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Ultrastructural characterisation of *Bacillus subtilis* TatA complexes suggests they are too small to form homooligomeric translocation pores

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ABSTRACT

Tat-dependent protein transport permits the traffic of fully folded proteins across membranes in bacteria and chloroplasts. The mechanism by which this occurs is not understood. Current theories propose that a key step requires the coalescence of a substrate-binding TatC-containing complex with a TatA complex, which forms pores of varying sizes that could accommodate different substrates. We have studied the structure of the TatAd complex from *Bacillus subtilis* using electron microscopy to generate the first 3D model of a TatA complex from a Gram-positive bacterium. We observe that TatAd does not exhibit the remarkable heterogeneity of *Escherichia coli* TatA complexes but instead forms ring-shaped complexes of 7.5–9 nm diameter with potential pores of 2.5–3 nm diameter that are occluded at one end. Such structures are consistent with those seen for *E. coli* TatE complexes. Furthermore, the small diameter of the TatAd pore, and the homogeneous nature of the complexes, suggest that TatAd cannot form the translocation channel by itself. Biochemical data indicate that another *B. subtilis* TatA complex, TatAc, has similar properties, suggesting a common theme for TatA-type complexes from *Bacillus*.

1. Introduction

The twin-arginine translocation (Tat) pathway is an unusual system that acts to translocate fully folded proteins across the bacterial plasma membrane, and the chloroplast thylakoid membrane [1–3]. The Tat system functions in parallel with the well characterised Sec pathway and is dependent on the presence of a proton-motive force across the membrane [4–6]. It derives its name from the highly conserved twin-arginine motif present in the N-terminal signal peptide of Tat substrates [7,8]. In Gram-negative bacteria the integral membrane proteins TatA (10 kDa), TatB (18 kDa) and TatC (30 kDa) comprise the minimal components required for translocation of Tat substrates [9–11], and these proteins are all expressed from a single operon. An additional *tat* gene, *tatE*, is expressed elsewhere in the genome of *Escherichia coli*. The *tatE* gene is thought to be a cryptic duplication of *tatA* due to its high degree of sequence similarity [9] and ability to functionally complement a *ΔtatA* mutant [12].

Under steady state conditions the TatABC core components are observed to form two primary types of integral membrane complexes: a TatABC substrate-binding complex of ~370 kDa and TatA complexes ranging in size from 50 kDa to over 500 kDa [13–15]. The TatABC complex and TatA complex or complexes are thought to transiently coalesce to form the active translocon, and TatA has been suggested to form pores of varying sizes that could accommodate different substrates. Recent studies on the function of TatC have refined this model, suggesting a role for TatC in inserting the twin-arginine signal sequence into the membrane and a combined role for TatB and TatC in substrate recognition [16,17]. In addition, information from a recent crystal structure of TatC suggests that TatA could bind within a large, concave face of Tat C [16,17]. Low resolution structures of purified TatA suggest that this protein assembles into ring-shaped particles of 9–13 nm in diameter, a small subset of which have an internal cavity that could be large enough to accommodate the larger Tat substrates [18]. On the other hand, recent investigations have shown that TatE, which can apparently fully substitute for TatA, forms complexes that are much smaller and more homogeneous. These complexes appear as rings of 6–8 nm [12] which are too small to accommodate large Tat substrates in a folded state. The precise nature and function of TatA-type complexes of *E. coli* is thus currently unclear.

In contrast to Gram-negative bacteria, almost all Gram-positive bacteria possess a 'minimalist' Tat system which lacks a TatB component. Interestingly some Gram-positive organisms contain multiple Tat systems which possess differing substrate specificities [19]. *Bacillus subtilis* is one such bacterium, containing two minimal TatAC-type complexes, termed TatAdCd and TatAyCy, along with a third TatA component, TatAc [20]. The *tatAd* and *tatCd* genes are expressed under phosphate-limiting conditions and are located together in an operon downstream of the *phoD* gene. The PhoD protein has both phosphodiesterase and

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alkaline phosphatase activity, and is the only known substrate of the TatAdCd translocase [21–23]. The absence of a TatB component is compensated for by the bifunctional role of the TatAd protein, which has been shown to complement both *E. coli tatA/E* and *tatB* null mutants [24]. As with the *E. coli* system, the *B. subtilis* Tat components have been shown to form two types of complexes; a TatC-containing complex that we assume to be functionally analogous to the TatABC complex, and a separate TatA complex. However, biochemical assays have suggested that the *B. subtilis* complexes are significantly smaller and more homogeneous than the *E. coli* versions. The TatAdCd complex runs at ~230 kDa during blue-native PAGE and the TatAd complex has been estimated to be ~160 kDa by gel filtration [24]. Similarly, both TatAyCy and TatAy have been reported to form ~200 kDa complexes as judged by gel filtration [25,26]. The earlier studies also showed that the TatAdCd system is able to export the large, cofactor-containing trimethylamine N-oxide (TMAO) reductase (TorA) substrate (~90 kDa) when expressed in an *E. coli tat* null mutant. These data therefore suggest that multiple-sized TatA complexes are

