ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1995, p. 714–719 Vol. 39, No. 3
0066-4804/95/$04.00+0

Mode of Action of the Lantibiotic Mersacidin: Inhibition of Peptidoglycan Biosynthesis via a Novel Mechanism?

HEIKE BRÖTZ,[1] GABRIELE BIERBAUM,[1] ASTRID MARKUS,[2] ERNST MOLITOR,[1] AND HANS-GEORG SAHL[1]*

Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, D-53105 Bonn,[1] and Allgemeine Pharma Forschung, Hoechst AG, D-65926 Frankfurt am Main,[2] Germany

Received 11 July 1994/Returned for modification 26 October 1994/Accepted 23 December 1994

Mersacidin is an antibiotic peptide produced by *Bacillus* sp. strain HIL Y-85,54728 that belongs to the group of lantibiotics. Its activity in vivo against methicillin-resistant *Staphylococcus aureus* strains compares with that of the glycopeptide antibiotic vancomycin (S. Chatterjee, D. K. Chatterjee, R. H. Jani, J. Blumbach, B. N. Ganguli, N. Klesel, M. Limbert, and G. Seibert, J. Antibiot. 45:839–845, 1992). Incubation of *Staphylococcus simulans* 22 with mersacidin resulted in the cessation of growth and slow lysis. Biosyntheses of DNA, RNA, and protein were not affected, whereas incorporation of glucose and D-alanine was inhibited and a regular reduction in the level of cell wall thickness was observed. Thus, unlike type A lantibiotics, mersacidin does not form pores in the cytoplasmic membrane but rather inhibits cell wall biosynthesis. Comparison with tunicamycin-treated cells indicated that peptidoglycan rather than teichoic acid metabolism is primarily affected. Mersacidin caused the excretion of a putative cell wall precursor into the culture supernatant. The formation of polymeric peptidoglycan was effectively inhibited in an in vitro assay, probably on the level of transglycosylation. In contrast to vancomycin, the activity of mersacidin was not antagonized by the tripeptide diacetyl-L-Lys–D-Ala–D-Ala, indicating that on the molecular level its mode of action differs from those of glycopeptide antibiotics. These data together with electron microscopy suggest that mersacidin acts on a novel target, which opens new perspectives for the treatment of methicillin-resistant *S. aureus*.

The term *lantibiotics* designates a group of lanthionine (and/or 3-methyllanthionine)-containing peptides with antibiotic activity against gram-positive bacteria (25). In contrast to peptide antibiotics like bacitracin or gramicidin S (10), lantibiotics are ribosomally synthesized and show unique structural properties, which arise from posttranslational modifications (for reviews, see references 3 and 9).

With a molecular mass of 1,825 Da, mersacidin is the smallest lantibiotic known so far. Its propeptide template consists of 20 amino acids, 8 of which are modified to yield 4 thioether amino acids: 3 3-methyllanthionines and 1 2-aminovinyl-2-methylcysteine. This leads to the formation of four intramolecular heterocyclic rings. Additionally, the unusual amino acid α,β-didehydroalanine is found. Mersacidin carries no net charge and has overall hydrophobic properties (5). It is produced by *Bacillus* sp. strain HIL Y-85,54728 and is active against several gram-positive bacteria such as streptococci, bacilli, and staphylococci including methicillin-resistant *Staphylococcus aureus* strains; enterococci are relatively insusceptible (4, 14). Although in vitro mersacidin is less active than vancomycin, teicoplanin, and daptomycin (17), its activity in vivo is at least comparable to that of vancomycin in the treatment of experimental staphylococcal infections (including those caused by methicillin-resistant *S. aureus* strains) in mice (4, 14).

In addition to its potential therapeutical value, mersacidin has been the subject of increased interest because of its position among the lantibiotics described so far, which have been classified into two groups (9). The type A lantibiotics (e.g., Pep5 and nisin) are screw-shaped, positively charged, amphipathic molecules which exert their primary bactericidal action by the formation of pores in the cytoplasmic membrane (20). The type B lantibiotics, as defined so far (e.g., duramycin and cinnamycin), possess a globular shape, carry no net charge or a negative charge, and are described as inhibitors of phospholipase A_2 or the angiotensin-converting enzyme (3). Mersacidin does not completely conform to either group because of atypical structural features (9, 11).

Here, we report that it is also different regarding its mode of bactericidal activity. We show that mersacidin inhibits peptidoglycan biosynthesis, probably on the level of transglycosylation. Furthermore, we demonstrate that on the molecular level its mode of action differs from those of glycopeptide antibiotics like vancomycin or teicoplanin. This opens the possibility of using a new target for the antibiotic treatment of methicillin-resistant *S. aureus* strains and makes mersacidin an interesting tool for the investigation of peptidoglycan biosynthesis of gram-positive bacteria.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Staphylococcus simulans* 22 (formerly *Staphylococcus cohnii* 22; Institute for Medical Microbiology and Immunology of the University of Bonn, Bonn, Germany [21]) and *Micrococcus luteus* ATCC 4698 (American Type Culture Collection) were used as indicator strains. Unless indicated otherwise all experiments were performed with early-log-phase cells (A_{600}, about 0.5) grown in half-concentrated Mueller-Hinton broth (Oxoid, Basingstoke, United Kingdom) at 37°C for *S. simulans* 22 and 30°C for *M. luteus* ATCC 4698. *Escherichia coli* JE 5684 and *Bacillus cereus* T were kindly provided by J.-V. Höltje (Tübingen, Germany) and were used in the in vitro peptidoglycan synthesis assay.

