Tryptophan-mediated Dimerization of the TssL Transmembrane Anchor Is Required for Type VI Secretion System Activity

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Abstract

The type VI secretion system (T6SS) is a multiprotein complex used by bacteria to deliver effectors into target cells. The T6SS comprises a bacteriophage-like contractile tail structure anchored to the cell envelope by a membrane complex constituted of the TssJ outer-membrane lipoprotein and the TssL and TssM inner-membrane proteins. TssJ establishes contact with the periplasmic domain of TssM whereas the transmembrane segments of TssM and its cytoplasmic domain interact with TssL. TssL protrudes in the cytoplasm but is anchored by a C-terminal transmembrane helix (TMH). Here, we show that TssL TMH dimerization is required for the stability of the protein and for T6SS function. Using the TOXCAT assay and point mutations of the 23 residues of the TssL TMH, we identified Thr194 and Trp199 as necessary for TssL TMH dimerization. NMR hydrogen–deuterium exchange experiments demonstrated the existence of a dimer with the presence of Trp185 and Trp199 at the interface. A structural model based on molecular dynamic simulations shows that TssL TMH dimer formation involves π–π interactions resulting from the packing of the two Trp199 rings at the C-terminus and of the six aromatic rings of Tyr184, Trp185 and Trp188 at the N-terminus of the TMH.

Introduction

The type VI secretion system (T6SS) is a molecular machine widely distributed in proteobacteria and responsible for the contact-dependent secretion of effector proteins into target cells [1,2]. Effectors delivered by this apparatus interfere with eukaryotic host cell physiology, or cause damages in bacterial cells [3–6]. The T6SS is therefore involved in pathogenesis and/or bacterial competition. At the molecular level, the T6SS could be represented as a nano-speargun [7–9] (Fig. 1a): an inner tube made of stacked Hcp hexameric rings capped by the VgrG spike complex and wrapped by a contractile sheath is propelled into the target cells during sheath contraction [10–12]. This tubular structure is assembled on a sub-membrane platform—or baseplate—that comprises the TssEFGK and VgrG subunits and is anchored to the cell envelope by the membrane complex [13–18].

In enteroaggregative *Escherichia coli* (EAEC), the T6SS membrane core complex is constituted of the outer membrane-associated TssJ lipoprotein and the TssL and TssM inner-membrane proteins [14,19,20] (Fig. 1b and c). TssJ folds as a classical transthyretin with an additional α-helix and an extended loop connecting β-strands 1 and 2 (L1–2) [21–23]. TssM comprises three transmembrane helices (TMH): TMH1 and TMH2 form a hairpin located close to the N-terminus, separated from the third helix, TMH3, by a 35-kDa cytoplasmic domain [16,24] (Fig. 1c). The TssM cytoplasmic portion comprises two subdomains with structural homologies to NTPases and the DPY-30 dimerization hairpin [16]. The TssM cytoplasmic domain makes contact with TssL, as well as with TssG and TssK, two components of the tail assembly platform [13,15,16,18]. The majority of the EAEC TssM subunit (750 amino acids) is located in the periplasm and its C-terminal extremity interacts with