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BACITRACIN-INDUCED CHANGES IN BACTERIAL PLASMA MEMBRANE STRUCTURE

UWE B. SLEYTR, THONDA C. OLIVER and KAREEN J.I. THORNE

Strangeways Research Laboratory, Wort's Causeway. Cambridge, CB1 4RN (U.K.)

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Summary

Bacitracin interacts with the plasma membranes of gram-positive and gram-negative bacteria to produce morphological changes which appear as rods, 25—35 nm in diameter, and of variable length, in freeze fractured preparations.

Bacitracin is an antibiotic and its main component is a cyclic polypeptide with a peptide side chain [8]. It inhibits bacterial cell wall biosynthesis by forming a complex with C_{55} prenol pyrophosphate and thereby preventing the regeneration of the prenol monophosphate required for peptidoglycan, lipopolysaccharide and polysaccharide biosynthesis [5, 8]. Since C_{55} prenol and its derivatives are located in the plasma membrane [9, 10] it seems likely that this is the site of bacitracin action. We therefore examined the effect of bacitracin on the appearance of the plasma membrane in freeze-etched preparations of gram-negative and gram-positive bacteria, including mesophilic and thermophilic species.

Staphylococcus aureus (NCTC 6571), *Acinetobacter* sp. MJT/F5/199A (NCIB 10885) and *Escherichia coli* (ML 30) were grown on a shaker in Difco heart infusion broth at 25—30°C as described previously [11]. *Clostridium thermosaccharolyticum* D 120-70 and *C. thermohydrosulfuricum* L 111-69 were grown in TSE medium at 60 and 70°C respectively [2]. Bacitracin (Sigma, Kingston-upon-Thames, Surrey, KT2 7BH, England) was added during the logarithmic phase of growth at a concentration of 100 μg/ml, except for *E. coli* which was treated with 5 mg/ml. Incubation varied from 1 to 2 h. For freeze-etching, cells were harvested, before or after bacitracin treatment, by centrifuging for 15 min at 5000 x *g*. The cells were either frozen immediately, or after 1.5 h infiltration with 25—30% glycerol, and examined as described earlier [7]. Erythrocytes from human blood were prepared by the method of Dodge et al. [1].

Logarithmically grown cells of all the species of bacteria examined showed

the typical concave and convex plasma membrane fracture faces found in most bacteria [4] whether or not they were pretreated with glycerol. Gram-negative bacteria also fractured in a plane in the outer membrane when glycerol was present [7]. In all the species of bacteria examined bacitracin treatment induced the formation of randomly distributed "rod-like" structures in the plasma membrane. These were seen as invaginations on the concave (p͡m) and elevations on the convex (p͡m) plasma membrane fracture face (Figs. 1—5). The diameter of these rods was remarkably uniform, measuring 25 to 35 nm, whereas their lengths varied from 80—400 nm within a single cell. These "rod-like" structures were devoid of typical membrane particles on both fracture faces. Especially in *C. thermosaccharolyticum* and *C. thermohydrosulfuricum*, the rods showed a tendency to align into closely-packed parallel formations (Figs. 4, 5). Higher concentrations of bacitracin were required to produce these structures in *E. coli* than in other bacteria; this was consistent with the high level of 5 mg/ml required to inhibit the growth of *E. coli*. Rod-like structures were not seen in the outer membranes of gram-negative bacteria (Fig. 3b).

Rod-shaped structures have so far only been observed in bacterial plasma membranes in *Micrococcus denitrificans*, *M. halodenitrificans* [6] and *M. diversus* [3]. Although these rods have similar demensions to those produced by bacitracin-treatment they represent invaginations and not extrusions and are observed in the plasma membranes of normal, untreated cells.