Chemicals and antibiotics. All standard chemicals were of analytical grade (Merck, Darmstadt, Germany). Diacetyl-L-lysine–D-alanine–D-alanine and tunicamycin were purchased from Sigma (Munich, Germany), penicillin G was purchased from Hoechst AG (Frankfurt, Germany), vancomycin was purchased from Eli Lilly (Giessen, Germany), chloramphenicol was purchased from Serva

* Corresponding author. Mailing address: Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, Sigmund-Freud-Str. 25, D-53105 Bonn, Germany. Phone: (49)228/2875704. Fax: (49)228/2876763.

(Heidelberg, Germany), and mersacidin was a gift from Hoechst AG. Radiochemicals were provided by Amersham-Buchler (Braunschweig, Germany) except for [^{14}C]D-alanine, which was obtained from ICN (Costa Mesa, Calif.).

Accumulation of radioactive compounds and estimation of the membrane potential. The influence of mersacidin on the uptake and retention of glutamate was investigated as described previously (19). Briefly, *S. simulans* 22 was grown in CPY medium (casein hydrolysate, peptone, yeast extract) and was resuspended in 0.2% Bacto Peptone–0.8% glucose–0.4% yeast extract in 20 mM potassium phosphate buffer (pH 7), which was supplemented with 100 μg of chloramphenicol per ml to prevent glutamate incorporation. After 10 min of preincubation the culture was separated into two parts. One aliquot was transferred into a flask that already contained mersacidin (92 μg/ml) to test its effect on the uptake of glutamate. Immediately afterward 2 μCi of [^{3}H]glutamate (59 Ci/mmol) per ml was added both to the mersacidin-pretreated and to the untreated culture. Eight minutes later the untreated culture was further subdivided into two aliquots, one of which received mersacidin (92 μg/ml) to follow its effect on preaccumulated, but not incorporated, amino acids. Samples of 100 μl were filtered and the intracellular [^{3}H]glutamate was measured in a beta-counter (1900CA; Packard). For the evaluation of the effect of mersacidin on the membrane potential, *M. luteus* ATCC 4698 was treated for 90 min at 50 times the MIC (5 μg/ml). Then, 0.33 μCi [^{14}C]tetraphenylphosphoniumbromide (31.4 mCi/mmol) per ml was added and the membrane potential was determined as described by Ruhr and Sahl (19).

Incorporation of radioactive metabolites. To study the effect of mersacidin on the synthesis of macromolecules, the incorporation of ^{14}C- or ^{3}H-labeled precursors into the acid-precipitable cell fraction of *S. simulans* 22 was measured. The protocol of Sahl and Brandis (22) was used, except that the medium contained 20 mM potassium phosphate. All radiolabeled compounds ([^{3}H]thymidine, 84 Ci/mmol; [^{3}H]glutamate, 59 Ci/mmol; [^{3}H]glucose, 35 Ci/mmol; [^{14}C]uridine, 54 mCi/mmol; [^{14}C]D-alanine, 46 mCi/mmol) were used at concentrations of 0.5 μCi/ml, and mersacidin and chloramphenicol were added at concentrations of 92 μg/ml (50 μM; 8.3 times the MIC) and 100 μg/ml (0.3 mM), respectively. Samples of 0.5 ml were added to 2 ml of ice-cold 10% trichloroacetic acid containing 1 M NaCl and 1 mM unlabeled metabolite.

Phosphate contents of purified cell walls. *S. simulans* 22 was incubated with different inhibitors of cell wall biosynthesis at 10 times the MIC for 90 min. Cell walls were enzymatically purified as described previously (24), with some modifications. Briefly, bacteria were resuspended at 0.1 g (wet weight) per ml in 10 ml of buffer (50 mM Tris HCl [pH 7]) and were heated for 10 min at 80°C to destroy autolytic activity. After mechanical disruption (MSK homogenizer; Braun, Melsungen, Germany) the cell homogenate was kept on ice and was immediately fractionated by centrifugation at 5°C. Glass beads were removed at a low speed (120 × *g*, 5 min) and were washed once to increase cell wall yields. The cell wall-containing fraction was spun down at 22,000 × *g* for 30 min and was purified enzymatically in the presence of 75 μg of DNase I (bovine pancreas, 40 Kunitz U), 225 μg of RNase A (bovine pancreas, 23 Kunitz U), 700 μg of chloramphenicol, 70 mg of EDTA, a few micrograms of NaN_3, and 10 μl of toluene in 5 ml of buffer. After digestion for 3 h at 37°C the mixture was incubated with 650 μg of trypsin for 17 h at room temperature. Finally, the cell walls were treated with 2% sodium dodecyl sulfate (SDS) (2 h at 37°C) and were then dialyzed. The cell walls were hydrolyzed (0.1 ml of 70% $HClO_4$, 2.5 h, 165°C), and their phosphate contents were measured as described by Chen et al. (6).

Detection of the cell wall precursor in the culture supernatant. *S. simulans* 22 was precultured to an A_{600} of 0.25 in 50 ml CYG medium (0.5% casein hydrolysate, 0.2% yeast extract, 0.5% glucose in 10 mM phosphate buffer [pH 7]) supplemented with 100 μM *N*-acetylglucosamine (GlcNAc). Cells were then harvested by centrifugation and were resuspended in 20 ml of fresh, prewarmed medium containing 0.5 mM glucose but no GlcNAc. The cell suspension was preincubated for 25 min to reestablish exponential growth and was then labeled with [^{14}C]GlcNAc for another 20 min. Then, the culture was subdivided into three portions, one of which was treated with mersacidin (110 μg/ml; 10 times the MIC), a second received vancomycin (4 μg/ml, 2.7 μM; 10 times the MIC), and a third was run as a control. After 30, 60, and 90 min samples of 150 μl were withdrawn and the cells were removed by centrifugation. The supernatant was heated (10 min, 80°C) to destroy autolytic activity, dried under vacuum, and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) on 25% gels as described by Laemmli (13) without further purification. The gels were then dried and exposed to an X-ray film (Medical X-ray film; Fuji) for 5 days at −70°C.

