

PRIORITY PAPER

## Purified components of the *Escherichia coli* Tat protein transport system form a double-layered ring structure

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The *Escherichia coli* twin arginine translocation (Tat) system mediates Sec-independent export of protein precursors bearing twin arginine signal peptides. The genes *tatA*, *tatB*, *tatC* and *tatE* code for integral membrane proteins that are components of the Tat pathway. Cells co-overexpressing *tatABCDE* show an increased rate of export of a signal peptide-defective Tat precursor protein and a complex containing the TatA and TatB proteins can be purified from the membranes of such cells. The purified TatAB complex has an apparent molecular mass of

600 kDa as measured by gel permeation chromatography and, like the membranes of wild-type cells, contains a large molar excess of TatA over TatB. Negative stain electron microscopy of the complex reveals cylindrical structures that may correspond to the Tat protein transport channel.

**Keywords:** *Escherichia coli*; Tat protein export pathway; twin-arginine motif; membrane proteins; electron microscopy.

The majority of bacterial periplasmic proteins are exported across the cytoplasmic membrane by the Sec pathway [1]. However, a subset of periplasmic proteins, including many that bind redox-active cofactors, are translocated by a distinct, Sec-independent, mechanism. Such proteins are synthesized with N-terminal signal sequences containing a consensus S-R-R-x-F-L-K motif in which the arginine residues are invariant [2]. These 'twin arginine' signal peptides target the precursor protein to the recently discovered twin arginine translocation (Tat) protein export system [3] which is mechanistically and structurally related to the  $\Delta$ pH-dependent thylakoid import pathway of chloroplasts [4]. Translocation by the Sec system occurs by a threading mechanism in which the substrate must adopt an extended conformation. In contrast the Tat system functions to transport folded proteins across the cytoplasmic membrane, a feat that must be achieved without rendering the membrane freely permeable to protons or other ions.

In *Escherichia coli* the integral membrane proteins TatA, TatB, TatC and TatE have been shown to be components of the Tat pathway [5–8]. TatA, TatB, and

TatE are sequence-related proteins that are each predicted to comprise a transmembrane N-terminal  $\alpha$  helix followed by an amphipathic  $\alpha$  helix at the cytoplasmic side of the membrane [6,9]. Genetic experiments have shown TatA and TatE to have overlapping functions on the Tat pathway while TatB is an essential Tat component with a distinct role in protein export [5,6,8]. Recent expression studies suggest that *tatE* may be a cryptic gene duplication of *tatA* [10]. TatC is also an essential component of the Tat system and is predicted to be a polytopic membrane protein with six transmembrane helices [7]. In *E. coli*, the genes encoding TatA, TatB, and TatC are arranged as an operon with a fourth gene, *tatD*, that has no discernible role in protein export [11]. No further membrane-bound or water-soluble factors required for Tat-dependent protein export have so far been identified.

The functions of each Tat protein in the export pathway are poorly understood. Thus the TatA/B/E proteins have variously been predicted to act as membrane receptors for Tat substrates [9,12,13] or to form the export channel itself [3] while both roles have also been argued for the TatC protein [3,13–15]. A recent study demonstrated coimmunoprecipitation of TatA and TatB proteins from detergent solubilized *E. coli* membranes strongly suggesting a physiological interaction between these components of the Tat pathway [16]. We have sought to define the interrelationships between the known Tat components in more detail by co-ordinately overexpressing the *E. coli* *tat* genes and then attempting to purify the overexpressed Tat proteins. Here we report the purification and characterization of a large TatAB complex. This complex has an annular shape when visualized by negative stain electron microscopy.

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**Abbreviations:** TMAO, trimethylamine N-oxide; Tat, twin arginine translocation; IPTG, isopropyl thio- $\beta$ -D-galactoside; CTF, contrast transfer function; MSA, multivariate statistical analysis; MRA, multiple reference alignment.

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## EXPERIMENTAL PROCEDURES

### Bacterial strains and growth conditions

Preprotein export and processing kinetics were studied in *E. coli* strain BL21(DE3) ( $F^-$ , *dcm*, *ompT*, *hdsS*(rB-, mB-), *gal*, DE3 [17]); cultured in M9 minimal medium [18]. The *E. coli* host strain used for protein production was M15 ( $F^-$ , *lac*, *ara*, *gal*, *mtl* [19]). During all genetic manipulations *E. coli* strains were cultured in Luria–Bertani medium [18]. Concentrations of antibiotics were as previously described [6]. Overproduction of Tat components was carried out aerobically in Luria–Bertani medium supplemented with 0.8% (w/v) glucose.

