started by addition of 0.5 μCi of ^3H-thymidine per ml; after 15 min at 37 C, the culture was divided into three equal parts: two received different amounts of the bacteriocin in 0.01 M phosphate buffer (pH 7.0), and one received only buffer to serve as the control. At various time intervals, the ^3H-thymidine incorporation into acid-precipitable material in 2-ml samples was measured. (B) Effect of staphylococcin on ^3H-uracil incorporation. The procedure was the same as under A but 0.5 μCi of ^3H-uracil was added per ml. (C) Effect of staphylococcin on ^{14}C-glutamic acid incorporation into hot-acid-precipitable material. The procedure was the same as under A, but 0.5 μCi of ^{14}C-glutamic acid was added per ml. Symbols: ●, control; △, 100 AU of staphylococcin per ml of incubation mixture; and ○, 1,000 AU per ml.*

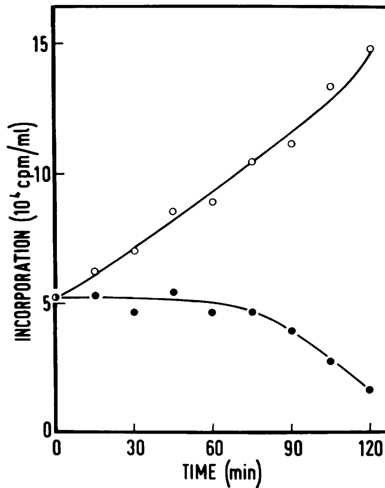

FIG. 2. *Degradation of RNA induced by staphylococcin 1580. The same conditions were used as described in Fig. 1B. Symbols:* ○, *without staphylococcin;* ●, *3,000 AU of staphylococcin per ml of incubation mixture.*

Rubidium efflux. A rapid efflux of preaccumulated rubidium was observed, resulting in a marked depletion of cellular rubidium (Fig. 5). More than 90% of the intracellular rubidium was lost within 4 min.

Respiration. The oxygen consumption of cells growing on glucose was inhibited by staphylococcin 1580 (Fig. 6). However, this effect increased on prolonged incubation and was less drastic than as was observed for macromolecular syntheses. Therefore, the effect on respiration does not seem to be the primary effect of the bacteriocin.

ATP level. The ATP level was determined according to the firefly assay. The assay was linearly on a full logarithmic scale from 10 to 500 pmoles of ATP per ml. In cells treated with staphylococcin, the ATP content decreased rapidly (Fig. 7), and reached 15% of the original value within 10 min.

ONPG transport. No hydrolysis of ONPG could be detected in whole cells, which were preinduced for this enzyme and transport system immediately after addition of staphylococcin (Fig. 8). No effect of the bacteriocin (3,400 AU/ml) was observed on the β-galactosidase in cell-free extracts, obtained by disintegration in a Braun homogenizer. No β-galactosidase leaked out of the cells; most probably the permeation of ONPG or the product of the enzymatic reaction, or both, through the membrane was markedly reduced.

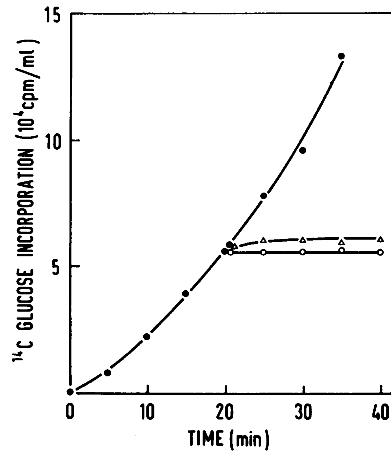

FIG. 3. *Effect of staphylococcin 1580 on the incorporation of ^{14}C-glucose. Cells of Staphylococcus aureus Oxford 209P were harvested in the exponential growth phase and incubated at 37 C in AJ1 medium supplemented with 1 μCi of ^{14}C-glucose per ml. After 20 min, the cell suspension was divided into three equal parts: two received staphylococcin in 0.01 M phosphate buffer (pH 7.0), and the third received only buffer. At various time intervals, samples were withdrawn, and the ^{14}C-glucose incorporation into acid-precipitable material was determined. Symbols:* ●, *control;* △, *100 AU of staphylococcin per ml of incubation mixture;* ○, *1,000 AU per ml.*

