Peptidoglycan layer and disruption processes in *Bacillus subtilis* cells visualized using quick-freeze, deep-etch electron microscopy

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Peptidoglycan, which is the main component of the bacterial cell wall, is a heterogeneous polymer of glycan strands cross-linked with short peptides and is synthesized in cooperation with the cell division cycle. Although it plays a critical role in bacterial survival, its architecture is not well understood. Herein, we visualized the architecture of the peptidoglycan surface in Bacillus subtilis at the nanometer resolution, using quick-freeze, deep-etch electron microscopy (EM). Filamentous structures were observed on the entire surface of the cell, where filaments about 11nmwide formed concentric circles on cell poles, filaments about 13 nm wide formed a circumferential mesh-like structure on the cylindrical part and a 'piecrust' structure was observed at the boundary. When growing cells were treated with lysozyme, the entire cell mass migrated to one side and came out from the cell envelope. Fluorescence labeling showed that lysozyme preferentially bound to a cell pole and cell division site, where the peptidoglycan synthesis was not complete. Ruffling of surface structures was observed during EM. When cells were treated with penicillin, the cell mass came out from a cleft around the cell division site. Outward curvature of the protoplast at the cleft seen using EM suggested that turgor pressure was applied as the peptidoglycan was not damaged at other positions. When muropeptides were depleted, surface filaments were lost while the rod shape of the cell was maintained. These changes can be explained on the basis of the working points of the chemical structure of peptidoglycan.

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