### Plasmid construction

Plasmids for *in vivo* synthesis of pre-SufI and pre-SufI-R6K were constructed as follows. The *sufI* coding sequence from pNR14 [20] was transferred as an *EcoRI*–*XbaI* fragment to plasmid pSU23 [21] to yield pNR66 (*sufI*<sup>+</sup> Cm<sup>R</sup>) which expresses preSufI. The mutant *sufI* sequence from pNR23 [20] was excised with *EcoRI*–*XbaI* and cloned into pSU23 to give pNR67 encoding preSufI-R6K.

Plasmid pFAT75 (*tatABCDE*<sup>+</sup> Amp<sup>R</sup>) was constructed as follows. The *tatA*, *tatB*, and partial *tatC* genes were excised from pFAT65 [6] by *EcoRI*–*HindIII* digestion and cloned into pQE60 (Qiagen) to give pFAT70. The *tatE* gene was amplified by PCR using primers: 5'-GCGCAATTCCTG CAGGGCGCGTTCTGTTGCCG-3' and 5'-GCGCAAG CTTGGATGGAAGTTAAGTAATCC-3', digested with *EcoRI*–*HindIII*, and cloned into pT7.5 [22] to give pFAT73. Plasmid pFAT73 was then digested with *PstI*–*ClaI* and the fragment harbouring *tatE* cloned into pFAT65 to yield pFAT74. Finally, a fragment containing the portion of *tatC* not found in pFAT70, *tatD*, and *tatE* was excised from pFAT74 by digestion with *HindIII* and cloned into pFAT70 to give pFAT75. The *cat* gene from pACYC184 [23] was amplified with primers 5'-GCGCCTCGAGCCA TAATGAAATAAGATCACTACC-3' and 5'-GCGCCG AAGGGCCCGCTTATTACACTTATTCAG-3', digested with *XhoI*–*BglI*, and cloned into pFAT75 predigested with the same enzymes (thus inactivating *bla*) to give pFAT75C (*tatABCDE*<sup>+</sup> Cm<sup>R</sup>). All clones constructed by PCR were verified by DNA sequencing.

### Pulse–chase analysis

Co-expression of plasmid-borne *tat* and *sufI* in strain BL21(DE3) was carried out essentially as described earlier [11]. Synthesis of T7 RNA polymerase and Tat proteins was induced by the addition of isopropyl thio- $\beta$ -D-galactoside (IPTG; 0.4 mM final concentration).

### Purification of TatAB

Strain M15 was transformed with plasmid pREP4 (Kan<sup>R</sup>, *lacI*<sup>+</sup>, Roche Molecular Biochemicals) and subsequently with plasmid pFAT75C. Over-expression of *tatABCDE* was achieved following the addition of IPTG (final concentration 2 mM) to an exponentially growing culture of M15[pREP4] + pFAT75C. Cultures were incubated

with IPTG for 3 h at 37 °C and 2 g (wet weight) of cell paste was routinely recovered from a 1-L induction. Cells were resuspended in 10 mL 20 mM Mops (pH 7.2), 500 mM NaCl, 2.5 mM Na<sub>2</sub>EDTA, including a protease inhibitor mixture (Complete<sup>TM</sup>, Roche Molecular Biochemicals), broken by passage through a French pressure cell and membranes isolated by differential centrifugation as described [6]. Membranes were washed once in 50 mM Tris.HCl (pH 8.0) and then resuspended in 10 mM Mes (pH 6.3) to a final protein concentration of 40 mg·mL<sup>-1</sup>. Membranes were dispersed by the addition of an equal volume of 10 mM Mes (pH 6.3), 100 mM Chaps with gentle stirring at 4 °C for 1 h. Insoluble material was removed by ultracentrifugation at 150 000 g for 90 min and the high-speed supernatant applied to a 1-mL Mono-Q HR 5/5 anion-exchange column (Amersham Pharmacia Biotech) equilibrated in 10 mM Mes (pH 6.3), 1 mM Chaps. Bound proteins were eluted at room temperature with a linear 60-mL gradient of 0–1 M NaCl in 10 mM Mes (pH 6.3), 1 mM Chaps, and fractions enriched in TatA and TatB as judged by SDS/PAGE were pooled and concentrated. The concentrated TatAB sample was adjusted to 250 mM NaCl, 4 mM Chaps, and applied to a Superose-6 HR 10/30 gel filtration column (Amersham Pharmacia Biotech) pre-equilibrated at room temperature in 20 mM Mops (pH 7.2), 250 mM NaCl, 4 mM Chaps, 10% glycerol. Fractions containing TatAB were identified by SDS/PAGE and retained. Typically, 1 mg of recombinant TatAB could be isolated from 80 mg of membrane protein by this method.

