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High-resolution Structure of the Major Periplasmic Domain from the Cell Shape-determining Filament MreC

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Bacterial cell shape is dictated by the cell wall, a plastic structure that must adapt to growth and division whilst retaining its function as a selectively permeable barrier. The modulation of cell wall structure is achieved by a variety of enzymatic functions, all of which must be spatially regulated in a precise manner. The membrane-spanning essential protein MreC has been identified as the central hub in this process, linking the bacterial cytoskeleton to a variety of cell wall-modifying enzymes. Additionally, MreC can form filaments, believed to run perpendicularly to the membrane. We present here the 1.2 Å resolution crystal structure of the major periplasmic domain of *Streptococcus pneumoniae* MreC. The protein shows a novel arrangement of two barrel-shaped domains, one of which shows homology to a known protein oligomerization motif, with the other resembling a catalytic domain from a bacterial protease. We discuss the implications of these results for MreC function, and detail the structural features of the molecule that may be responsible for the binding of partner proteins.

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Introduction

The bacterial cell wall is conceptually simple, yet is believed to be responsible for the widely differing shapes of bacteria.[1] The cell wall is mostly comprised of peptidoglycan (murein), a polymer of *N*-acetylglucosamine and *N*-acetylmuramic acid that gains its strength through a mesh-like network of peptide substituent crosslinks. The role of the cell wall as a strong, selectively permeable barrier must also be counterbalanced with the need for a malleable structure that adapts to respond to both cellular growth and division. Therefore, synthesis and insertion of new cell wall material needs to be tightly regulated, and it has been postulated that this may be achieved through a multi-protein peptidoglycan-processing holoenzyme.[2] The holoenzyme would need to contain hydrolytic enzymes to cleave existing cell wall material (e.g. lytic transglycosylases and endopeptidases) and polymerases to insert new cell wall material (e.g. monofunctional/bifunctional peptidoglycan glycosyltransferases and transpeptidases). Additionally, it is believed that peptidoglycan synthesis (and therefore the components of the holoenzyme) is regulated spatially, and that bacterial shape is governed by separate processes for peptidoglycan turnover along the length of the cell and at the division septum.[3]

A search for bacteria possessing defects in cell shape, but without mutation of the peptidoglycan-processing enzymes, led to the discovery of the mreBCD operon (murein region e genes).[4] The finding that mreB encoded a bacterial homolog of actin[5,6] led to a widespread reappraisal of bacterial physiology, and we are now beginning to understand how complex the prokaryotic cytoskeleton may be.[7] The MreC and MreD protein products have predicted transmembrane sequences, with a single span for MreC (Figure 1(a)) and five putative transmembrane helices for mreD. Mutations in MreC confer the same physiological effects as mutations in MreB, resulting in a breakdown of regular cell shape to a more spherical morphology.[8] Subsequent experiments have shown that MreC forms a helical periplasmic filament,[9,10] and interacts with a vast array of proteins, including itself, MreB, MreD, and PBP2.[10–12] Indeed, the mreBCD operon is often located in close proximity to genes responsible for peptidoglycan synthesis (PBP2) or putative cell wall precursor transport (rodA).[13] These observations have led to a model in which MreC bridges cytoplasmic and periplasmic ultrastructure, acts as a scaffold for proteins involved in peptidoglycan turnover, and ultimately governs the

