INFECTION AND IMMUNITY, Aug. 1995, p. 2886–2891 Vol. 63, No. 8
0019-9567/95/$04.00+0
Copyright 1995, American Society for Microbiology

Morphology of Defensin-Treated *Staphylococcus aureus*

MASAKO SHIMODA,[1,2] KAZUNORI OHKI,[1] YOSHINORI SHIMAMOTO,[2] AND OSAMU KOHASHI[1]*

Department of Microbiology[1] and Department of Internal Medicine,[2] Saga Medical School, Saga 849, Japan

Received 21 February 1995/Returned for modification 23 March 1995/Accepted 17 May 1995

Defensins are a family of broad-spectrum antimicrobial peptides found abundantly in the cytoplasmic granules of mammalian neutrophils and Paneth cells of the small intestine. Defensins are known to form ion channels on the membranes of target cells. These channel formations and the cytotoxicity of defensins are intimately linked. We showed the morphological effects of defensins on the cytoplasmic membranes of *Staphylococcus aureus* by transmission electron microscopy. *S. aureus* exposed to defensins developed characteristic mesosome-like structures but did not show remarkable changes in cell walls. Defensins induced such structural changes not only at high concentration but also at low concentrations that were not bactericidal. We also showed that increasing the concentration of NaCl in the reaction mixture completely inhibited the occurrence of membranous changes of target cells exposed to defensins. These findings are, to our knowledge, the first report of morphological changes in gram-positive bacteria treated with defensins. Our results indicate that the first effect of defensins in *S. aureus* is to damage cytoplasmic membranes directly; they also support previous reports that the cell membrane is the principal target of defensins.

Polymorphonuclear leukocytes (PMN) contribute to the host defense against infection by a variety of microorganisms. In general, two mechanisms, often called oxygen dependent and oxygen independent, are involved in the killing of invading microorganisms. Various antimicrobial polypeptides released from PMN granules into phagolysosomes are involved in the oxygen-independent mechanism. Among these polypeptides, defensins are most abundant and account for 30 to 50% of the azurophil granule proteins (6). Defensins are small, cationic, and relatively arginine-rich peptides composed of 29 to 34 amino acids. Their unique structural motifs are characterized by six invariant cysteines and three intramolecular disulfide bonds. Defensins have been identified in rabbit lung macrophages (22) and in PMN of humans (7), rabbits (26), rats (4), and guinea pigs (24) and were recently demonstrated in mouse (3) and human (10, 13) small intestinal Paneth cells. Defensins exert antimicrobial activity against many gram-positive and gram-negative bacteria (7, 11, 24, 26), fungi (1, 25) and some enveloped viruses (2, 4, 15, 24) and cytotoxic activity against normal and malignant cells (17, 18). The antimicrobial and cytotoxic activities of defensins are related to their ability to increase the permeability of membranes of microorganisms and mammalian cells (14, 17, 27). In addition, the voltage-dependent channels formed by defensins are thought to be involved in making membranes permeable (12). Some reports suggest that the cell membrane is the principal target of defensins. Although there have been many studies on the bactericidal effects of defensins, there is little information about morphological changes in bacteria exposed to them (14, 23), and no information is presently available on morphological changes in gram-positive bacteria treated with defensins. In the present study, we examined by transmission electron microscopy morphological changes in the cell membrane of *Staphylococcus aureus* exposed to defensins.

* Corresponding author. Mailing address: Department of Microbiology, Saga Medical School, 5-1-1 Nabeshima, Saga 849, Japan. Phone: 81-952-31-6511, ext. 2252. Fax: 81-952-30-6629.

MATERIALS AND METHODS

Purification of rat defensins. Rat defensins were purified as described previously (4, 11). Briefly, PMN were purified from peritoneal exudate cells of *rnu*/+ rats that had been injected intraperitoneally with thioglycolate broth (Eiken Chemical Co., Tokyo, Japan). After 6 h, peritoneal exudate cells were obtained by lavaging the peritoneal cavity with cold phosphate-buffered saline (PBS). The lavage fluids were centrifuged at 200 × *g* for 10 min at 4°C, and the pelleted cells were suspended in PBS. Erythrocytes were removed by brief hypotonic lysis in distilled water. After centrifugation, the PMN were suspended in ice-cold 0.05 M sodium phosphate buffer (pH 6.6) containing 2 mM *p*-amidinophenylmethanesulfonyl fluoride hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 10 μM leupeptin (Sigma Chemical Co., St. Louis, Mo.), 10 μM pepstatin (Sigma), and 2 mM EDTA (Wako). This mixture was sonicated and kept on ice for 30 min. Cold 20% acetic acid was added, and the mixture was sonicated again, stirred for 1 h at 4°C, and then centrifuged at 27,000 × *g* for 20 min. The pellet was extracted as before. The supernatants obtained from these extractions were concentrated by ultrafiltration with an Amicon YC-05 filter (molecular weight cutoff, 500). The acid extracts were loaded onto a Sephadex G-100 (Pharmacia, Uppsala, Sweden) column (2.7 by 95 cm) and eluted with 5% (vol/vol) acetic acid. Defensin fractions were pooled and lyophilized. Working solutions of defensins were prepared in 0.01% acetic acid, and their purity was evaluated by acid-urea–polyacrylamide gel electrophoresis (PAGE) (21) and sodium dodecyl sulfate-PAGE.