MIC determinations. MICs were determined by broth microdilution. Serial twofold dilutions of mersacidin or vancomycin were prepared in Mueller-Hinton broth. Diacetyl-L-Lys–D-Ala–D-Ala was diluted along with the antibiotics and was kept in a constant 100-fold molar excess. Bacteria were added to give a final inoculum of 10^5 CFU/ml in a volume of 0.2 ml. After incubation for 16 h at 37°C for *S. simulans* 22 or for 26 h at 30°C for *M. luteus* ATCC 4698 the MIC was read as the lowest concentration of antimicrobial agent resulting in the complete inhibition of visible growth.

In vitro murein biosynthesis. The membrane fraction was prepared from *E. coli* JE 5684 by mechanical disruption of bacteria in the exponential growth phase. After removing the glass beads by centrifugation, the membranes were separated from the supernatant at 100,000 × *g*. The membranes were then washed and resuspended in 50 mM Tris HCl (pH 7.4) supplemented with 0.1 mM $MgCl_2$ and 1 mM mercaptoethanol to give a protein concentration of 20 mg/ml. UDP–*N*-acetylmuramylpentapeptide (UDP-MurNAc-pentapeptide) was isolated from *B. cereus* T. We used the protocol of Kohlrausch and Höltje (12), with slight modifications. In order to accumulate murein precursors, cells in the exponential growth phase were supplemented with 5 μg of vancomycin per ml. After 30 min of incubation the cells were centrifuged, resuspended in distilled water (0.1 g/ml), and extracted by stirring the suspension slowly into 2.5 volumes of boiling water. After 15 min of boiling the suspension was cooled to room temperature and centrifuged (100,000 × *g*), and the supernatant was lyophilized. The lyophilized material was then dissolved in water and was adjusted to pH 2 with 20% H_3PO_4. After acidification the precipitated material was removed by centrifugation and the supernatant was immediately subjected to fractionation by high-pressure liquid chromatography. The pentapeptide obtained after this step was lyophilized and dissolved in distilled water to a concentration of 10 mM on the basis of the molar extinction coefficient for uridine ($\varepsilon = 10^7$ cm^2 mol^{-1}). For in vitro murein biosynthesis UDP-MurNAc-pentapeptide (0.5 mM), [^{14}C]UDP-GlcNAc (0.01 mM), and membranes (0.2 mg) were mixed with different concentrations of the compound to be tested (0.1 to 100 μg/ml) in a total volume of 40 μl of the buffer (50 mM Tris HCl [pH 8], 10 mM $MgCl_2$, 1 mM mercaptoethanol). Incubation proceeded for 20 min at 30°C before the reaction was stopped by heating the mixture to 100°C. In order to measure the rate of incorporation of the radioactive material into murein, the samples were applied to 20-cm filter strips (LKB electrophoresis strips) and the strips were developed by paper chromatography in isobutyric acid-water-ammonia (50/28.25/1.75) for 16 h. Polymeric murein remained at the starting position, and radioactivity was determined with a beta-counter (LS 2800; Beckman).

Electron microscopy. After incubation of *S. simulans* 22 with different inhibitors of cell wall biosynthesis at 10 times the MIC for 90 min, 10-ml samples were taken and the bacteria were harvested by centrifugation. Samples were fixed in Sörensen's phosphate solution (SPS; 50.8 mM KH_2PO_4, 49.2 mM Na_2HPO_4 [pH 6.8]) containing 3% glutaraldehyde for 4 h at 4°C, washed several times with SPS supplemented with 0.1 M sucrose, and incubated with 1% tannic acid in SPS for 30 min at 4°C. After removal of the phosphate by washing the cells with 0.9% NaCl, the samples were stained with saturated aqueous uranyl acetate for 3 h at room temperature in the dark. Excess uranyl acetate was removed with 0.9% NaCl, and the samples were dehydrated in a graded series of ethanol and embedded in LR-White (London Resin Co.) by thermal polymerization. After cutting on a LKB Ultratome III, sections were examined in a Zeiss EM 902 transmission electron microscope.

RESULTS

Effects on growth and viability of *S. simulans* 22. The addition of mersacidin (110 μg/ml; 10 times the MIC) to exponentially growing cells did not have an immediate effect. The cell count as well as the turbidity at 600 nm increased for another 50 min, which corresponds approximately to one generation time under the experimental conditions that were used. Then cell viability decreased, and slow lysis set in as indicated by a reduction in turbidity. Resting cells deprived of a carbon source were not affected, indicating that mersacidin interferes with a growth process.

Effect on uptake and retention of low-molecular-weight metabolites. Type A lantibiotics primarily act via pore formation in the cytoplasmic membrane which leads to deenergization of bacterial cells and an inability to perform active transport. Moreover, accumulated ions or amino acids leak out of the cells upon the addition of pore-forming peptides (20). In contrast mersacidin-pretreated cultures took up labeled glutamate at the same rate as untreated controls (Fig. 1A). Furthermore, cells that had preaccumulated the label remained intact upon the addition of mersacidin, and no release of intracellular glutamate was observed (Fig. 1B). In addition, treated cells kept a constantly high membrane potential (data not shown).

