SHORT COMMUNICATION

Regular Arrangement of Wall Polymers in Staphylococci

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Concentric circular structures were observed on the newly exposed surface of the wall of *Staphylococcus epidermidis* and on its isolated cross-wall. These structures were removed by treatment with trichloroacetic acid. Chemical analysis revealed that after treatment with trichloroacetic acid most of the wall phosphorus was extracted but more than half of the *N*-acetylglucosamine remained associated with the wall. These observations suggest that polysaccharides are likely to be arranged circularly on the surface of the wall.

INTRODUCTION

Staphylococcal walls are composed of peptidoglycan, non-peptidoglycan polysaccharides, such as teichoic acids, teichuronic acids or neutral polysaccharides, and several species of proteins. Currently, little is known about the topological localization of these polymers in the wall.

The wall of *Staphylococcus* is synthesized as a cross-wall inside the cell. This cross-wall then splits in the middle and separates into two leaflets, each of which forms part of the wall of a newly generated daughter cell (Giesbrecht *et al.*, 1976). These two wall parts are discernible in electron micrographs (Amako & Umeda, 1977*a*).

Previously we have demonstrated the presence of concentric circular structures on the surface of a newly exposed cell (Amako & Umeda, 1978). The presence of these structures on the cell surface suggests there is a regular arrangement of the wall polymers in the cross-wall. In this paper, we describe the results of an electron microscopic examination of the cross-wall showing the regular circular structures and discuss the nature of these structures.

METHODS

Bacterial strain and growth conditions. *Staphylococcus epidermidis* strain KD (Amako & Umeda, 1978) was used throughout. It was grown in nutrient broth with continuous shaking at 37 °C for 6 h. The bacteria were then collected by centrifugation (2000 *g*, 30 min) and suspended in distilled water.

Preparation of wall fractions. The bacterial suspension was mixed vigorously with small glass beads in a Dyno-Mill cell disintegrator (Willy A. Bachofen, Basel, Switzerland) at 15 °C for 3 min. The glass beads and unbroken bacteria were removed by centrifugation (2000 *g*, 10 min). The wall fraction was then sedimented by centrifugation (10000 *g*, 20 min) and suspended in distilled water. To remove the membranes associated with the wall preparation, it was treated with 2 % (w/v) sodium dodecyl sulphate (SDS) at 37 °C for 30 min and then washed four times with distilled water (SDS-wall). To remove the wall polysaccharides other than peptidoglycan, the SDS-wall fraction was treated with 10 % (w/v) trichloroacetic acid (TCA) at 90 °C for 10 min (Tsien *et al.*, 1978) and then washed four times with distilled water (TCA-wall).

Chemical analyses. The phosphorus content of the wall preparations was determined by the method of Allen (1940), and the *N*-acetylglucosamine content was measured by a modification of the Morgan-Elson procedures (Reissig *et al.*, 1955).

Electron microscopy. Scanning electron microscopy (SEM) was done as described previously (Amako & Umeda, 1977*b*), the specimens being fixed only with a 1 % (w/v) solution of glutaraldehyde prepared in a 0.15 M-sodium cacodylate buffer. A Hitachi HFS-2 field emission source instrument was used.

For transmission electron microscopy (TEM), the wall fraction was stained by mixing with a 1.0 % (w/v) solution of uranyl acetate on an electron microscope grid covered with a carbon-coated Formvar film. Before use, the grid was rendered hydrophilic by glow discharging. Specimens were examined in a Hitachi HU-12A electron microscope.

The dimensions of the specimens were calibrated from the magnification. In the transmission-type electron microscope the lattice spacing of a catalase crystal was used as the internal standard for the magnification (Wrigley, 1968).

RESULTS AND DISCUSSION

The concentric circular structure on the newly exposed surface of a staphylococcal cell is shown in the scanning electron micrograph presented in Fig. 1(*a*). The circular structures were seen as complete circles on newly exposed surfaces, and about 20 such circles could be counted. The width of the 'line' of each circle was about 24 nm. On old surfaces, the structures were generally obscure and half circles rather than complete circles were seen. This is due to the fact that cell separation usually occurred along a plane which transects the centre of the circles (Fig. 1*b*). The wall is thought to grow and increase in thickness even after cell separation, thus obscuring the regular circular structure in aged walls.

The cross-walls present in SDS-wall fractions were examined by TEM after staining with uranyl acetate. A cross-wall could be easily recognized by its relative position in the wall preparation and by the presence of a central round or oval-shaped hole. The cross-wall in Fig. 1(*c*) shows similar concentric circular structures to those observed by SEM. Such circular structures were more clearly visible on cross-walls having a central hole than on completely closed walls.

It was difficult to count the number of circles on a plane of the cross-wall in these micrographs as only a part of each circle could be followed. Close examination revealed that each circle appeared to be a trilamellar structure, with an electron-transparent layer sandwiched between two electron-dense lines (arrowed in Fig. 1*c*). The width of this trilamellar 'line' was approximately 12 nm. From this width and the radius of the cross-wall, the number of the circles on a plane of the cross-wall was calculated to be about 40, suggesting that the circular structures observed by SEM might have been produced by the joining of two 'lines' on the cross-wall. The morphological differences between the circular structures observed by SEM, and those observed by TEM cannot be satisfactorily explained. Some of the chemical or physical treatment inflicted during the processing for SEM observation might have induced a regular aggregation of polymers on the cell surface.

The circular structures were removed from the cross-wall (Fig. 1*d*) and cell surface (not shown) by treatment with 10 % TCA at 90 °C. Chemical analysis of the wall polymers revealed that the SDS-wall contained 20 % (w/w) *N*-acetylglucosamine and 2.1 % (w/w) phosphorus, and that hot-TCA treatment extracted all the phosphorus and 42.5 % of the *N*-acetylglucosamine.

Examination by SEM of whole bacteria treated with 10 % TCA at 37 °C for 14 h showed that this milder acid treatment also removed the circular structures.

The concomitant removal of the circular structures and wall polysaccharides from the SDS-wall by TCA treatment suggests that wall polysaccharides such as teichoic acids or teichuronic acids are likely to be arranged circularly. Since these wall polymers are thought to be covalently linked to the peptidoglycan layer in the wall (Coley *et al.*, 1978), a circular arrangement of peptidoglycan in the wall is feasible.

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Fig. 1. Scanning electron micrographs of *Staphylococcus epidermidis* fixed with glutaraldehyde (a, b) and transmission electron micrographs of wall preparations stained with uranyl acetate (c, d). All bar markers represent 100 nm.

(a) The newly exposed surfaces of cells showing the concentric circular structures.

(b) An early stage of cell separation, showing separation occurring in a plane through the centre of the circular structure; thus in the second generation, the circular structure would be divided into half circles.

(c) The cross-wall in an SDS-wall preparation showing many concentric circles. The arrow indicates a 'line' where the trilamellar structure is visible.

(d) The cross-wall in a TCA-wall preparation; no circular structures are visible.

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