Human neutrophil peptides. Synthetic human defensin 1 (human neutrophil peptide 1 [HNP-1]) was purchased from Peptide Institute, Inc., Osaka, Japan. The peptide was dissolved in 0.01% acetic acid solution before use.

Bacteria and growth conditions. *S. aureus* 209P (JCM 2151) was obtained from the Japan Collection of Microorganisms. The organism was cultivated for 18 h at 37°C in trypto-soy broth (Eiken), and a portion of this culture was diluted 1:50 with fresh trypto-soy broth and incubated for 3 h at 37°C to provide mid-logarithmic-phase organisms. The concentration of CFU per milliliter was quantified by spectrophotometry at 600 nm, with reference to previously determined standards.

Antibacterial assays. The assay mixture contained approximately 10^5 or 10^8 CFUs and a specific concentration of defensins in a final volume of 100 μl of 10 mM sodium phosphate buffer (pH 7.4) without any nutrient. The control mixture contained 0.01% acetic acid instead of the defensin solution. Incubations were carried out in a shaking water bath for up to 120 min at 37°C. At timed intervals, aliquots of the incubation mixtures were diluted, and colony counts on trypto-soy agar (Eiken) were performed in duplicate.

Preparation of staphylococcal protoplasts and treatment with defensins. Staphylococcal protoplasts were prepared by a method described earlier (28). These protoplasts were treated with defensins (200 μg/ml) in Tris-HCl buffer (0.05 mol/liter; pH 7.4) containing NaCl (0.045 mol/liter) for 20 min at 37°C. The mixture contained 30% sucrose (final concentration) to stabilize the protoplasts.

Preparations for transmission electron microscopy. Approximately 10^8 CFU of *S. aureus* 209P cells was treated with purified rat defensins (either 600 μg/ml or 3.2 mg/ml) and HNP-1 (200 μg/ml) in 10 mM sodium phosphate buffer (pH 7.4), and the mixtures containing each concentration of defensins were kept for 20 and 120 min at 37°C. In experiments to ascertain the effects of incubation temperature and ionic strength on morphological changes caused by defensins,

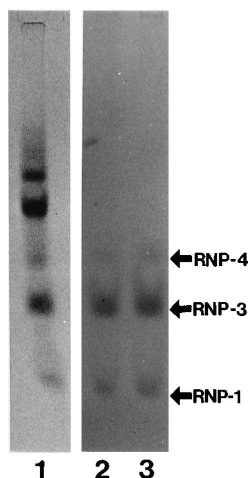

FIG. 1. Acid-urea–PAGE of rat defensins. Lanes: 1, 50 μg of rat neutrophil extract; 2, 10 μg of purified defensins; 3, 15 μg of purified defensins. Arrows indicate rat neutropeptides 1, 3, and 4 (RNP-1, -3, and -4).

assay mixtures were incubated at 0°C and supplemented with 140 mM NaCl, respectively. Protoplasts were prepared and treated with defensins as described above. After being treated with defensins, organisms were fixed with 1% glutaraldehyde and postfixed in 1% osmium tetroxide in 0.05 M cacodylate buffer (pH 7.2). The agar blocks were dehydrated in a graded series of alcohols. The organisms were embedded in Spurr resin (TAAB Co., Aldermaston, England) at 70°C. Ultrathin sections (thickness, 60 to 90 nm) were doubly stained with uranyl acetate and lead citrate.

Statistics. Results are presented as means ± standard deviations of five to six independent experiments. Data were evaluated by analysis of variance, and multiple comparisons were performed by the method of least significant difference. A level of $P < 0.05$ was accepted as statistically significant.

RESULTS

Purity of peptides. Acid-urea–PAGE revealed that the fraction used in this study contained three major cationic peptides that were designated rat neutropeptides 1, 3, and 4 (arrows in Fig. 1). This finding agrees with a previous report (4).