### Protein analysis

Protein sequencing services, including cyanogen bromide cleavage, were provided by Alta Biosciences (Birmingham, UK). SDS/PAGE and immunoblotting analyses were as previously described [24,25]. For immunoblotting experiments a cytoplasmic membrane fraction was purified from the crude membrane fraction by density gradient centrifugation [26]. This protocol removes outer membrane fragments and aggregated proteins. Anti-TatA and anti-TatB sera were raised against proteins electroeluted from the purified TatAB preparation [16]. The antisera were affinity purified using recombinant TatA and TatB proteins immobilized on NHS-activated Sepharose HiTrap columns (Amersham Pharmacia BioTech). The putative N-terminal transmembrane helix of the recombinant proteins had been replaced by a hexahistidine tag to allow isolation by Ni(II)-affinity chromatography (E. de Leeuw, F. Sargent & B. C. Berks, unpublished data). As a consequence the purified antisera do not crossreact with the transmembrane regions of TatA and TatB. The TatA:TatB molar ratio was estimated by quantitative immunoblotting using the recombinant TatA and TatB proteins as standards. The concentration of the standards was determined by amino-acid analysis (Alta Biosciences Birmingham, UK) in duplicate. The anti-TatC serum was a gift from M. Müller, University of Freiburg, Germany. Radiolabeled Tat proteins were synthesized as described earlier [6]. Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry was carried out on a Ciphergen Biosystems instrument in the presence of sinapinic acid. Trimethylamine *N*-oxide (TMAO) reductase activity was measured as described [27].

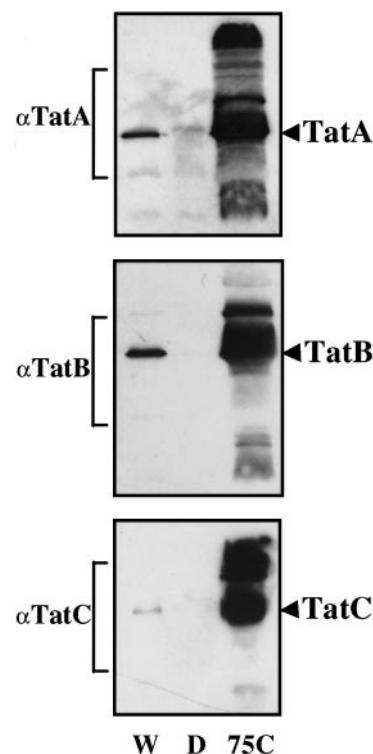
## Electron microscopy

Prior to analysis, TatAB samples were dialysed against excess 20 mM Mops (pH 7.2), 50 mM NaCl, 4 mM Chaps. Samples at a protein concentration of  $0.015 \text{ mg}\cdot\text{mL}^{-1}$  were then applied to freshly glow-discharged carbon-coated copper grids and stained with 2% (w/v) uranyl acetate. Micrographs were taken with a JEOL 1200EX transmission electron microscope operating at 120 kV at a nominal magnification of  $50\,000\times$ . Five suitable negatives were scanned with a LeafScan 45 CCD scanner (Ilford) with a step size of  $10 \mu\text{m}$ . The images were then reduced by  $2 \times 2$  pixel averaging which resulted in a pixel size of 4 Å at the specimen level. Individual particles were selected interactively with XIMDISP [28] and extracted ( $76 \times 76$  pixels) using SPIDER [29]. Subsequent image processing was performed within IMAGIC 5 [30]. First, the defocus for all images originating from a particular micrograph was determined by examining the total sum of their Fourier transforms. The defocus range was determined to be  $1.46\text{--}2.30 \mu\text{m}$  for the five micrographs used in the reconstruction. After band-pass filtering to suppress unwanted low and high spatial frequencies, the images were individually corrected for effects of the contrast transfer function (CTF) by flipping negative values according to the calculated CTF at a given defocus. The corrected images were then combined in a single data set (1928 images), mass-centred and analysed by multivariate statistical analysis (MSA) and classification into 100 classes. Characteristic views of the class averages were chosen as references for a multiple reference alignment (MRA). In total, four rounds of MSA/classification/MRA were performed.