Motility. Cells of staphylococcin-resistant *B. cereus* V5003 and -sensitive *B. subtilis* NCTC 60015 were used. The cells are actively motile in the early exponentional phase, when grown in AJ2 medium. The effect of staphylococcin 1580 on motility was quantitatively determined by the method of Shoesmith (34) and by observation of small hanging drops of control and staphylococcin-treated cells (1,000 AU of staphylococcin per ml) under a phase-contrast microscope. Control cells remained vigorously motile during the tests, whereas the motility of treated sensitive cells slowed down (Fig. 9). The motility of cells of *B. cereus* was not affected by the staphylococcin.

Phospholipase activity. In view of the action of megacin A, which has been identified as phospholipase A (31), the phospholipase activity was investigated. A mixture of ^{32}P-phosphatidyl glycerol, ^{32}P-diphosphatidyl glycerol, and ^{32}P-lysylphosphatidyl glycerol was incubated at 37 C in the presence and absence of staphylococcin 1580 (2,000 AU per ml) for 3 hr. No evidence of hydrolysis of the phospholipids was found. In both cases, only three spots could be observed on the autoradiogram, and the percentages of phosphatidyl glycerol (69%), diphosphatidyl glycerol

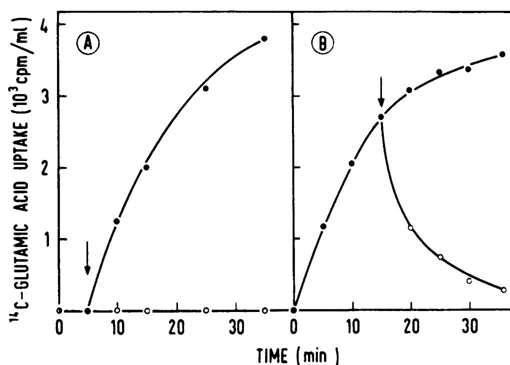

FIG. 4. *Effect of staphylococcin 1580 on amino acid transport. Cells of Staphylococcus aureus Oxford 209P were grown in AJ1 medium. When the culture reached an optical density of 0.5 at 600 nm, chloramphenicol (100 μg/ml) was added. (A) After 15 min at 37 C, the culture was divided into two parts: one received staphylococcin 1580 in 0.01 M phosphate buffer (pH 7.0) and the other received only the buffer. After 5 min, 0.1 μCi of ^{14}C-glutamic acid was added per ml. At various time intervals, samples were removed and the uptake of ^{14}C-glutamic acid was measured. (B) To the chloramphenicol-pretreated cell suspension 0.1 μCi of ^{14}C-glutamic acid was added per ml. After 15 min, the suspension was split out into two flasks, one containing staphylococcin 1580 in 0.01 M phosphate buffer (pH 7.0) and the other containing only the buffer. Samples were taken at the times indicated, and intracellular ^{14}C-glutamic acid was measured. Symbols: ●, control; ○, 1,000 AU of staphylococcin per ml of incubation mixture.*