Effects of defensins on viability of *S. aureus*. The bactericidal activities of purified rat defensins and of the synthetic defensin, HNP-1, against *S. aureus* 209P were similar when tested at 50 μg/ml under our incubation conditions (Fig. 2). In electron microscopic studies, we tested the bactericidal activity of purified defensins against 10^8 CFU of bacteria per ml (Fig. 3). *S. aureus* (10^8 CFU/ml) exhibited no loss in viability until the concentration of defensins exceeded 1.6 mg/ml. These results suggested that the bactericidal activity of defensins was dependent on the size of the inoculum of target cells. We chose two concentrations of rat defensins, 600 μg/ml and 3.2 mg/ml, for use in electron microscopic observations of bacteria.

Ultrastructural changes in *S. aureus* 209P after treatment with defensins. The appearance of bacteria cultured without defensins is shown in Fig. 4A. In defensin-free mixtures, we observed bacterial populations with a substantially preserved morphology. Figures 4B to D showed the characteristic morphological changes of bacteria treated with defensins for 2 h. In the presence of purified rat defensins, staphylococci showed structural changes mainly at the cell membrane level. Some portions of the membrane were lamellar mesosome-like structures (arrows in Fig. 4B and C), and some peeled off the cell walls (arrow in Fig. 4D). These membranous structures were prominent at the periphery of the cells. The cytoplasmic membranes of approximately 25% of the defensin-treated cells were distorted. There were no leaking or burst cells when bacteria were treated for 2 h with defensins at 600 μg/ml. After 20 min of treatment with this concentration of defensins, these membranous changes were observed, but they did not occur when incubation lasted only 5 min. A difference in the complexity of mesosome-like structures was observed when two cell populations were treated with different concentrations of defensins; cells treated with 3.2 mg/ml showed more complex structural changes in the cytoplasmic membrane than did those treated with 600 μg/ml. Fewer than 10% of untreated *S. aureus* cells possessed simple mesosome-like structures. The mesosome-like structures of defensin-treated bacteria were obviously more complex and more frequently observed than were those of untreated bacteria, and the peeled cytoplasmic membrane

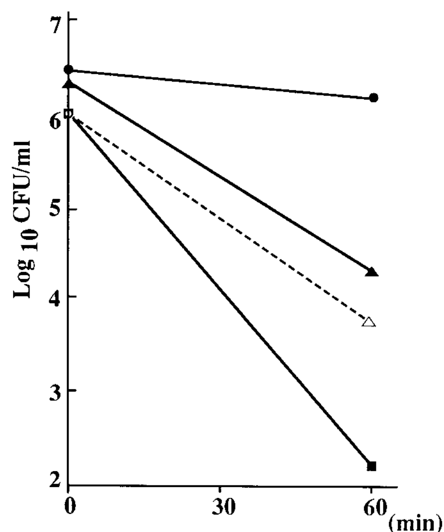

FIG. 2. Bactericidal activities of rat defensins and HNP-1 against *S. aureus* 209P. Mid-log-phase organisms (10^6 CFU/ml) were incubated with various concentrations of purified rat defensins (solid lines) and HNP-1 (dashed line) (50 μg/ml) in 10 mM sodium phosphate buffer (pH 7.4) for 60 min at 37°C. Symbols: ●, control; ▲ and △, 50 μg/ml; ■, 100 μg/ml.

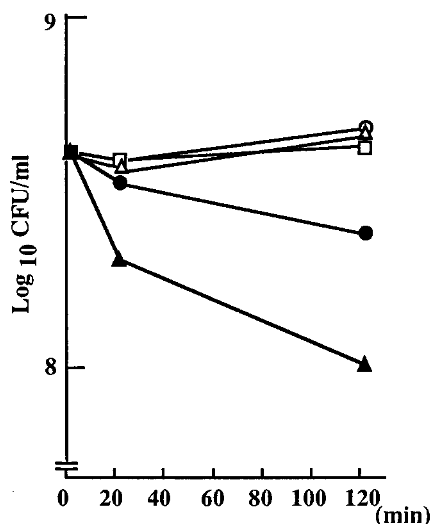

FIG. 3. Bactericidal activities of rat defensins against *S. aureus* 209P. Mid-log-phase organisms (10^8 CFU/ml) were incubated with various concentrations of rat defensins in 10 mM sodium phosphate buffer (pH 7.4) at 37°C. Symbols: ○, control; △, 400 μg/ml; □, 800 μg/ml; ●, 1.6 mg/ml; ▲, 3.2 mg/ml.

