doi:10.1016/j.jmb.2010.08.050 J. Mol. Biol. (2010) 403, 270–285

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

TolA Modulates the Oligomeric Status of YbgF in the Bacterial Periplasm

Anne Marie Krachler, Amit Sharma, Anna Cauldwell, Grigorios Papadakos and Colin Kleanthous*

Department of Biology, University of York, Heslington, PO Box 373, York YO10 5YW, UK

Received 24 June 2010; received in revised form 18 August 2010; accepted 26 August 2010
Available online 15 September 2010

Edited by R. Huber

Keywords:
protein–protein interaction; periplasm; Tol–Pal complex; quaternary structure; outer membrane

The trans-envelope Tol complex of Gram-negative bacteria is recruited to the septation apparatus during cell division where it is involved in stabilizing the outer membrane. The last gene in the *tol* operon, *ybgF*, is highly conserved, yet does not seem to be required for Tol function. We have addressed this anomaly by characterizing YbgF from *Escherichia coli* and its interaction with TolA, which, based on previous yeast two-hybrid data, is the only known physical link between YbgF and the Tol system. We show that the stable YbgF trimer undergoes a marked change in oligomeric state on binding TolA, forming a one-to-one complex with the Tol protein. Through a combination of pull-down assays, deletion analysis, and isothermal titration calorimetry, we map the TolA–YbgF interface to the C-terminal tetratricopeptide repeat domain of YbgF and 31 residues at the C-terminal end of TolA domain II ($TolA^{280–313}$). We show that TolB, which binds TolA domain III close to the YbgF binding site, has no impact on the YbgF–TolA association. We also report the crystal structures of the two component domains of YbgF, the N-terminal coiled coil from *E. coli* YbgF, which forms a stable trimer and controls the oligomeric status of YbgF, and the monomeric tetratricopeptide repeat domain from *Xanthomonas campestris* YbgF, which is also able to trimerize. Although the coiled coil is not directly involved in TolA binding, we demonstrate that the regular hydrophilic patterning of its otherwise hydrophobic core is a prerequisite for the TolA-induced oligomeric-state transition of YbgF. We postulate that rather than YbgF affecting Tol function, it is the change in YbgF oligomeric status (with an accompanying change in its function) that likely explains the necessity for tight co-regulation of the *ybgF* and *tol* genes in Gram-negative bacteria.

*Corresponding author. E-mail address: ck11@york.ac.uk.

Present addresses: A. M., Krachler, Department of Molecular Biology, UT Southwestern Medical Center, 5323 Harry Hines, MC 9148, Dallas, TX 75390, USA; A. Sharma, Department of Biophysics, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA; A. Cauldwell, Centre for Molecular Microbiology and Infection, Imperial College, London SW7 2AZ, UK.

Abbreviations used: AUC, analytical ultracentrifugation; TPR, tetratricopeptide repeat; NTD, N-terminal domain (of YbgF); SEC-MALLS, size-exclusion chromatography–multi-angle laser light scattering; ITC, isothermal titration calorimetry; OM, outer membrane.

Introduction

The Tol–Pal system is a periplasmic protein complex transiently spanning the cell wall of Gram-negative bacteria in a proton motive force-dependent manner that is required for maintaining outer membrane (OM) integrity and correct septation during the late stages of cell division.[1,2] Consequently, deletion of *tol* genes or disruption of interactions between Tol proteins leads to loss of OM integrity, which is accompanied by increased susceptibility towards antibiotics, detergents, and killing of pathogenic strains by the host complement system, as well as aberrant cell division and concomitant cell chaining.[3–5] In addition, the Tol assembly is exploited by bacteriocins and filamentous phages that recruit either TolA or TolB to promote their uptake into the cytoplasm of host cells.[6–8] Thus, malfunctioning of the Tol system renders cells tolerant to bacteriocins and phage infection.