Effects on macromolecular synthesis. As shown in Fig. 2, mersacidin immediately inhibited the incorporation of glucose into the cellular macromolecules of *S. simulans* 22, whereas the biosynthesis of DNA, RNA, and protein, measured as the incorporation of thymidine, uridine, and glutamate, respectively, was not affected. Incorporation of the cell wall-specific amino acid D-alanine was also strongly reduced in the presence of mersacidin (Fig. 2), suggesting that it does not merely inhibit glucose uptake or utilization but interferes with cell wall biosynthesis. Controls treated with a high concentration of chlor-

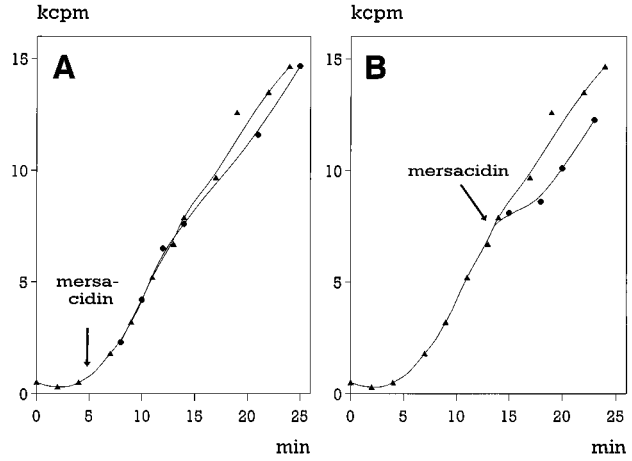

FIG. 1. Influence of mersacidin on [^{3}H]glutamate uptake (A) and retention (B) by *S. simulans* 22. ●, mersacidin-treated cells; ▲, untreated cells. Both experiments were performed with the same culture in the presence of 100 μg of chloramphenicol per ml to prevent glutamate incorporation; kcpm, 1,000 cpm.

amphenicol (100 μg/ml, 0.3 mM) confirmed that D-alanine was mainly incorporated into the cell wall.

Inhibition of peptidoglycan biosynthesis in vivo. During incubation with mersacidin increasing numbers of staphylococcal cells stained gram negative, additionally supporting the hypothesis that mersacidin interferes with cell wall metabolism. Therefore, we investigated its effect on the ultrastructure of staphylococci (Fig. 3). Mersacidin-treated cells showed regular reductions in their cell wall diameters from 30 to 34 nm in control cells to 17 to 20 nm in mersacidin-treated cells. Multiple lesions were observed all over the cell wall, most likely as a consequence of osmotic cell rupture. Cross sections in the septal area showed a striking reduction in the cell wall width after the addition of mersacidin. Another interesting feature was that the chromosome, indicated by the less electron-dense area, was spread out in the cytoplasm, whereas it was restricted to a distinct area in control cells.

We also compared the morphological effects of mersacidin with those of other cell wall inhibitors (data not shown [15]). Similarities appeared after the incubation with vancomycin, but neither penicillin nor tunicamycin caused a reduction in the cell wall diameter. Unlike mersacidin, tunicamycin triggered the formation of an electron-lucent cell wall, and penicillin-treated cells possessed characteristically thickened septa, as reported by Giesbrecht et al. (7). Since comparison with tunicamycin by electron microscopy pointed to peptidoglycan biosynthesis rather than teichoic acid biosynthesis as the primary target for mersacidin, the phosphate contents of purified cell walls of *S. simulans* 22 were determined as a measure of teichoic acid content. In comparison with the untreated control (100%), the phosphate content was reduced to 72% by mersacidin, to 64% by vancomycin, to 58% by penicillin, and to 21% by tunicamycin. In this assay the effect of mersacidin equaled those of vancomycin and penicillin, both of which are inhibitors of peptidoglycan biosynthesis.

Inhibition of murein polymerization in vitro. Mersacidin also inhibited peptidoglycan synthesis in an in vitro assay with crude *E. coli* membranes. In this assay the incorporation of [^{14}C]GlcNAc into polymeric murein was determined by starting with UDP-MurNAc-pentapeptide as a precursor (Fig. 4). Mersacidin caused 98% inhibition at a concentration of 100 μg/ml, thereby exceeding the effect of vancomycin, which