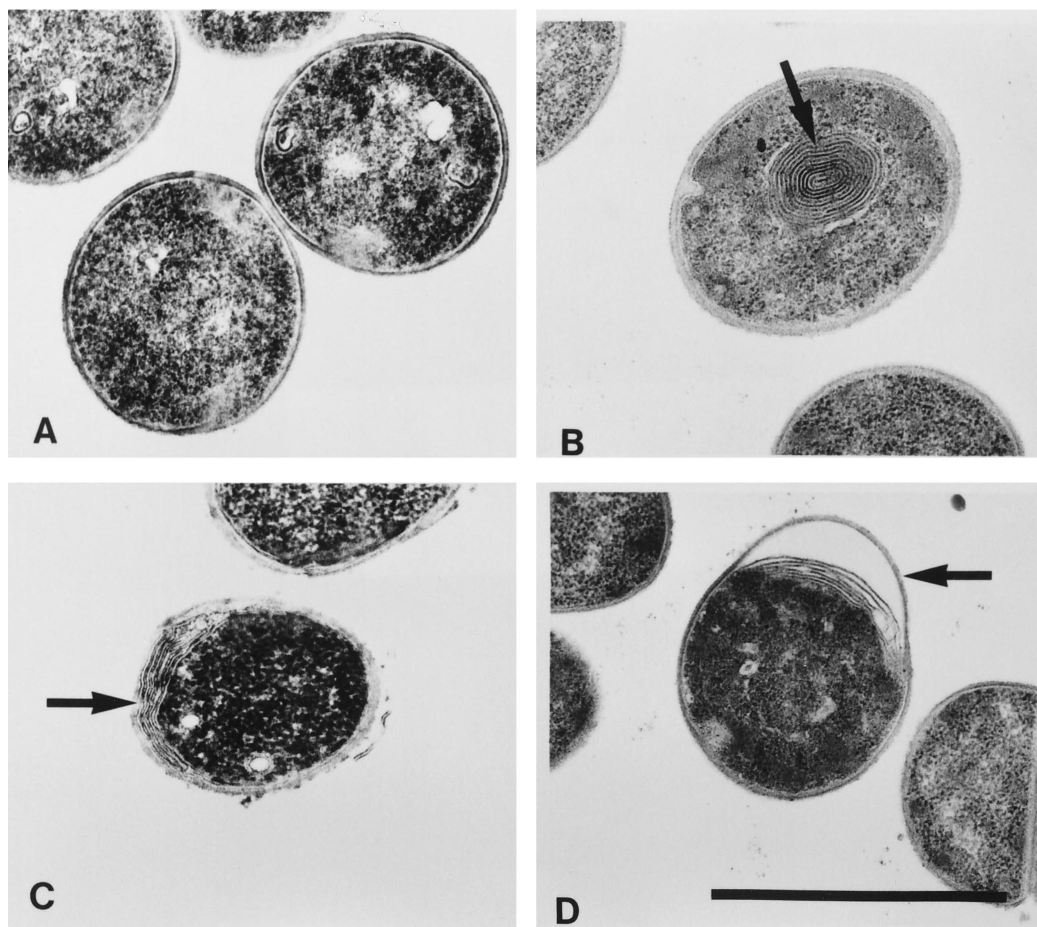

FIG. 4. Ultrastructure of untreated and defensin-treated *S. aureus*. (A) Untreated control; (B to D) bacteria treated with defensins (600 μg/ml) for 120 min. Bar = 1 μm.

was not observed in untreated cells. These findings indicate that the mesosome-like structures were not fixation artifacts but reflected the effects of defensins. The same strain treated with synthetic human defensin (HNP-1) showed the same ultrastructural changes that had been observed when the bacteria were treated with purified rat defensins (Fig. 5). The mesosome-like structures occupied about one-third of the area of defensin-treated bacteria (arrows in Fig. 5). Although we found no reduction of colony formation in cells treated with defensins at 600 μg/ml, more than 20% of bacteria exposed to this concentration of defensins showed the characteristic membranous changes. Although colony counts of cells treated with defensins at 3.2 mg/ml showed a 77% reduction after 120 min of treatment (Fig. 3), the frequency of occurrence of cells with remarkable membranous changes was the same as that found with defensins at 600 μg/ml.

Effects of ionic strength and incubation temperature on morphological changes caused by defensins. The ability of rat defensins to kill *S. aureus* used in our experiments depended on ionic strength and incubation temperature; the bactericidal activity of defensins was inhibited by an increased concentration of NaCl and by a low incubation temperature (data not shown). These results were consistent with previous reports (25, 26). The effects of these inhibitory conditions on the membranous changes caused by defensins are shown in Fig. 6. Whereas the addition of 140 mM NaCl completely inhibited the occurrence of the membranous changes, a low incubation temperature was less inhibitory.

Ultrastructural changes in staphylococcal protoplasts treated with defensins. Staphylococcal protoplasts were prepared from *S. aureus* in an effort to visualize the direct effect of defensins on the cytoplasmic membranes of bacteria. Defensins nearly broke the protoplasts directly (Fig. 7B). No bleb was seen on the membrane surfaces, nor was separation of the double structure of the membranes (28) observed under our conditions. The mesosome-like structures that had been seen in defensin-treated whole bacteria also were not observed.