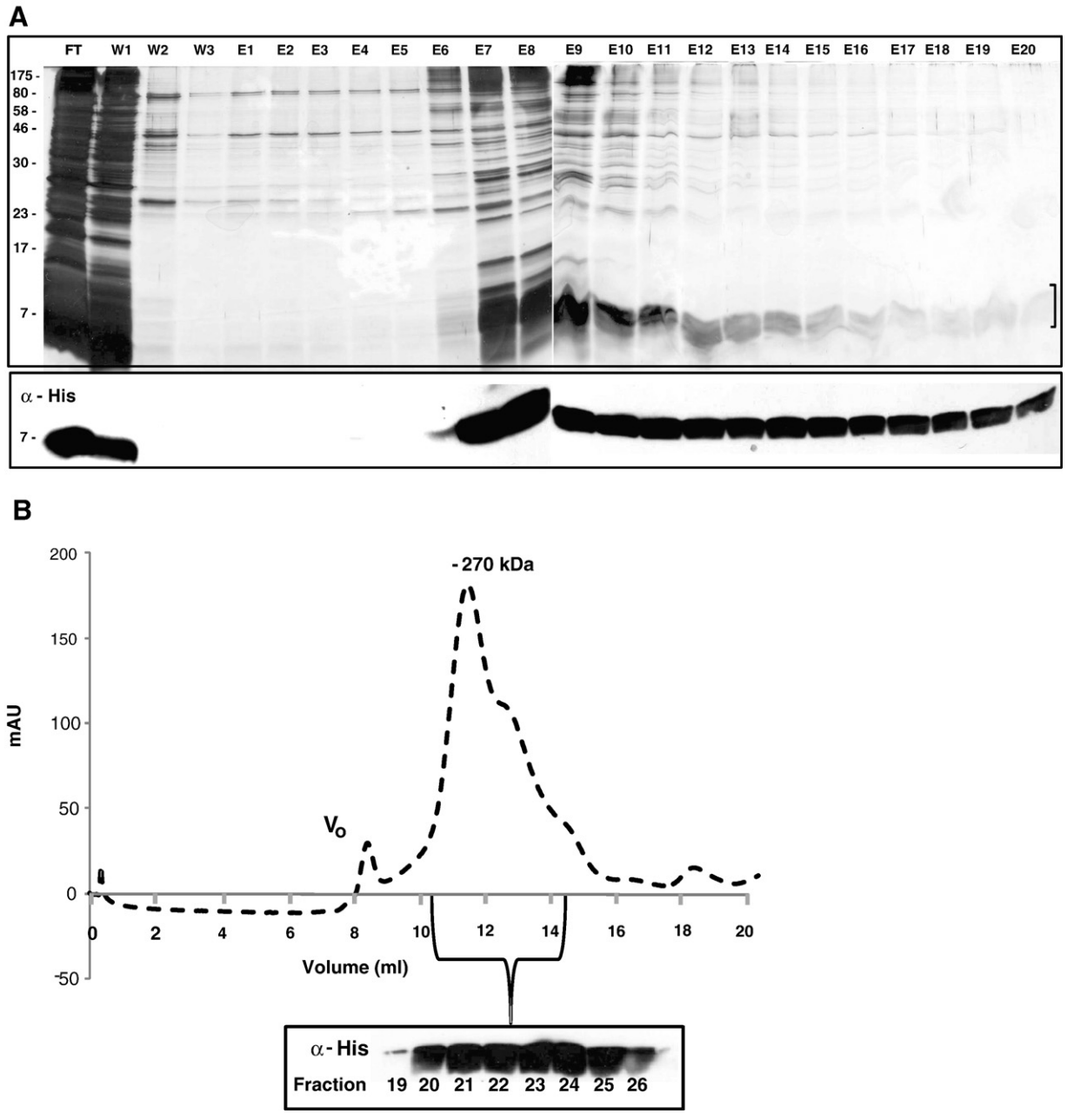

Fig. 1. Purification of *B. subtilis* TatAd. (A) Membranes were isolated from *E. coli Δtat* cells expressing *B. subtilis* TatAd with a C-terminal His-tag, solubilised in DDM and applied to a Talon affinity column. The proteins in all elution fractions were separated by SDS-PAGE and the gels were analysed using silver staining or immunoblotting with antibodies against the His-tag. Talon column fractions: FT = flow-through, W1-3 = wash fractions, E1-E20 = elution fractions. The bracket indicates the position of TatAd which runs as a diffuse band. (B). A sample of the TatAd concentrate was applied to a Superdex 200 GL 30/100 gel filtration column. The run (240 μl sample, 0.5 ml/min flow, 0.02% DDM in buffer) shows a major peak at ~270 kDa with a shoulder towards lower molecular weights. The corresponding Western blot shows that TatAd elutes across fractions 19–26. V^o = void volume. (C) Silver-stained gel of elution fractions which contain TatAd for different stages in column purification. T = pooled Talon column elution fractions, C = pooled Talon fractions after concentration, GF = gel filtration elution fraction selected for electron microscopy studies. Positions of TatAd are indicated on the right of the figure and molecular weight markers are shown on the left.