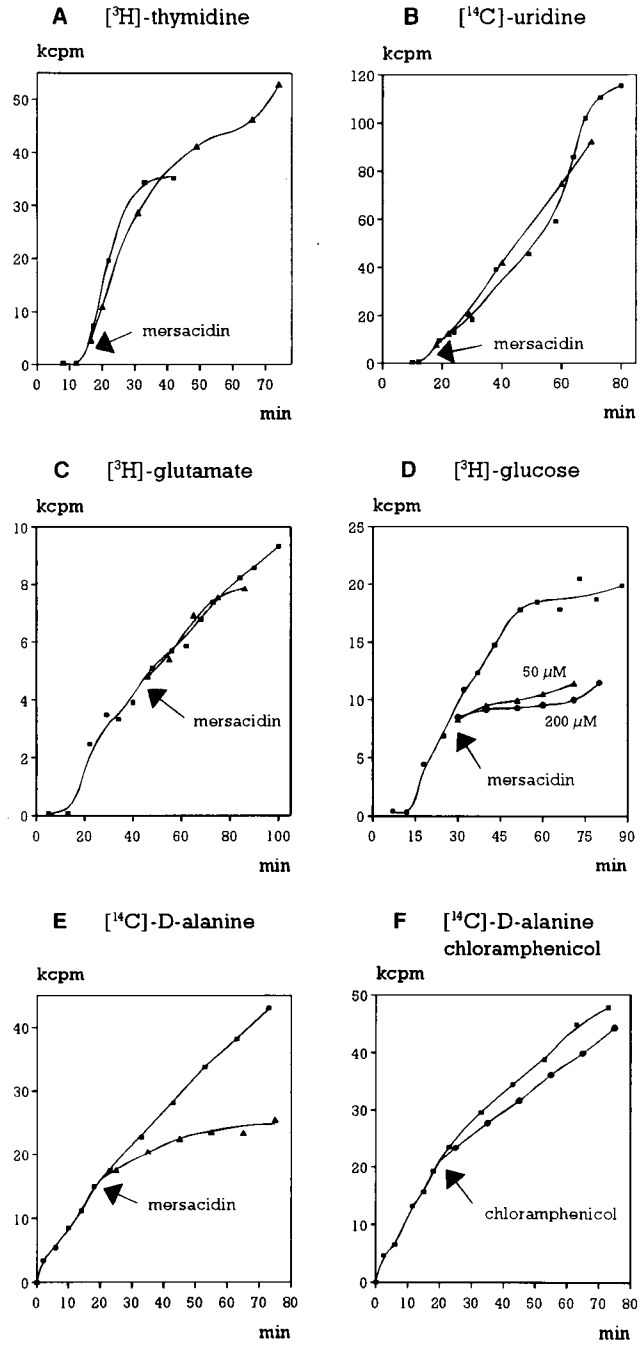

FIG. 2. Influence of mersacidin on the synthesis of biopolymers by *S. simulans* 22 determined by incorporation of radioactive metabolites. Incorporation of [^{3}H]thymidine (A), [^{14}C]uridine (B), [^{3}H]glutamate (C), [^{3}H]glucose (D), and [^{14}C]D-alanine (E and F). Results from the addition of mersacidin at 92 μg/ml (50 μM) (▲) or 368 μg/ml (200 μM) (●), of chloramphenicol at 100 μg/ml (0.3 mM) (●; panel F), and the untreated control (■) are shown.

caused only 77% inhibition at the same concentration. Moenomycin was much more effective and gave rise to 89% inhibition at 1 μg/ml.

Comparison with vancomycin. Since mersacidin and vancomycin had shown some parallel effects, we tried to obtain additional information on whether they act on the same target. When the culture supernatant of *S. simulans* 22, which had been treated with 110 μg of mersacidin per ml for 30 min,

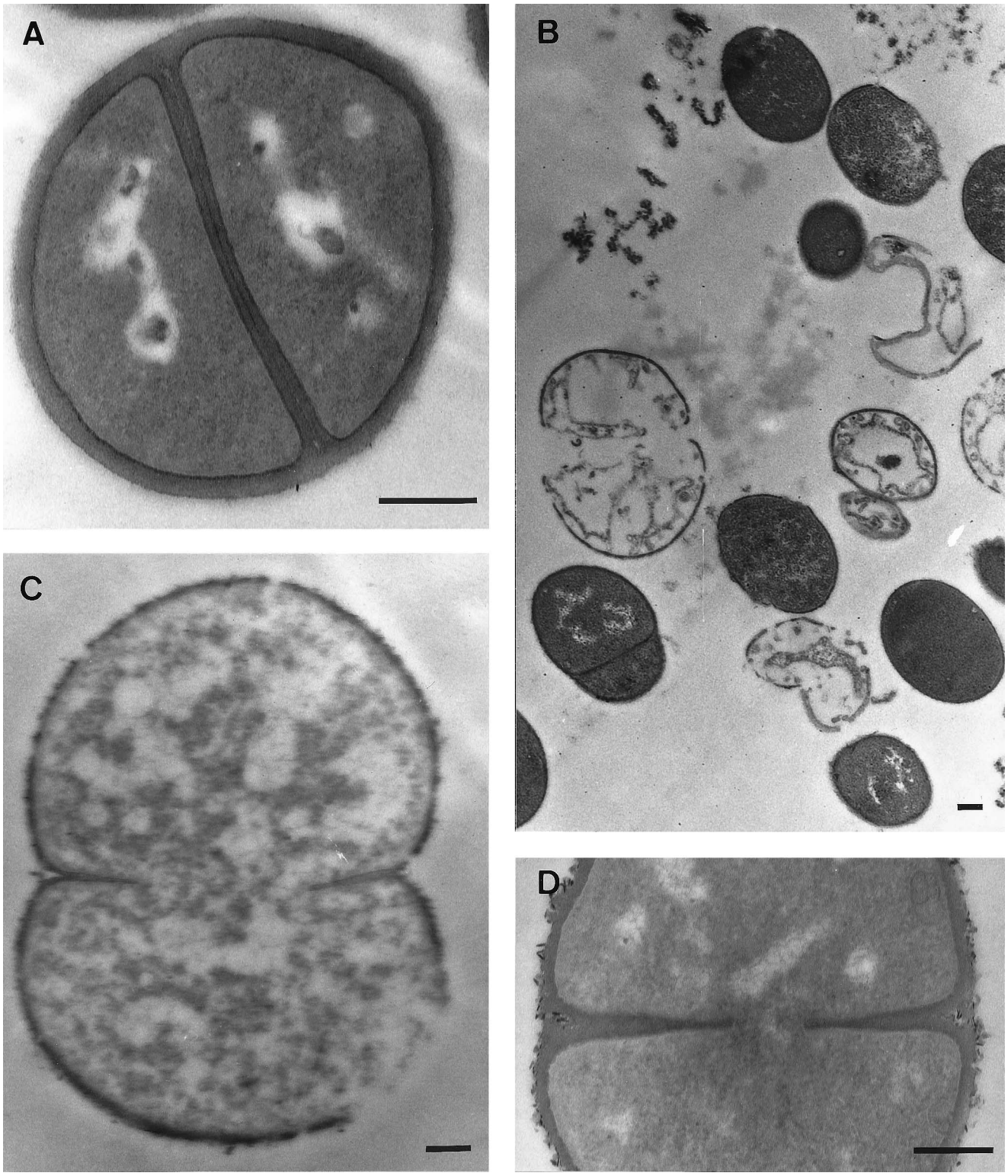

FIG. 3. Electron micrographs of *S. simulans* 22 grown in the presence or absence of mersacidin. (A) control; (B to D) mersacidin-treated cells; (B) overview; (C) enlarged single cell; (D) magnified septal area. Bars, 200 nm.