DISCUSSION

In this report, we demonstrated remarkable changes in the ultrastructure of defensin-treated *S. aureus*. Transmission electron microscopy of defensin-treated whole bacteria showed remarkable changes in the cytoplasmic membrane. Some portions of the membranes were folded like mesosomes, but changes in the cell walls were not as prominent as those observed in staphylococci exposed to some antibiotics. Beta-lactam antibiotics that inhibit cell wall synthesis cause marked thickening of the cross walls of staphylococci (19). Chloramphenicol, tetracycline, and rifampin produce thickening of the peripheral cell walls (19); all three drugs inhibit protein synthesis that is related to cell wall synthesis. From our observa-

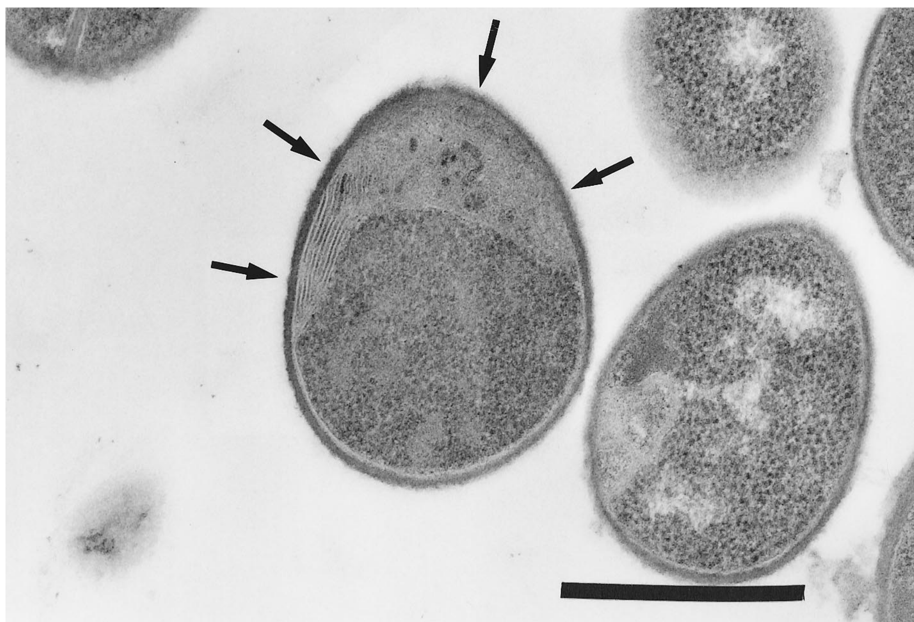

FIG. 5. Ultrastructure of *S. aureus* treated with HNP-1 (200 μg/ml) for 120 min. Bar = 0.5 μm.

tions, defensins do not seem to inhibit peptidoglycan and protein synthesis related to cell wall synthesis. The relationship between the morphological changes caused by defensins and the viability of defensin-treated bacteria is very interesting. Lehrer et al. (15) reported that the synchronous inhibition of RNA, DNA, and protein synthesis in defensin-treated *Escherichia coli* was a consequence of inner membrane permeabilization and that permeabilization of the inner membrane was essential for bacterial death. They also reported morphological

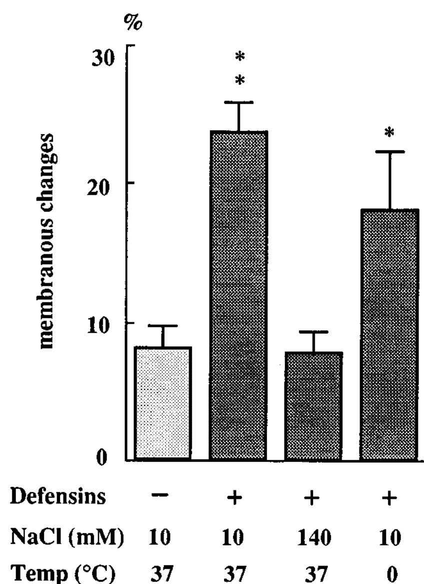

FIG. 6. Effects of ionic strength and incubation temperature on morphological changes caused by defensins. *S. aureus* (10^8 CFU/ml) was incubated for 2 h with or without defensins (600 μg/ml) under the conditions described in the text. The percentage of cells that possessed a mesosome-like structure is shown (1,000 cells were examined in each group). The data are expressed as means ± standard deviations of five to six independent experiments. *, $P < 0.005$; **, $P < 0.0001$ compared with the defensin-free control.