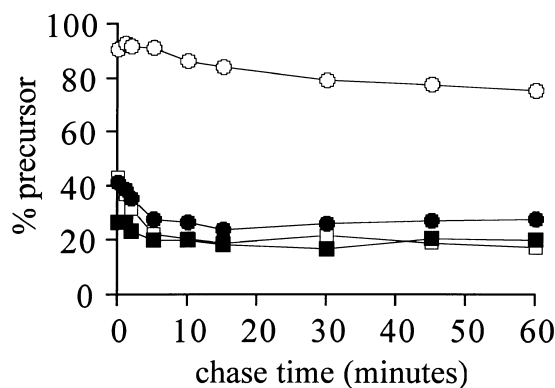
## RESULTS

### Co-ordinate overproduction of TatABCDE increases Tat pathway capacity *in vivo*

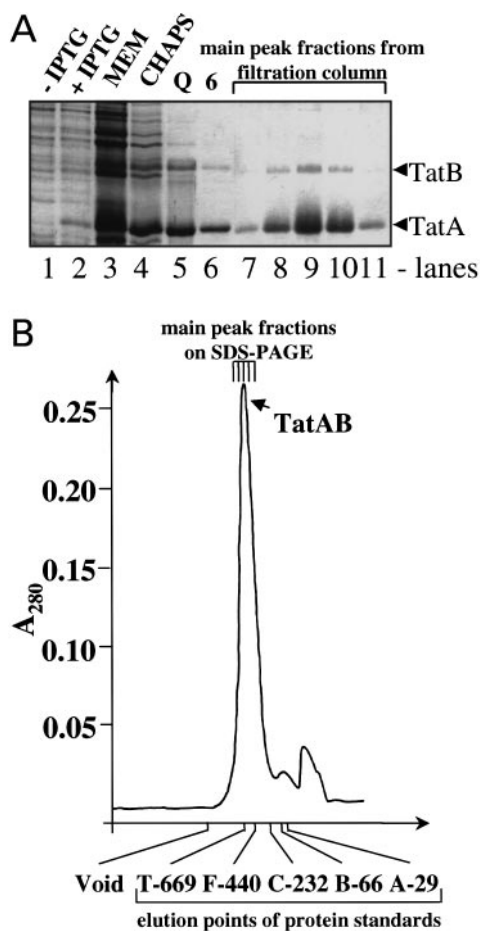
*In vivo* radiolabelling and immunological studies indicate that the TatA, TatB and TatC components of the *E. coli* Tat pathway are localized to the cytoplasmic membrane [11,16]. It is therefore possible that these proteins associate within the membrane to form a complex, or complexes, corresponding to the Tat preprotein translocase. A major aim of our laboratory is the purification of sufficient quantities of such translocases for biophysical and biochemical analysis. As an initial step towards this goal we have sought to determine whether the Tat translocase content of *E. coli* membranes can be enhanced by co-ordinate overexpression of the known *tat* genes. In order to maximize the chances of functional overproduction of the Tat pathway it is desirable to overexpress the Tat components in a ratio that reflects as closely as possible the stoichiometry of synthesis of the proteins in wild-type cells. We reasoned that this goal might best be achieved by placing the intact *tatA* operon under the control of a strong inducible promoter as it has been shown that the essential *tatABC* genes are transcribed as a single mRNA [10,11]. Any variation in the levels of protein produced by these genes thus arises from translational signals within the mRNA.



**Fig. 1.** Overproduction of TatA, TatB and TatC. Purified inner membrane vesicles of *E. coli* strains M15[pREP4] (*tatABCDE*<sup>+</sup> W), DADE (MC4100  $\Delta$ *tatABC* $\Delta$ *tatE*; D) and M15[pREP4, pFAT75C] (overexpressing *tatABCDE*; 75C) were analysed by SDS/PAGE and Tat proteins visualized by immunoblotting using the indicated anti-subunit sera. Equal quantities of membrane protein were loaded in each lane.