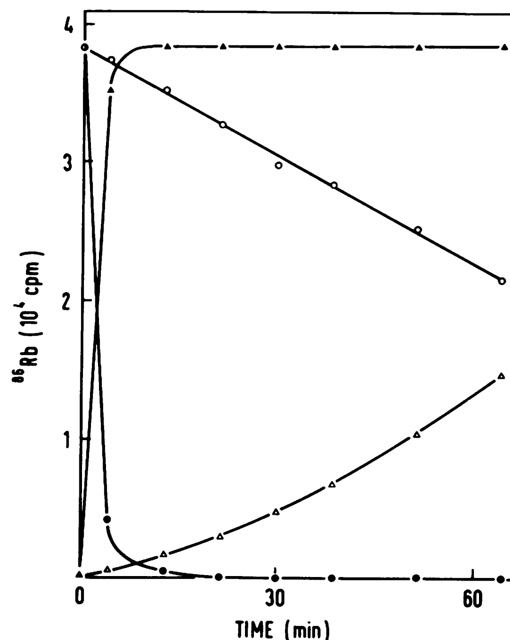

FIG. 5. *Effect of staphylococcin 1580 on rubidium transport. Cells of Staphylococcus aureus Oxford 209P were grown for 4 hr at 37 C in 100 ml of nutrient broth supplemented with 0.1 ml of ^{86}RbCl (2 to 10 mCi/ml). When the culture reached an optical density of 0.5 at 600 nm, the cells were centrifuged, washed, and resuspended in 0.3 M sucrose solution buffered with 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.0). One part was treated at 37 C with staphylococcin 1580 (final concentration of 1,000 AU per ml of incubation mixture) in buffered sucrose solution (closed symbols), and the control received only buffered sucrose solution (open symbols). At the time intervals indicated, intracellular (circles) and extracellular (triangles) radioactivity of 2-ml samples was determined.*

(12%), and lysylphosphatidyl glycerol (19%) remained unaltered in the presence of staphylococcin. In a separate test with lecithin we observed no degradation products after incubation in the presence of staphylococcin.

DISCUSSION

Staphylococcin 1580 is bactericidal to sensitive staphylococci, many other gram-positive bacteria, and stable staphylococcal L-forms.

The effects of staphylococcin 1580 on the various biochemical processes in the cell are very similar to those observed for colicins Ia, Ib (27), A (29), E1, and K (30), and resemble in many aspects the mode of action of the staphylococcin of the phage-type 71 staphylococci (14). Like these bacteriocins, staphylococcin 1580 inhibited DNA, RNA, and protein synthesis. The inhibition of the three processes seems to occur simultaneously and independently. After 1 hr of treatment with staphylococcin, extensive degradation of RNA was initiated, but no extensive degradation of DNA was observed. Therefore, degradation of RNA seems not to be the primary effect of the staphylococcin but may be due to structural changes induced by the observed leakage of monovalent cations, rendering ribosomes susceptible to the attack of nucleases, as suggested for colicin K (25). Like megacin A (31), colicins E1, K, G, A, and Q (8–10), and the staphylococcin of phage-type 71 staphylococci (12), staphylococcin 1580 caused leakage of ultraviolet-absorbing material which most probably contains degradation products of RNA.

Furthermore, staphylococcin 1580 exerted a marked effect on the transport of various compounds, e.g., glutamic acid, glucose, Rb ions, and ONPG.

These effects on biochemical processes indicate that at least three mechanisms of primary action may be involved, namely, inhibition of the energy supply, an alteration of the membrane perme-

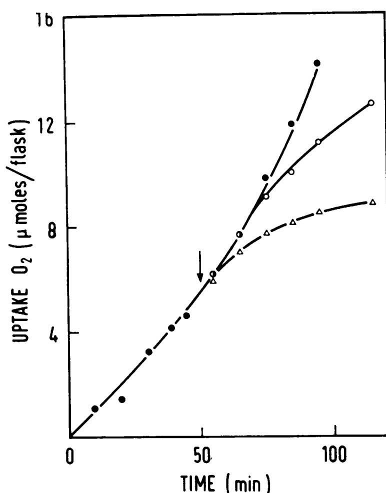

FIG. 6. *Effect of staphylococcin 1580 on the uptake of oxygen. Cells of Staphylococcus aureus Oxford 209P were harvested in the exponential phase and resuspended in 2 ml of AJ1 medium to an optical density of 0.6 at 600 nm. The uptake of oxygen was measured in a Warburg apparatus. After 50 min (arrow), staphylococcin in 0.01 M phosphate buffer (pH 7.0) or phosphate buffer was added from a side arm of the Warburg flask. Symbols:* ●, *control;* ○, *800 AU of staphylococcin per ml of incubation mixture;* △, *1,500 AU per ml.*