alterations of defensin-treated *E. coli*. Namely, *E. coli* that had been killed by defensins showed the presence of striking electron-dense deposits in the periplasmic space and on the surface of their outer membranes. The authors suggested that loss of bacterial viability was followed by the appearance of these morphological alterations and that posthumously bound defensins might be involved in these deposits. They reported no mesosome-like changes in defensin-treated *E. coli*. In our study, more than 20% of defensin-treated *S. aureus* cells showed mesosome-like structures, without a reduction in colony formation. In contrast to previous observations, our findings for defensin-treated *S. aureus* suggest that membranous changes, such as a mesosome-like structure, may appear before the bacteria lose their viability, and it is likely that membranous changes per se are not lethal for staphylococci. Additionally, our findings suggest the presence of heterogeneity of subpopulations with respect to susceptibility to defensins, even in a single strain. The size of this sensitive subpopulation was not proportional to the duration of exposure to defensins. In this study, we also demonstrated that defensin-mediated morphological changes were completely inhibited by increasing the concentration of NaCl in the incubation medium but were relatively independent of a lowering of the incubation temperature. These results differed in part from those of previous studies which showed that the bactericidal and candidacidal effects of defensins were markedly inhibited by an increased NaCl concentration and a low incubation temperature (25, 26). Lehrer et al. (16) reported that the rabbit peptide neutropeptide 1 bound to *Candida albicans* with biphasic kinetics. The primary phase of binding was temperature independent and occurred even at 0°C but was inhibited by an increase of the salt concentration in the incubation medium. This primary binding did not directly affect cell death. The authors concluded that the secondary phase of binding mediated candidacidal activity. This secondary phase is temperature dependent. The effects of NaCl concentration and low temperature on the primary phase were similar to our results of morphological studies. On the basis of those findings and our observations, we speculate that the first target of defensins is the