**Fig. 2.** Overexpression of *tatABCDE* increases preprotein flux through the Tat system. SufI precursor was expressed in BL21(DE3), pulse labelled for 5 min by addition of [<sup>35</sup>S]methionine, and then chased from time zero with unlabelled methionine. Precursor synthesis and processing were assessed by SDS/PAGE of whole cells followed by autoradiography. The mean percentage of total SufI protein remaining in the precursor form at each of the indicated time points is plotted ( $n = 3$ ). Expression of native SufI precursor from plasmid pNR66 in the presence (■) or absence (□) of pFAT75 (*tatABCDE*<sup>+</sup>). Expression of pre-SufI-R6K from plasmid pNR67 in the presence (●) or absence (○) of pFAT75.



**Fig. 3. Purification of a TatAB complex.** (A) Proteins were separated by SDS/PAGE and visualized by Coomassie Blue staining. Samples analysed are: whole cell extracts of M15[pREP4, pFAT75C] either with (lane 2; + IPTG) or without (lane 1; - IPTG) IPTG induction; the crude membrane fraction (lane 3; MEM) derived from the lane 2 sample; the high speed supernatant fraction following solubilization of the membranes with Chaps (lane 4; CHAPS); pooled TatA-containing fractions from the anion exchange (lane 5; Q) and gel filtration (lane 6; 6) columns; fractions across the TatA peak of the gel filtration column (lanes 7–11). (B) Elution of TatAB complex from a Superose-6 gel filtration column monitored at 280 nm. The chromatography conditions are detailed in Experimental procedures. The elution positions of water-soluble molecular mass marker proteins are indicated under the chromatographic trace. The marker proteins used were thyroglobulin 669 kDa (T-669), apoferritin 440 kDa (F-440), catalase 232 kDa (C-232), bovine serum albumin 66 kDa (B-66), and carbonic anhydrase 29 kDa (A-29). Fractions analysed by SDS/PAGE in panel A are indicated above the main elution peak.

The *tatABCD* and *tatE* operons were arranged in series behind the LacI-repressed T5 promoter of plasmid pQE60 yielding plasmid pFAT75C. Following induction of *tatABCDE* expression with IPTG the cytoplasmic membranes of an *E. coli* strain bearing pFAT75C contain grossly elevated levels of the three essential Tat proteins TatA, TatB and TatC as assessed by immunoblotting (Fig. 1).

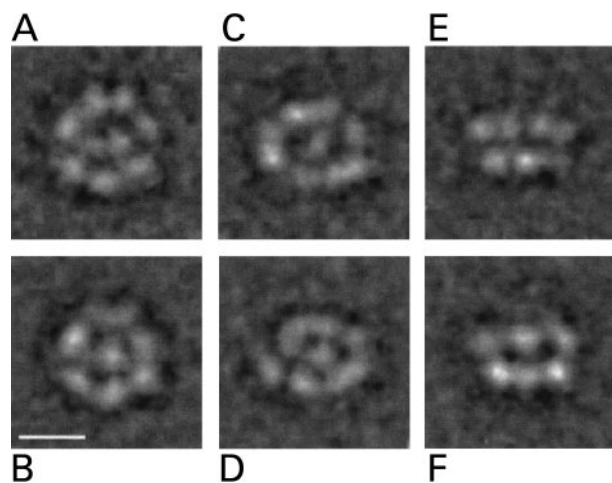
We sought to establish whether these overproduced proteins could form functional Tat translocases. Transport of the enzyme TMAO reductase to the periplasm depends

on the Tat pathway [6]. Thus no TMAO reductase activity is found in the periplasmic fraction of the  $\Delta$ *tatABCD* $\Delta$ *tatE* strain DADE [11]. Introduction of plasmid pFAT75C into strain DADE restores wild-type levels of periplasmic TMAO reductase activity (data not shown) indicating that pFAT75C is able to direct synthesis of a functional Tat pathway.