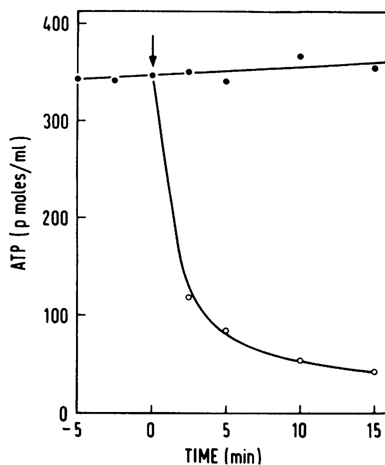

FIG. 7. *Effect of staphylococcin 1580 on the intracellular ATP level. Cells of Staphylococcus aureus Oxford 209P were grown in AJ2 medium at 37 C. At time zero, the cell suspension was divided into two parts: one received staphylococcin 1580 (final concentration, 1,500 AU per ml of incubation mixture) in 0.01 M phosphate buffer, pH 7.0* (○)*; the other received only buffer* (●). *At the time intervals indicated, samples were removed and assayed for ATP.*

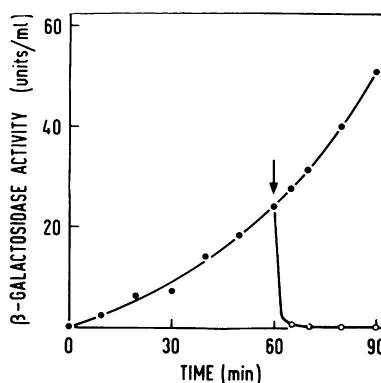

FIG. 8. *Effect of staphylococcin 1580 on β-galactosidase activity of whole cells. β-Galactosidase was induced by galactose in Staphylococcus aureus Oxford 209P cells. After 60 min (arrow), the cell suspension was divided into two parts: one was treated with staphylococcin 1580 (final concentration of 1,000 AU per ml of incubation mixture) in 0.01 M phosphate buffer, pH 7.0* (○), *and the other received only buffer* (●). *Samples were removed, and β-galactosidase activity was assayed in whole cells.*

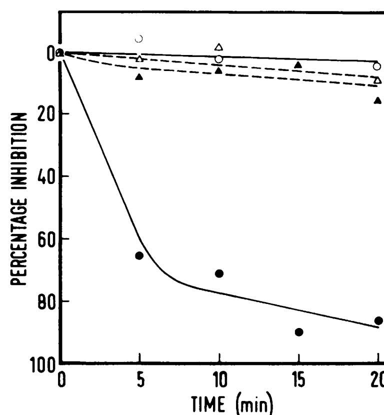

FIG. 9. *Effect of staphylococcin on motility. Bacillus cereus V 5003 (broken lines) and Bacillus subtilis NCTC 60015 (solid lines) were grown in AJ1 medium and treated in the exponential phase with staphylococcin 1580 (final concentration of 1,000 AU per ml of incubation mixture) in 0.01 M phosphate buffer, pH 7.0 (closed symbols), or with buffer (open symbols). Samples were taken at the times indicated, and the motility was assayed quantitatively according to Shoesmith (34).*

ability, or drastic changes in the membrane structure. These possibilities are not mutually exclusive.