was analyzed by SDS-PAGE, it was observed that a low-molecular-weight compound was excreted into the medium. This compound was radiolabeled when the cells were grown on [^{14}C]GlcNAc (Fig. 5). In samples taken 60 and 90 min after the addition of the antibiotics, no further excretion of the compound was found. When vancomycin was studied in parallel, no excretion was caused by the glycopeptide at a concentration of 4 μg/ml, corresponding to 10 times the MIC (Fig. 5). When it

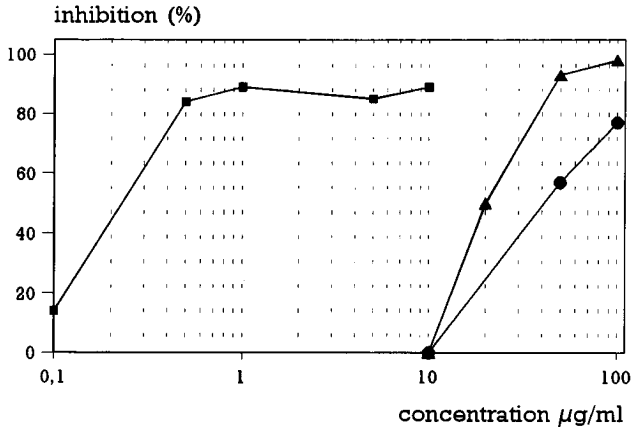

FIG. 4. Inhibition of peptidoglycan synthesis in vitro by mersacidin (▲), vancomycin (●), and moenomycin (■).

was used at the same concentration as mersacidin (110 μg/ml), a similar effect was observed.

To obtain information on whether mersacidin interacts with the D-alanyl–D-alanine terminus of the peptidoglycan precursor like vancomycin does, we determined the MICs of both compounds in the presence of synthetic diacetyl-L-Lys–D-Ala–D-Ala, assuming that this peptide should antagonize the activity in the case of complex formation with the C-terminal portion of the precursor. Whereas the activity of mersacidin was not affected by the tripeptide, vancomycin was effectively antagonized, and even at the highest concentration tested, unhindered growth was still recorded (Table 1). The same result could be observed when the purified cell wall of *M. luteus* ATCC 4698 digested with lysozyme was used as the antagonizing agent.

DISCUSSION

Several lines of evidence indicate that mersacidin exerts its bactericidal action by inhibition of cell wall biosynthesis rather than through pore formation. As shown for type A lantibiotics pore formation depolarizes the cytoplasmic membrane (23). This leads to an immediate and complete cessation of biosynthetic processes such as DNA, RNA, protein, and polysaccharide synthesis (22). In addition, low-molecular-weight intracellular compounds efflux rapidly through these pores (23; for a review, see reference 20). In contrast, mersacidin does not affect the energy-transducing cytoplasmic membrane of target cells (Fig. 1). Furthermore, the incorporation of glucose and

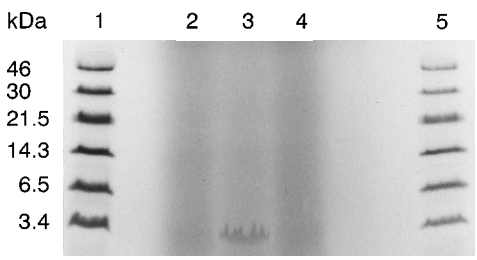

FIG. 5. Mersacidin-induced excretion of putative cell wall precursors by *S. simulans* 22. Autoradiogram of supernatants obtained from mersacidin-treated (lane 3), vancomycin-treated (lane 4), or untreated (lane 2) cultures. A total of 100 μl of supernatant was subjected to SDS-PAGE without purification. The standard was a ^{14}C-methylated Rainbow molecular mass marker (Amersham-Buchler, Braunschweig, Germany). Lane 1, 5 μl; lane 5, 2.5 μl.

TABLE 1. MICs for *S. simulans* 22 and *M. luteus* ATCC 4698 in the presence or absence of diacetyl-L-Lys–D-Ala–D-Ala

Indicator strain	Drug	MIC (μg/ml)	
		Without (Ac_2)KAA[a]	With (Ac_2)KAA
M. luteus	Vancomycin	0.1	>20
	Mersacidin	0.1	0.1
S. simulans	Vancomycin	0.4	>20
	Mersacidin	11	11

[a] (Ac_2)KAA, diacetyl-L-Lys–D-Ala–D-Ala.

D-alanine in macromolecules is selectively inhibited, while DNA, RNA, and protein synthesis proceeds unhindered (Fig. 2).

Electron micrographs of treated staphylococci revealed a drastic reduction in the cell wall diameter, eventually leading to cell lysis. The regular thinning of the cell wall in the septum as well as in the peripheral parts does not give any hint of a localized effect. Autolysis induced by type A lantibiotics in *S. simulans* 22 as a secondary effect following pore formation is clearly localized at the septal area (2). In this case, cell wall-degrading enzymes are indirectly activated by the cationic lantibiotics, in that these replace the basic enzymes from teichoic and teichuronic acids, which act as intrinsic cell wall inhibitors of cell wall autolysins (1).