We next investigated whether overproduction of the Tat components resulted in an increase in Tat translocation activity. The effects of Tat protein overproduction were studied in whole cells by pulse-chase analysis of the export of the Tat substrate SufI [20]. Export of the wild-type SufI precursor in *E. coli* strain BL21(DE3) was substantially completed during the labeling period even in the absence of plasmid pFAT75 (Fig. 2) making it difficult to judge whether expression of the *tat* genes enhanced the rate of export. To circumvent this problem we took advantage of the observation that certain amino-acid substitutions in the SufI signal peptide drastically slow export [20]. In variant pre-SufI-R6K the second arginine of the twin arginine motif has been conservatively substituted with a lysine. This substitution results in a very low rate of SufI export in wild-type Tat backgrounds (Fig. 2; [20]). In contrast, induction of expression of the *tatABCDE* genes on pFAT75 leads to export of pre-SufI-R6K at rates similar to those of wild-type pre-SufI (Fig. 2). Thus our overexpression system enhances export of a Tat substrate with a defective signal peptide suggesting that there is either an increase in the number of functional translocation sites in the membrane or that the rate-limiting step in the transport of this substrate has changed. It has previously been reported that a SufI variant in which both consensus arginine residues were replaced by lysines (preSufI-R5K,R6K) was not translocated in a wild-type Tat background [20]. Overexpression of *tatABCDE* does not restore translocation of this variant preprotein (data not shown) confirming that a Tat signal peptide lacking both of the consensus arginine residues is nonfunctional.

### The TatA and TatB proteins co-purify

Having established that our overexpression system increases an activity of the Tat pathway we attempted to isolate and characterize the overproduced Tat components. Overexpression of *tatABCDE* from plasmid pFAT75C results in a large increase in the abundance of a protein that has an identical electrophoretic mobility to radiolabeled TatA under SDS/PAGE [6] and an apparent molecular mass by this technique of 18 kDa (Fig. 3, lane 2). Following subcellular fractionation the overproduced protein partitioned with the membrane fraction and could be solubilised by treating the membranes with the zwitterionic detergent Chaps (Fig. 3A, lanes 3 and 4). The overexpressed protein was subject to purification by anion-exchange and gel permeation chromatography. The overexpressed 18-kDa protein was observed to copurify with a second protein having an apparent molecular mass by SDS/PAGE of 30 kDa (Fig. 3A, lane 6) and an identical electrophoretic mobility to radiolabeled TatB [6]. Attempts to directly sequence the two copurifying proteins failed indicating that both proteins had blocked N-termini. Following cyanogen bromide treatment the 30-kDa protein gave a mixture of three sequences that could be interpreted



**Fig. 4. Average projections of the TatAB complex.** Six characteristic views (density projections) out of 100 classes are shown. Assuming (A,B) to be top views of the complex as seen from above the membrane plane, it follows that (C,D) are oblique views resulting from the complex being rotated around the horizontal axis, and (E,F) are side views, rotated 90° from (A,B), as seen from within the membrane plane. The number of images averaged in each class is 18 (A), 23 (B), 25 (C), 19 (D), 18 (E), and 21 (F). The scale bar has a length of 100 Å.

as FDIGF, DELRQ, and KRSYV confirming this protein to be TatB. Similar treatment of the 18-kDa protein yielded two sequences interpreted as XGISIW and XDDEPK (where X is an unidentifiable residue) that positively identified the protein as TatA. Mass spectrometry of the purified TatAB sample detected a single mass peak at 9694 Da. This corresponds to an 89-residue *tatA* gene product (predicted mass 9692 Da) commencing fMet-Gly-Gly-Ile-Ser and indicates that the second of two potential *tatA* start codons is utilized [6]. It also demonstrates that the previously noted [6] anomalously high molecular mass of TatA during SDS/PAGE is an artefact of the electrophoretic method rather than a consequence of the formation of a covalent TatA dimer. Such aberrant electrophoretic migration is commonly observed for hydrophobic integral membrane proteins. No mass peak corresponding to TatB was detected in the mass spectrometry experiment.

The TatA and TatB proteins coelute on a Superose-6 gel-filtration column with an apparent molecular mass of approximately 600 kDa (Fig. 3B). As judged by densitometry of Coomassie-stained gels the molar ratio of the two proteins is constant in fractions across the gel filtration peak (Fig. 3A, lanes 7–11) strongly suggesting that the TatA and TatB proteins form a complex. Immunoblotting suggests that the TatC protein is apparently not present in our TatAB preparation, even though it is clearly overproduced and active in our expression system (Figs 1 and 2). It is possible the TatC protein is either unstable under the experimental conditions used or only transiently associated with this TatAB complex. Nevertheless, the copurification of essential Tat system components as a stable complex points to important structural and functional interrelationships between the two proteins.