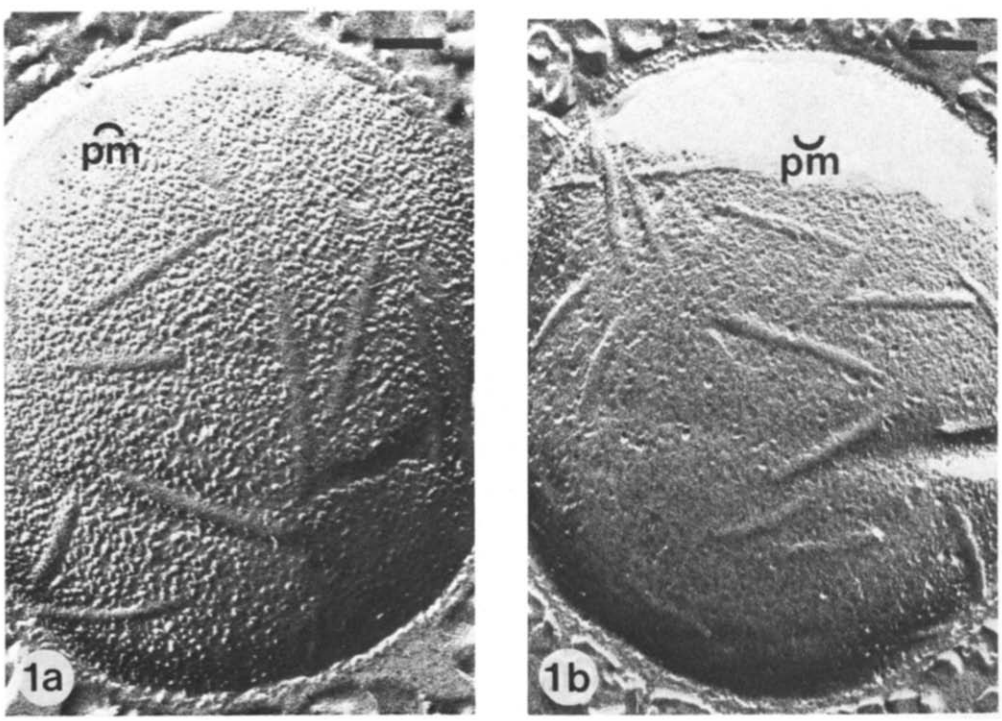


Fig. 1. Fracture faces of the plasma membrane of *Staphylococcus aureus* grown with bacitracin (100 μg/ml for 2 h). (a) Convex fracture face (p͡m). Numerous randomly oriented "rod-like" elevations devoid of membrane particles are present. (b) complementary fracture face to a. The rod-like structures appear as invaginations on the concave fracture face (p͡m) of the plasma membrane. Bar 100 nm.

Fig. 2. *E. coli* grown with bacitracin (5 mg/ml for 1 h). Convex fracture face of the plasma membrane (p͡m) with rod-like elevations. Bar 100 nm.

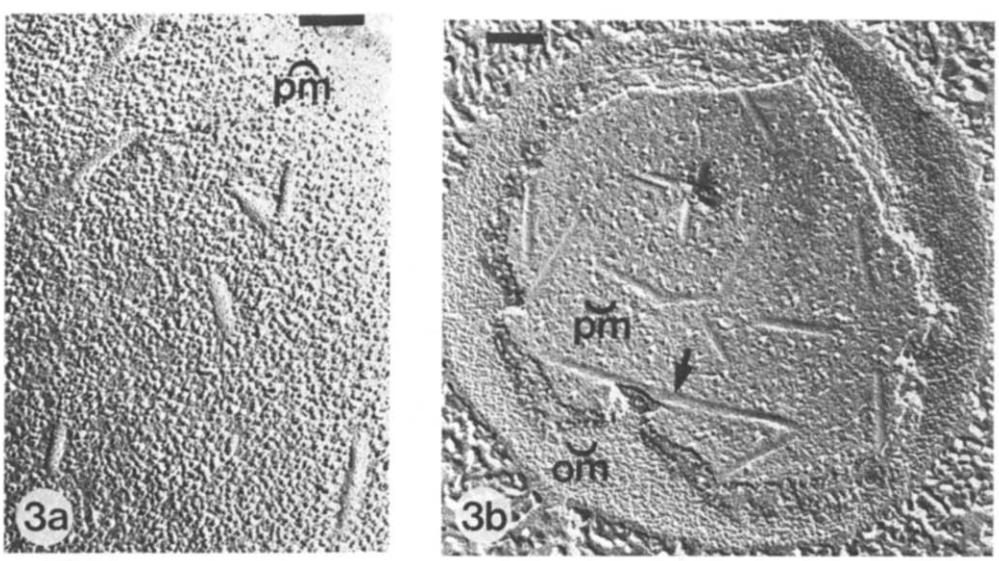
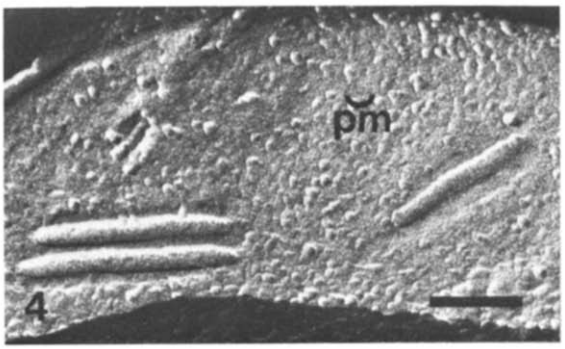