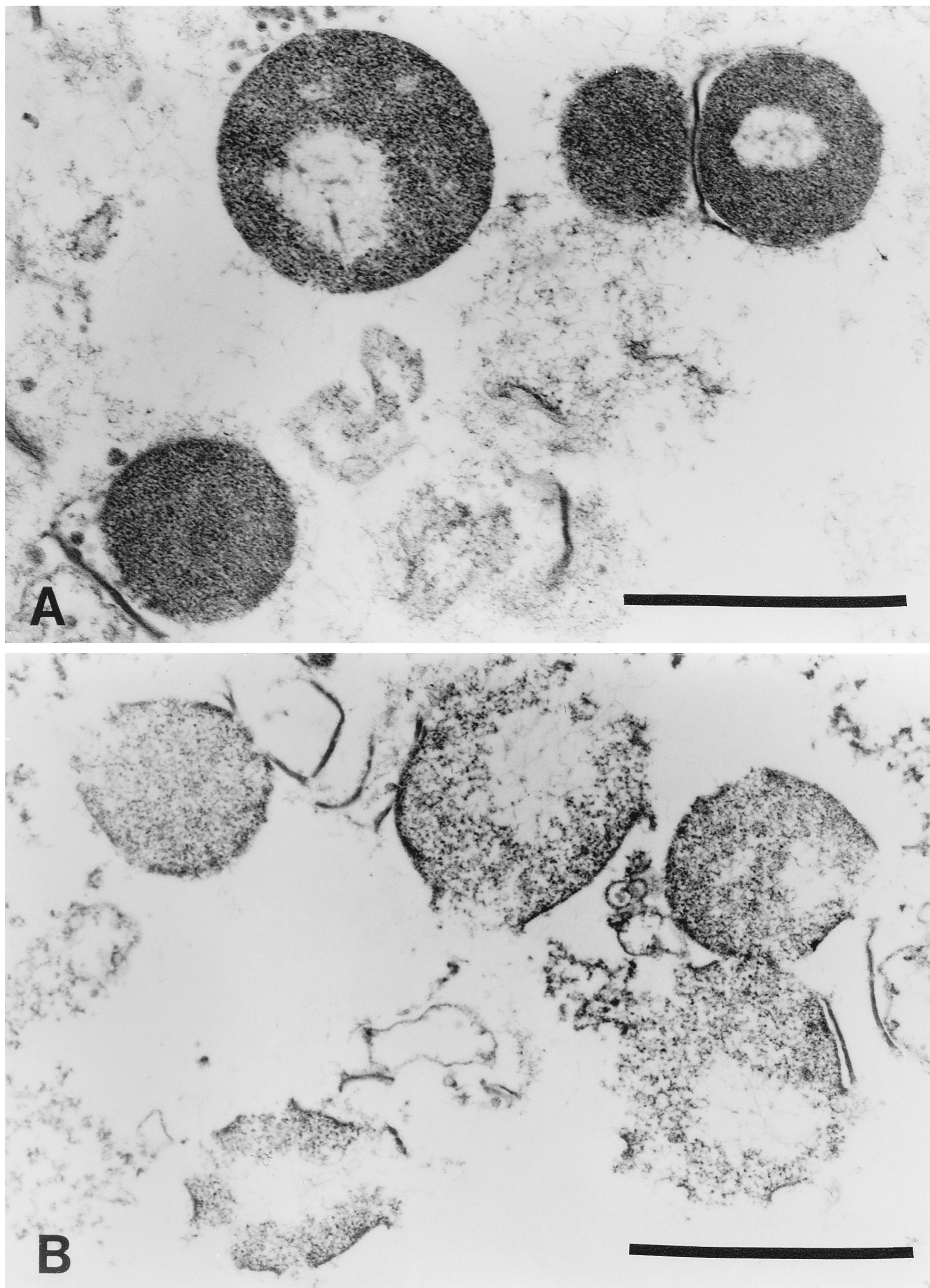

FIG. 7. Ultrastructure of staphylococcal protoplasts. (A) Untreated protoplasts; bar = 1 μm. (B) Protoplasts treated with defensins (200 μg/ml) for 20 min; bar = 1 μm.

cytoplasmic membrane of *S. aureus* and that defensins do not exert a direct effect on the bacterial cell wall.

In view of the ability of the defensins to injure the DNA of target cells (8), it is interesting that such inhibitors of nucleic acid synthesis as rifampin (9), trimethoprim (20), and mitomycin (20) induce mesosome-like structures in *S. aureus* similar to those observed in defensin-treated bacteria.

Walton demonstrated characteristic blebs on both the inner and outer membrane surfaces of spheroplasts exposed to cationic fractions purified from rabbit PMN (28). In our study, however, rat defensins did not induce such structural changes in protoplasts. In the presence of defensins, most of the protoplasts were broken under our conditions, and only bursting cells and fragments of the cells were observed. We did not observe ultrastructural changes in the double membrane of the defensin-treated protoplasts.

This appears to be the first report to describe the morphological changes in *S. aureus* produced by defensins. Our results support previous reports that the main targets of defensins are the cytoplasmic membranes (5, 12, 14, 17).

ACKNOWLEDGMENTS

We thank Kazunobu Amako and Akiko Umeda (Department of Bacteriology, Faculty of Medicine, Kyushu University) for helpful suggestions and Shinichi Nakahara and Toshimi Tabata for skilled technical assistance.

These studies were supported in part by a grant from Yakult Honsha Co. Ltd., Tokyo, Japan.