Quantitative immunoblotting was used to compare the TatA : TatB stoichiometry of the purified TatAB complex with that of purified cytoplasmic membranes

from wild-type *E. coli* cells containing the native Tat translocation pathway. The TatA to TatB molar ratio was determined to be  $15 \pm 4$  for the purified complex and  $19 \pm 4$  for the wild-type membranes. Thus both purified complex and wild-type membranes contain similarly large excesses of TatA protein over TatB protein.

### Large TatAB complexes are revealed by electron microscopy

Negative stain electron microscopy of single particles was used to image the purified TatAB complexes. A total of 1928 individual particle images were aligned and then sorted into 100 classes. Almost all particles appear to be structurally related as judged by their dimensions and features. Of those classes which displayed uniform staining and a good signal/noise ratio, six are shown in Fig. 4. The low number of images per class (18–25 in Fig. 4) makes it unlikely that unrelated views are grouped together but also results in relatively noisy images. However, within the limits of the negative stain technique, certain basic features of the TatAB complex can still be inferred as follows.

The examples shown in Fig. 4 are three pairs of similar views of the complex. As these views are two-dimensional projections of the same three-dimensional structure, one can make some deductions about their relative orientations. If the images in Fig. 4A,B are arbitrarily designated 'top' views, then c and d are 'oblique' and e and f are 'side' views of an asymmetric, approximately cylindrical structure. The 'top' views show an annular structure with outer dimensions of approximately 155–160 Å. Six or seven globular densities of roughly 35 Å diameter form the periphery, while the central region of  $\approx 65$  Å diameter contains 1–2 density features. The 'side' projections show double layered structures ( $\approx 90$  Å thick). The position of the membrane is not defined relative to the detergent solubilized structures. We note, however, that whatever the orientation of the particles in the membrane, the complex is considerably larger than the  $\approx 50$  Å necessary to span the membrane bilayer. This is not unexpected, given the fact, that considerable parts of both TatA and TatB are predicted to extend outside the membrane [3,9].

### DISCUSSION

In this work we determined that the TatA component of the Tat pathway is present in the *E. coli* cytoplasmic membrane at a roughly 20 : 1 molar excess over the homologous TatB component. This finding is in accord with a recent study in which the relative translational activities measured by single copy reporter gene fusions of *tatA* : *tatB* : *tatC* : *tatD* : *tatE* were shown to be approximately 25 : 1 : 0.5 : 0.1 : 0.2 [10]. Taken together these data suggest that the TatA protein is present at considerably higher stoichiometry than the other currently identified components of the *E. coli* Tat pathway.

A complex between the TatA and TatB proteins has been purified from a strain co-ordinately overexpressing the known *tat* genes. The purification of a TatAB complex confirms the existence of protein-protein interactions between the TatA and TatB components of the Tat pathway. The observation that both the purified complex and wild-type *E. coli* membranes contain a similar large excess of

TatA protein over TatB protein argues for the physiological relevance of the TatAB complex. It also raises the possibility that TatA may be the major structural subunit of the Tat translocase. The estimated molecular mass of the purified *E. coli* TatAB complex (600 kDa) is close to that inferred on the basis of Blue Native PAGE analysis (620 kDa) for the functional Tat transporter of plant thylakoid membranes [31]. The subunit composition of the thylakoid complex has, however, not been determined.

Views of the TatAB complex in Fig. 4 are compatible with a cylindrical structure in which a central cavity is sealed at one or both ends. In the 'top' views (Fig. 4A,D) this cavity is apparent as a partially occluded stain-filled region within the main annulus of density. The central densities may be associated with one or both ends of the particle as the 'side views' (Fig. 4C,F) have a low density in their central region. In the top view, the unblocked opening would have a diameter of  $\approx 60$  Å. As the largest *E. coli* Tat substrates have diameters in the range of 60–70 Å [3] this internal cavity could be large enough to accommodate the proteins transported by the Tat translocase. The structures blocking the ends might then be involved in controlling access to this cavity. Indeed, in the absence of substrate proteins the translocation pathway must be sealed to maintain the ionic integrity of the bacterial cytoplasmic membrane. Consistent with this requirement, initial studies indicate that the purified TatAB complex does not form large ion-conducting channels when reconstituted into planar lipid bilayers (E. J. A. Lea, F. Sargent & B. C. Berks, unpublished data).

The studies reported here are an initial step in the molecular level analysis of the Tat translocation pathway.

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