To ensure co-regulation of the Tol complex components, the proteins are encoded in an operon consisting of seven genes, *ybgC*, *tolQ*, *tolR*, *tolA*, *tolB*, *pal*, and *ybgF*. The five core components form two subcomplexes, an inner-membrane-bound TolQRA complex and an OM-anchored TolB–Pal complex.[9,10] TolA, which is thought to span the periplasm, transiently interacts with TolB, and this interaction is mutually exclusive of the TolB interaction with Pal at the OM. Hence, TolB is thought to shuttle between an active (TolA-bound) and an inactive (Pal-bound) state.[11] To date, no evidence for a functional role of *ybgC* or *ybgF* has been found. Neither YbgC nor YbgF (both of which are soluble proteins) is required for Tol functionality since deletion strains do not display the characteristic *tol* phenotype of OM perturbation, leakiness, or colicin tolerance.[12] YbgC, which is the least conserved of the Tol proteins and is expressed in the cytoplasm, is only present in the subgroup of γ-, δ-, and ε-proteobacteria (Fig. 1) and possesses thioesterase activity *in vitro*, but how this is related to the role of the Tol assembly remains to be elucidated.[13,14] No function has yet been identified for periplasmic YbgF, which is conserved in the *tol* operon in all five subgroups of proteobacteria and most other Gram-negative organisms with the exception of intracellular parasites such as *Chlamydiae* (Fig. 1).[13]

This leaves the question why these genes are retained within the *tol* operon. The only evidence of a functional link between YbgF and the Tol system was provided by yeast two-hybrid screening, which identified a putative interaction between YbgF and TolA.[15] However, this link remains to be validated. The aim of the present study was to characterize YbgF alone and in complex with TolA in more detail to shed light on the possible reason for their co-regulation. Here, we verify the interaction between TolA and YbgF *in vitro* and demonstrate that YbgF is a highly elongated protein that undergoes a dramatic change in oligomeric state on binding TolA. We also identify the interacting regions of the two proteins. We further report the X-ray structures of the two constituent domains of YbgF and describe their roles in TolA complex formation. Our findings suggest that TolA modulates the function of YbgF by controlling its oligomeric state.

Results

Interaction with TolA *in vitro* disrupts the quaternary structure of YbgF

Based on a yeast two-hybrid study testing pairwise interactions between members of the *tol* operon, a potential interaction between TolA and YbgF was identified by Walburger *et al.*[15] We set out to investigate this protein–protein interaction using a variety of biophysical methods. YbgF is a 25-kDa protein that forms a stable trimer in solution, as shown by chemical cross-linking, size-exclusion chromatography–multi-angle laser light scattering (SEC-MALLS), and analytical ultracentrifugation (AUC).[16] Sedimentation velocity experiments have also shown the protein to be an elongated molecule with a frictional ratio of 1.95 ± 0.08 (data not shown). The interaction between YbgF and TolA was first investigated using SEC-MALLS. TolA$^{74–421}$, corresponding to the two soluble periplasmic domains of TolA, TolAII–III, and excluding its transmembrane domain, eluted as a monomer in SEC-MALLS experiments (theoretical monomer mass, 36.5 kDa; detected mass, 32.8 ± 0.7 kDa; Fig. 2a) while YbgF eluted as a trimer as previously reported. When YbgF and TolA$^{74–421}$ were mixed at high concentration (~100 μM), they formed a stable complex. Surprisingly, however, the molar mass of the complex corresponded to that of a TolA$^{74–421}$/YbgF heterodimer (theoretical mass, 61.9 kDa; detected mass, 57.5 ± 1.2 kDa; Fig. 2a). Thus, these experiments not only verified the proposed interaction between YbgF and TolA *in vitro* but also demonstrated that binding to TolA disrupts the otherwise stable YbgF homotrimer.

We investigated the possibility that this unusual binding stoichiometry was specific to the *Escherichia coli* system by analyzing the equivalent complexes from *Salmonella enterica* serovar Typhimurium (90% and 80% similarity to *E. coli* YbgF and TolA, respectively) and *Xanthomonas campestris* (70% and 50% similarity to *E. coli* YbgF and TolA, respectively), both of which showed the same change in oligomeric status for YbgF on binding TolA (data not shown). That this effect is species-independent is emphasized by the fact that the same changes in stoichiometry occur for non-cognate complexes for