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Biochemical Characterization of a Bacterial Outer Membrane Porin TdeA from Actinobacillus Actinomycetecomitans

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TolC is an outer membrane channel protein as an essential component of drug efflux and type-I secretion systems in gram negative bacteria. TolC comprises a periplasmic alpha-helical barrel domain and a membrane-embedded beta-barrel domain, which can be solubilized by detergents since it has a transmembrane domain. TdeA, a functional and structural homologue of TolC, is required for toxin and drug export in a pathogenic oral bacteria Actinobacillus actinomycetecomitans. Herein we report expression of the periplasmic alpha-helical domain of TdeA as a soluble protein by substitution of the membrane-embedded domain with soluble linkers, which enabled us to purify the protein in the absence of a detergent. We confirmed that the recombinant protein shows the expected three-dimensional structure by a size exclusion chromatography, circular dichroism, and electron microscopy. Subsequently, we reveal that the periplasmic domain of TdeA has a property intercalating into the bacterial cell wall component peptidoglycan, which suggests that peptidoglycan exhibits a vertically porous structure accommodating the alpha helical barrel domain of TolC or its homologues.

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Crystal Structure of the Periplasmic Component of Multidrug Efflux Pump from Gram-negative Bacteria

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Periplasmic membrane fusion proteins (MFPs) are essential components of the type I protein secretion system and drug efflux pump in Gram-negative bacteria. They play a crucial role in bridging between the outer membrane porin TolC(-like) protein and inner membrane transporter proteins. To date, two crystal structures were reported revealing that MFP consists of α-helical hairpin, lipoyl, and β-barrel domains. However, the functional form of MFP is poorly understood. MFP MacA is associated with the resistant to macrolides through MacA-MacB-TolC efflux pump in E.coli and through MacA-MacB-TdeA efflux pump in Actinobacillus Actinomycetemcomitans. Here, we report the crystal structure of A. actinomycetemcomitans MacA, in which MacA forms a hexameric assembly with a central channel lined by the long 12 α-helices in analogy to the periplasmic domain of TolC. The hexameric assembly of MacA explains how MFPs connect the inner membrane transporter and the outer membrane channel protein. Based on the hexamer-hexamer packing interaction observed in the crystal, we suggest a molecular mechanism on how MFPs open the channel of the outer membrane porin protein.