In contrast to mersacidin, β-lactam antibiotics also induce morphological aberrations in staphylococci that are most obvious in the septal area (7). Comparison of the ultrastructures of mersacidin- and tunicamycin-treated cells as well as determination of the phosphate contents of purified cell walls showed that teichoic acid biosynthesis cannot be the primary target for mersacidin. In contrast to tunicamycin, which inhibits teichoic acid biosynthesis (29), mersacidin reduced the phosphate content only slightly, and the reduction equaled those caused by vancomycin and penicillin, both of which are inhibitors of peptidoglycan synthesis. The reduction in the teichoic acid contents in these cell walls should be a consequence of the close association of teichoic acid and peptidoglycan metabolism, e.g., the involvement of the isoprenoid carrier in both pathways.

Mersacidin-treated cells excrete a low-molecular-weight compound into the culture supernatant that contains GlcNAc, and this compound may therefore represent a cell wall precursor. Prolonged incubation with mersacidin caused no further excretion, which makes it more likely that this substance is indeed a peptidoglycan precursor and does not derive from cell wall-degrading processes.

In addition, we could demonstrate the effect of mersacidin on peptidoglycan synthesis in an in vitro assay with crude *E. coli* membranes, which involves three distinct enzyme activities: (i) transport of MurNAc-pentapeptide from UDP to the isoprenoid carrier, (ii) attachment of GlcNAc to this intermediate, and (iii) formation of polymeric peptidoglycan via transglycosylation. Mersacidin prevented the synthesis of polymeric murein effectively and even better than vancomycin, although the MICs are lower for the glycopeptide (4, 14, 17). Nevertheless, the concentration of mersacidin necessary for full inhibition of peptidoglycan synthesis in vitro is in the range of that of vancomycin but is 100-fold greater than that of moenomycin. Moenomycin interferes with transglycosylation directly as a competitive inhibitor (28), whereas vancomycin forms a complex with the terminal D-alanyl–D-alanine residue of the muropeptide, thus exerting a steric inhibition on the transglyco-

sylases (18; for a review, see reference 16). Our data, obtained from the *E. coli* system, suggest that mersacidin, similarly to the glycopeptides, inhibits transglycosylation indirectly, although it might as well act as a competitive inhibitor of transglycosylases, with a 100-fold lower efficiency compared with that of moenomycin; also, the affinity of mersacidin for staphylococcal enzymes could be considerably higher than that for the *E. coli* enzymes tested.

MIC determinations in the presence of a diacetyl-L-Lys–D-Ala–D-Ala or of lysozyme-digested *M. luteus* cell wall showed striking differences between mersacidin and vancomycin. The tripeptide competes with the D-alanyl–D-alanine residue of the peptidoglycan precursor for the binding site of vancomycin, thus strongly antagonizing its activity. The finding that the MIC of mersacidin was not affected suggests that on the molecular level its mode of action differs from those of the glycopeptide antibiotics. Furthermore, because it certainly differs from β-lactams, mersacidin most likely interacts with a target which is not attacked by chemotherapeutic drugs currently in use, particularly against methicillin-resistant *S. aureus*. The threatening situation with these staphylococci, which eventually become resistant to vancomycin (26), makes it necessary to search for novel targets, and mersacidin has considerable potential in this respect.

Our results demonstrate that the mode of action of mersacidin differs not only from those of type A lantibiotics but also from those of type B lantibiotics, as defined so far with respect to the thioether bridging pattern and biological target, although its overall properties regarding globular structure, hydrophobicity, and charge agree with those of the cinnamycin-duramycin type of lantibiotics. In this context it is interesting to mention that actagardine, formerly designated gardimycin, which is the second lantibiotic that does not correctly fit into the type A and type B categories (9), was also reported to inhibit peptidoglycan synthesis (27), although molecular details are not known. There are defined structure-function relationships between the different types of lantibiotics, and the prospect of finding novel targets extends the potential application of this group of antibacterial peptides beyond their current use as food preservatives, with nisin as the prototype lantibiotic (8).

ACKNOWLEDGMENTS

We gratefully acknowledge László Vértesy (Hoechst AG, Frankfurt, Germany) for the preparation of MurNAc-pentapeptide and Monika Eschbach-Bludau, Michaele Josten, and Christiane Ruland for expert technical assistance.

This work was partially supported by the Deutsche Forschungsgemeinschaft (grant Bi 504/1-1).