REFERENCES

1. **Alcouloumre, M. S., M. A. Ghannoum, A. S. Ibrahim, M. E. Selsted, and J. E. Edwards, Jr.** 1993. Fungicidal properties of defensin NP-1 and activity against *Cryptococcus neoformans* in vitro. Antimicrob. Agents Chemother. **37:**2628–2632.
2. **Daher, K. A., M. E. Selsted, and R. I. Lehrer.** 1986. Direct inactivation of viruses by human granulocyte defensins. J. Virol. **60:**1068–1074.
3. **Eisenhauer, P. B., S. S. L. Harwig, and R. I. Lehrer.** 1992. Cryptdins: antimicrobial defensins of the small intestine. Infect. Immun. **60:**3556–3565.
4. **Eisenhauer, P. B., S. S. L. Harwig, D. Szklarek, T. Ganz, M. E. Selsted, and R. I. Lehrer.** 1989. Purification and antimicrobial properties of three defensins from rat neutrophils. Infect. Immun. **57:**2021–2027.
5. **Fujii, G., M. E. Selsted, and D. Eisenberg.** 1993. Defensins promote fusion and lysis of negatively charged membranes. Protein Sci. **2:**1301–1312.
6. **Ganz, T.** 1987. Extracellular release of antimicrobial defensins from human polymorphonuclear leukocytes. Infect. Immun. **55:**568–571.
7. **Ganz, T., M. E. Selsted, D. Szklarek, S. S. L. Harwig, K. A. Daher, D. F. Bainton, and R. I. Lehrer.** 1985. Defensins: natural peptide antibiotics of human neutrophils. J. Clin. Invest. **76:**1427–1435.
8. **Gera, J. F., and A. Lichtenstein.** 1991. Human neutrophil peptide defensins induce single strand DNA breaks in target cells. Cell. Immunol. **138:**108–120.
9. **Gottfredsson, M., H. Erlendsdottir, R. Kolka, A. Gudmundsson, and S. Gudmundsson.** 1993. Ultrastructural alterations of bacteria during the post-antibiotic effect. Chemotherapy **39:**153–162.
10. **Jones, D. E., and C. L. Bevins.** 1992. Paneth cells of the human small intestine express an antimicrobial peptide gene. J. Biol. Chem. **267:**23216–23225.
11. **Jones, D. E., and C. L. Bevins.** 1993. Defensin-6 mRNA in human Paneth cells: implications for antimicrobial peptides in host defense of the human bowel. FEBS Lett. **315:**187–192.
12. **Kagan, B. L., M. E. Selsted, T. Ganz, and R. I. Lehrer.** 1990. Antimicrobial defensin peptides from voltage-dependent ion-permeable channels in planar lipid bilayer membranes. Proc. Natl. Acad. Sci. USA **87:**210–214.
13. **Kohashi, O., T. Ono, K. Ohki, T. Soejima, T. Morita, A. Umeda, Y. Meno, K. Amako, S. Funakoshi, M. Maeda, and N. Fujii.** 1992. Bactericidal activities of rat defensins and synthetic rabbit defensins on staphylococci, *Klebsiella pneumoniae* (chedid, 277, and 8N3), *Pseudomonas aeruginosa* (mucoid and nonmucoid strains), *Salmonella typhimurium* (Ra, Rc, Rd, and Re of LPS mutants) and *Escherichia coli*. Microbiol. Immunol. **36:**369–380.
14. **Lehrer, R. I., A. Barton, K. A. Daher, S. S. L. Harwig, T. Ganz, and M. E. Selsted.** 1989. Interaction of human defensins with *Escherichia coli*: mechanism of bactericidal activity. J. Clin. Invest. **84:**553–561.
15. **Lehrer, R. I., K. A. Daher, T. Ganz, and M. E. Selsted.** 1985. Direct inactivation of viruses by MCP-1 and MCP-2, natural peptide antibiotics from rabbit leukocytes. J. Virol. **544:**467–472.
16. **Lehrer, R. I., D. Szklarek, T. Ganz, and M. E. Selsted.** 1985. Correlation of binding of rabbit granulocyte peptides to *Candida albicans* with candidacidal activity. Infect. Immun. **49:**207–211.
17. **Lichtenstein, A.** 1991. Mechanism of mammalian cell lysis mediated by peptide defensins. Evidence for an initial alteration of the plasma membrane. J. Clin. Invest. **88:**93–100.
18. **Lichtenstein, A., T. Ganz, M. E. Selsted, and R. I. Lehrer.** 1986. In vitro tumor cell cytolysis mediated by peptide defensins of human and rabbit granulocyte. Blood **68:**1407–1410.
19. **Lorian, V., and C. G. Gemmell.** 1991. Effect of low antibiotic concentrations on bacteria: effects on ultrastructure, virulence, and susceptibility to immunodefenses, p. 493–555. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore.
20. **Nishino, T., T. Wecke, D. Kruger, and P. Giesbrecht.** 1987. Trimethoprim-induced structural alterations in *Staphylococcus aureus* and recovery of bacteria in drug-free medium. J. Antimicrob. Chemother. **19:**147–159.
21. **Panyim, S., and R. Chalkley.** 1969. High resolution acrylamide gel electrophoresis of histones. Arch. Biochem. Biophys. **130:**337–346.
22. **Patterson-Delafield, J., R. J. Martinez, and R. I. Lehrer.** 1980. Microbicidal cationic protein in rabbit alveolar macrophages: a potential host defense mechanism. Infect. Immun. **30:**180–192.
23. **Sawyer, J. G., N. L. Martin, and R. E. W. Hancock.** 1988. Interaction of macrophage cationic proteins with the outer membrane of *Pseudomonas aeruginosa*. Infect. Immun. **56:**693–698.
24. **Selsted, M. E., and S. S. L. Harwig.** 1987. Purification, primary structure, and antimicrobial activities of a guinea pig neutrophil defensin. Infect. Immun. **55:**2281–2286.
25. **Selsted, M. E., D. Szklarek, T. Ganz, and R. I. Lehrer.** 1985. Activity of rabbit leukocyte peptides against *Candida albicans*. Infect. Immun. **49:**202–206.
26. **Selsted, M. E., D. Szklarek, and R. I. Lehrer.** 1984. Purification and antibacterial activity of antimicrobial peptides of rabbit granulocyte. Infect. Immun. **45:**150–154.
27. **Viljasen, P., P. Koski, and M. Vaara.** 1988. Effect of small cationic leukocyte peptides (defensins) on the permeability barrier of the outer membrane. Infect. Immun. **56:**2324–2329.
28. **Walton, E.** 1978. The preparation, properties and action on *Staphylococcus aureus* of purified fractions from the cationic proteins of rabbit polymorphonuclear leukocytes. Br. J. Exp. Pathol. **59:**416–431.