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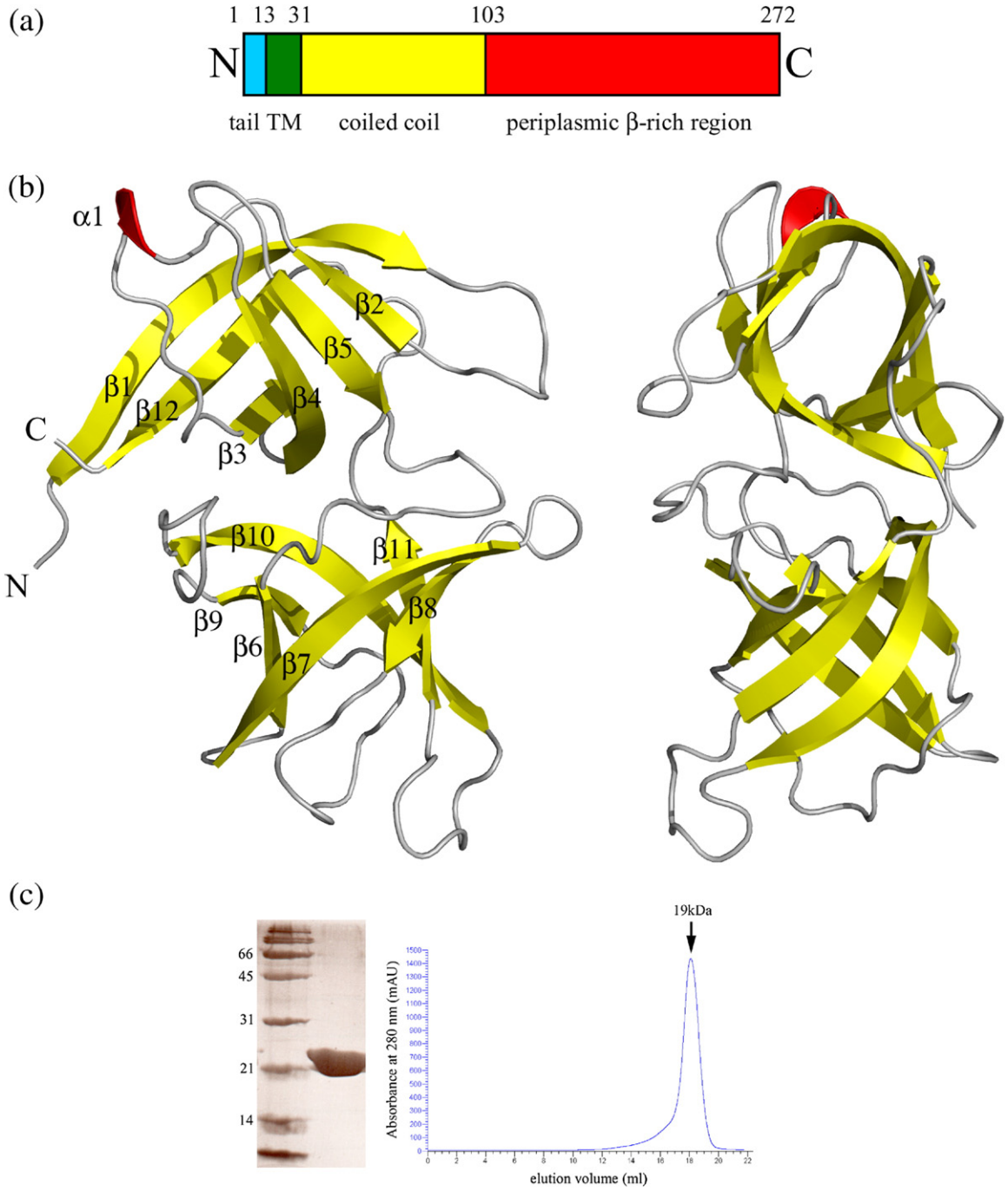

Figure 1. (a) Domain arrangement of *S. pneumoniae* MreC. The putative coiled coil region is located immediately after the transmembrane domain (TM), and contains typical leucine-rich heptad repeats, interspersed with three residue "stammer" insertions.[28] The construct crystallized in this study encompasses amino acid residues 104–272. (b) Overall fold of MreC major periplasmic domain. For clarity, two orthogonal views are shown. The structure is composed of two six-stranded β-barrels, joined at approximate right-angles. The fold inserts barrel 2 (β6 to β11) into a loop between strands 5 and 6 of the first barrel (β5 and β12, respectively). The domain is predominantly β-sheet, with a 3_{10} helix at residues 136–138, and a helical turn in the loop between β1 and β2. (c) SDS-PAGE and size-exclusion chromatography profile of the purified MreC construct. Lane 1, molecular mass markers (in kDa); lane 2, purified MreC construct. The observed approximate molecular mass of 19 kDa agrees with that of a monomer. Results with a longer construct (*S. pneumoniae* enzyme, residues 33–272, data not shown; the *L. monocytogenes* enzyme structure indicated a dimer, consistent with the hypothesis that the coiled coil region is responsible for MreC dimerization.[18]