REFERENCES

1. **Bierbaum, G., and H.-G. Sahl.** 1987. Autolytic system of *Staphylococcus simulans* 22: influence of cationic peptides on activity of *N*-acetylmuramoyl-L-alanine amidase. J. Bacteriol. **169:**5452–5458.
2. **Bierbaum, G., and H.-G. Sahl.** 1991. Induction of autolysis of *Staphylococcus simulans* 22 by Pep5 and nisin and influence of the cationic peptides on the activity of the autolytic enzymes, p. 386–396. *In* G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. ESCOM, Leiden, The Netherlands.
3. **Bierbaum, G., and H.-G. Sahl.** 1993. Lantibiotics—unusually modified bacteriocin-like peptides from gram-positive bacteria. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **278:**1–22.
4. **Chatterjee, S., D. K. Chatterjee, R. H. Jani, J. Blumbach, B. N. Ganguli, N. Klesel, M. Limbert, and G. Seibert.** 1992. Mersacidin, a new antibiotic from *Bacillus*, *in vitro* and *in vivo* antibacterial activity. J. Antibiot. **45:**839–845.
5. **Chatterjee, S., S. Chatterjee, S. J. Lad, M. S. Phansalkar, R. H. Rupp, B. N. Ganguli, H.-W. Fehlhaber, and H. Kogler.** 1992. Mersacidin, a new antibiotic from *Bacillus*, fermentation, isolation, purification and chemical characterization. J. Antibiot. **45:**832–838.
6. **Chen, P. S., Jr., T. Y. Toribara, and H. Warner.** 1956. Microdetermination of phosphorus. Anal. Chem. **28:**1756–1758.
7. **Giesbrecht, P., T. Kersten, and J. Wecke.** 1992. Fan-shaped ejections of regularly arranged murosomes involved in penicillin-induced death of staphylococci. J. Bacteriol. **174:**2241–2252.
8. **Hurst, A.** 1981. Nisin. Adv. Appl. Microbiol. **27:**85–123.
9. **Jung, G.** 1991. Lantibiotics—ribosomally synthesized biologically active polypeptides containing sulfide bridges and α,β-didehydroamino acids. Angew. Chem. Int. Ed. Engl. **30:**1051–1068.
10. **Kleinkauf, H., and H. van Döhren.** 1987. Biosynthesis of peptide antibiotics. Ann. Rev. Microbiol. **41:**259–289.
11. **Kogler, H., M. Bauch, H.-W. Fehlhaber, C. Griesinger, W. Schubert, and V. Teetz.** 1991. NMR-spectroscopic investigations on mersacidin, p. 159–170. *In* G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. ESCOM, Leiden, The Netherlands.
12. **Kohlrausch, U., and J.-V. Höltje.** 1991. One-step purification procedure for UDP-*N*-acetylmuramyl-peptide murein precursors from *Bacillus cereus*. FEMS Lett. **78:**253–258.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
14. **Limbert, M., D. Isert, N. Klesel, A. Markus, G. Seibert, S. Chatterjee, D. K. Chatterjee, R. H. Jani, and B. N. Ganguli.** 1991. Chemotherapeutic properties of mersacidin *in vitro* and *in vivo*, p. 448–456. *In* G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. ESCOM, Leiden, The Netherlands.
15. **Molitor, E., C. Ruland, H. Brötz, G. Bierbaum, and H.-G. Sahl.** Unpublished data.
16. **Nagarajan, R.** 1991. Antibacterial activities and modes of action of vancomycin and related glycopeptides. Antimicrob. Agents Chemother. **35:**605–609.
17. **Niu, W.-W., and H. C. Neu.** 1991. Activity of mersacidin, a novel peptide, compared with that of vancomycin, teicoplanin, and daptomycin. Antimicrob. Agents Chemother. **35:**998–1000.
18. **Perkins, H. R.** 1969. Specificity of combination between mucopeptide precursors and vancomycin or ristocetin. Biochem. J. **111:**195–205.
19. **Ruhr, E., and H.-G. Sahl.** 1985. Mode of action of the peptide antibiotic nisin and influence on the membrane potential of whole cells and on cytoplasmic and artificial membrane vesicles. Antimicrob. Agents Chemother. **27:**841–845.
20. **Sahl, H.-G.** 1991. Pore formation in bacterial membranes by cationic lantibiotics, p. 347–358. *In* G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. ESCOM, Leiden, The Netherlands.
21. **Sahl, H. G., and H. Brandis.** 1981. Production, purification and chemical properties of an antistaphylococcal agent produced by *Staphylococcus epidermidis*. J. Gen. Microbiol. **127:**377–384.
22. **Sahl, H.-G., and H. Brandis.** 1982. Mode of action of the staphylococcin-like peptide Pep5 and culture conditions effecting its activity. Zentral. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. A **252:**166–175.
23. **Sahl, H.-G., and H. Brandis.** 1983. Efflux of low-M_r substances from the cytoplasm of sensitive cells caused by the staphylococcin-like agent Pep 5. FEMS Lett. **16:**75–79.
24. **Sahl, H.-G., C. Hahn, and H. Brandis.** 1985. Interaction of the staphylococcin-like peptide Pep5 with cell walls and isolated cell wall components of gram-positive bacteria. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. A **260:**197–205.
25. **Schnell, N., K.-D. Entian, U. Schneider, F. Götz, H. Zähner, R. Kellner, and G. Jung.** 1988. Prepeptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings. Nature (London) **333:**276–278.
26. **Schwalbe, R. S., W. J. Ritz, P. R. Verma, E. A. Barranco, and P. H. Gilligan.** 1990. Selection for vancomycin resistance in clinical isolates of *Staphylococcus haemolyticus*. J. Infect. Dis. **161:**45–51.
27. **Somma, S., W. Merati, and F. Parenti.** 1977. Gardimycin, a new antibiotic inhibiting peptidoglycan synthesis. Antimicrob. Agents Chemother. **11:**396–401.
28. **van Heijenoort, J., Y. van Heijenoort, and P. Welzel.** 1988. Moenomycin: inhibitor of peptidoglycan polymerization in *Escherichia coli*, p. 549–557. *In* P. Actor, L. Daneo-Moore, M. L. Higgins, H. R. J. Salton, and G. D. Shockman (ed.), Antibiotic inhibition of bacterial cell surface assembly and function. American Society for Microbiology, Washington, D.C.
29. **Wyke, A. W., and J. B. Ward.** 1977. Biosynthesis of wall polymers in *Bacillus subtilis*. J. Bacteriol. **130:**1055–1063.