Fig. 3. *Acinetobacter* sp. grown with bacitracin (100 μg/ml for 2 h). (a) Convex fracture face (p͡m) with rod-like elevations. (b) Concave fracture face of the plasma membrane (p͡m) and outer membrane (o͡m). Note the long rod on the plasma membrane (arrow) and the absence of rods in the outer membrane. Bar 100 nm.

When isolated erythrocytes (Fig. 6) and corneal fibroblasts (not illustrated) were incubated with bacitracin (300 μg/ml) no rod-like structures were seen in any of the membranes. Since bacitracin leads to the appearance of rods in all the bacterial species examined we assume that this is a general phenomenon characteristic of bacterial plasma membranes. The observation that the rods always represent elevations of the plasma membrane and not invaginations suggests that there is an asymmetric distribution of the membrane components involved in their formation.



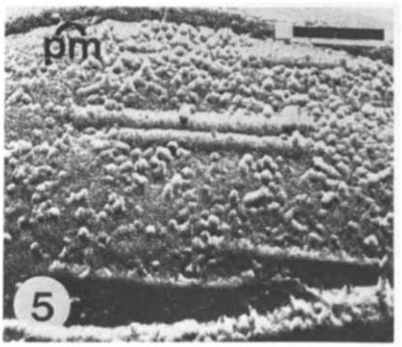
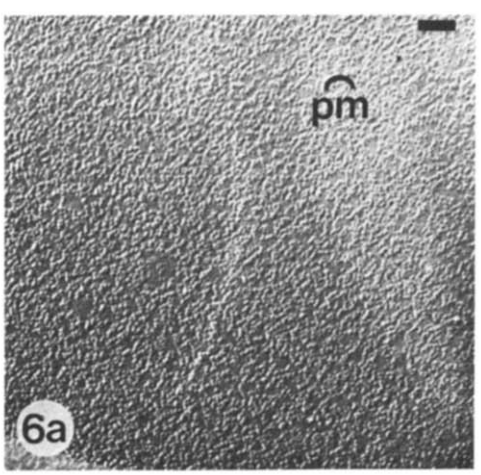


Fig. 4. Concave view of the plasma membrane (p͡m) of *Clostridium thermosaccharolyticum* grown with bacitracin (100 μg/ml for 2 h) showing parallel-aligned rod-like structures. Bar 100 nm.

Fig. 5. Convex fracture face of the plasma membrane (p͡m) of *C. thermohydrosulfuricum* grown with bacitracin (100 μg/ml for 2 h). Note the parallel rod-like elevations. Bar 100 nm.



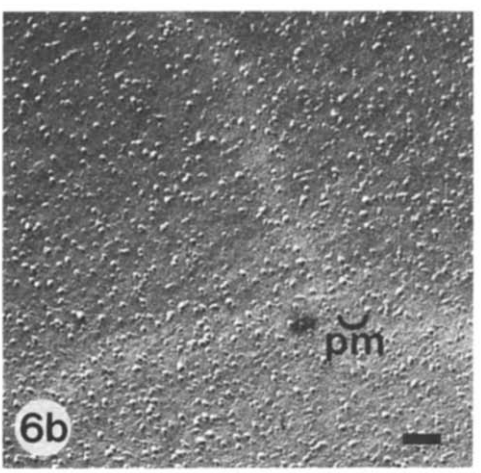


Fig. 6. Erythrocytes incubated in Dodge buffer [1] with bacitracin (300 μg/ml for 2 h) (a) convex (p͡m) and (b) concave (p͡m) fracture faces showing no rod-like elevations or invaginations, respectively. Bar 100 nm.

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