The effects caused by colicins E1 and K (17, 18), Ia and Ib (27), and A (29) were interpreted as an interference with the supply of energy in the af-

fected *Escherichia coli* cells. This interference may be a selective inhibition of the oxidative phosphorylation or an activation of membrane-bound adenosine triphosphatase. Results of Feingold (16) indicated that the inhibition of active transport and macromolecular syntheses was not caused by cessation of the ATP production. Like the above-mentioned colicins, staphylococcin 1580 gradually inhibited the oxygen uptake of sensitive cells. The ATP level was reduced rapidly by staphylococcin to a level 15% of the original one. This effect may explain the inhibition of motility of the sensitive *B. subtilis* cells, but seems not to be the primary action of the staphylococcin, since a distinct amount of ATP remains available to the cells. Moreover, the effect on the various transport phenomena reported here cannot be explained on basis of ATP depletion, since ATP is not directly involved in them.

An effect of a bacteriocin on transport phenomena was previously shown for colicin E1 (3) and colicin A (29). The transport of amino acids and some β-galactosides in *E. coli* cells under aerobic conditions is tightly coupled to a membrane-bound D-lactate dehydrogenase and to a smaller extent to a succinate dehydrogenase, which both are coupled to the electron transport chain (23). Stable high-energy phosphate compounds are not involved in this transport. Recently, it was demonstrated that colicin E1 and K uncouple both D-lactate and succinate dehydrogenases from proline transport in isolated membrane vesicles (J. P. Kabat and S. E. Luria, Bacteriol. Proc., p. 62, 1970).

The transport of glutamic acid and various other amino acids, measured in isolated membrane vesicles of *S. aureus*, seems to be almost exclusively coupled to a membrane-bound α-glycerolphosphate dehydrogenase, which is coupled to the electron transport chain (26). Since oxygen uptake is only gradually inhibited, the staphylococcin, like colicin E1, may uncouple the electron transport chain from amino acid transport rather than block the electron transport chain itself. In accordance with this view, streptococci and hemin-negative mutants of *E. coli*, both of which lack cytochromes, are still sensitive to staphylococcin 1580 (21) and colicin E1 (17), respectively. Alternatively, the staphylococcin interferes directly with potassium, rubidium, or proton transport like valinomycin or 2,4-dinitrophenol, both of which abolish aspartate uptake in *S. aureus* (19). However, the inhibition of the transport of ONPG, which is mediated by a phosphoenolpyruvate phosphotransferase system in *S. aureus* (24), is not in accordance with both alternatives.

It must be emphasized also that the effect of staphylococcin on the synthesis of DNA, RNA, protein, and glycogenlike substances can be merely a result of inhibition of the transport of the radioactive precursors used in our experiments.

Various membranous and transport activities are dependent on lipid-protein interactions in the membrane (28). The stimulation of aspartate uptake by several lipids may be interpreted as a physical change in membrane structure (19). Inhibition of transport and other processes caused by staphylococcin 1580 could be due to alterations of lipid composition and lipid-protein interactions. This hypothesis can explain most of the effects caused by the staphylococcin. The bacteriocin may adsorb to the cell membrane, inducing conformational changes in the proteins or lipids, or both, of the membrane. These changes may be allosteric in nature, and a model has been suggested by Changeux and Thiéry (11) to explain how such alterations are spread throughout the membrane. Alternatively, the staphylococcin interferes directly with phospholipid metabolism, causing an altered lipid composition, followed by conformational changes over the membrane. Preliminary experiments presented here show that staphylococcin 1580 exposes no phospholipase activity on phospholipids in vitro; neither did colicin E1, K, and A, though the latter cause changes in phospholipid composition of the membrane (9). In this aspect these bacteriocins differ from megacin A, which acts like phospholipase A on the cell membrane and several phospholipids (31).

Theories similar to those proposed here for the mode of action of colicins and staphylococcin 1580 have been suggested for the mode of action of phage ghosts (15) and several morphine derivatives (19).

Studies on the effect of staphylococcin 1580 on phospholipid metabolism and transport through membrane vesicles are in progress and may further elucidate the primary action.

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