The TatA component of the twin-arginine protein transport system forms channel complexes of variable diameter

Ulrich Gohlke^{*†}, Lee Pullan^{*‡}, Christopher A. McDevitt[§], Ida Porcelli[§], Erik de Leeuw[§], Tracy Palmer[¶], Helen R. Saibil^{*}, and Ben C. Berks[§]

^{*}Institute of Structural Molecular Biology, School of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, United Kingdom; [§]Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom; [¶]School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom; and [¶]Department of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH, United Kingdom

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The Tat system mediates Sec-independent transport of folded precursor proteins across the bacterial plasma membrane or the chloroplast thylakoid membrane. Tat transport involves distinct high-molecular-weight TatA and TatBC complexes. Here we report the 3D architecture of the TatA complex from *Escherichia coli* obtained by single-particle electron microscopy and random conical tilt reconstruction. TatA forms ring-shaped structures of variable diameter in which the internal channels are large enough to accommodate known Tat substrate proteins. This morphology strongly supports the proposal that TatA forms the protein-conducting channel of the Tat system. One end of the channel is closed by a lid that might gate access to the channel. On the basis of previous protease accessibility measurements, the lid is likely to be located at the cytoplasmic side of the membrane. The observed variation in TatA diameter suggests a model for Tat transport in which the number of TatA protomers changes to match the size of the channel to the size of the substrate being transported. Such dynamic close packing would provide a mechanism to maintain the membrane permeability barrier during transport.

conical tilt reconstruction | electron microscopy | Tat protein transport | three-dimensional structure | twin-arginine signal peptide

Many proteins function in extracytoplasmic compartments such as the periplasm in Gram-negative bacteria or the chloroplast in plants. In bacteria, most exported proteins are moved across the cytoplasmic membrane by the Sec pathway (1). This translocation occurs by a threading mechanism in which the substrate adopts an extended conformation. By contrast, the Tat system exports folded proteins across the cytoplasmic membrane (2). Substrates of the Tat pathway have N-terminal signal sequences containing an S-R-R-x-F-L-K consensus motif in which the arginine residues are almost invariant (3). Tat transport is energized by the transmembrane proton electrochemical gradient (4). The Tat system is vital for many bacterial processes, including energy metabolism, formation of the cell envelope, biofilm formation, heavy metal resistance, nitrogen-fixing symbiosis, and bacterial pathogenesis (5). A Tat system is also found in the thylakoid membrane of plant chloroplasts, where it plays an essential role in the biogenesis of the photosynthetic electron transport chain (6).

In *Escherichia coli*, the minimal components of the Tat translocation system are the integral membrane proteins TatA, TatB, and TatC (7–11). Attempts to purify the Tat components from *E. coli* membranes have led to the identification of two distinct high-molecular-mass complexes, one corresponding to TatA and the other containing predominantly TatB and TatC (12–15). Both types contain multiple subunits per complex. Analogous complexes have been described in de-energized thylakoids (16). Projection maps of Tat complexes obtained by negative stain electron microscopy show that each type of complex forms particles of ≈ 90 – 160 Å (1 Å = 0.1 nm) in

diameter (15, 17). TatBC has been shown to act as the receptor element of the translocation pathway (13, 16, 18), and TatC has been shown to contain the primary binding site for the signal peptide (18). The function of TatA is less clear. It is known that TatA is required subsequent to substrate recognition by the TatBC complex (16, 18, 19), and this has led to the suggestion that TatA constitutes the protein-conducting channel of the Tat system. Recent chemical crosslinking studies suggest that TatA transiently associates with the TatBC–substrate complex during active protein translocation (18, 19).

Like other protein translocation systems, the Tat pathway is presumed to form an aqueous transmembrane channel for protein transport. It is vital that there is no ion leakage through this channel, because the bacterial cytoplasmic membrane has to maintain transmembrane proton and other ion electrochemical gradients to drive essential cellular functions. To preserve the ionic permeability barrier of the membrane, opening of the Tat channel must be gated by substrate. In addition, a mechanism is required to prevent ion leakage around the translocating substrate protein during transport. These considerations also apply to the Sec channel present in the same membrane. However, the mechanistic challenges posed for the Tat channel are considerably greater than for the Sec channel. Because some folded *E. coli* Tat substrates are close to 70 Å in diameter (20), the Tat system must be able to form a channel of this size as opposed to the 12 –Å channel needed to thread unfolded chains by the Sec translocase (1). Concerted helix tilting in the membrane may be sufficient to gate the pore in the Sec apparatus (1), but a radically different mechanism seems necessary to provide the huge conformational change required to gate the Tat channel. An additional challenge for the Tat system is that, in contrast to the Sec pathway, substrate proteins vary widely in size. If the channel needs a diameter of 70 Å to accommodate the largest substrates, how is ion leakage between the substrate protein and the walls of the channel prevented when the smallest substrates (20 – 30 Å in diameter) (21) are being transported?

In this report we present the 3D density maps of the TatA complex from *E. coli* obtained by random conical tilt electron microscopy of negatively stained specimens. This is the first 3D structure determination for any component of the Tat translocation pathway. It strongly supports the hypothesis that TatA forms the protein-conducting channel of the Tat system and suggests possible mechanisms by which the channel can transport

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[†]To whom correspondence should be sent at the present address: PSF Biotech AG, Heubnerweg 6, 14059 Berlin, Germany. E-mail: gohlke@psf-ag.com.

^{*}Present address: Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, TX 77030.

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Fig. 3. Comparison of observed views with reprojections of the 3D maps. (A and B) Averages of the classes outlined in Fig. 2B (A) for comparison with reprojections of the corresponding 3D maps of TaTA after refinement (B). (C and D) Raw images of tilted views selected from different size groups (C) for comparison with reprojections of the 3D maps at the corresponding orientations (D).

asymmetry was subsequently confirmed by an independent analysis of a different data set, which showed clear asymmetry without the use of a masked reference. From the realigned images, final 3D reconstructions were computed and filtered to 25 Å.

Results

Electron Microscopy of TatA. Electron microscopy of TatA complexes solubilized in the detergent nona-polyoxyethylene dodecyl ether (C₁₂E₉) and negatively stained reveals mainly ring-shaped particles (Fig. 1A). Preliminary analysis indicated that the particles vary in size, that most adopt a preferred orientation, and that no symmetry could be identified. Therefore, we used a strategy of size separation followed by random conical tilt analysis (26) to obtain the different views required for 3D reconstruction of the complex.

A micrograph of the area in Fig. 1A tilted at an angle of 45° is shown in Fig. 1B. The particles appear elliptical, with the long axis lying parallel to the tilt axis. Approximately 1,800 particles were selected from 11 pairs of tilted and untilted views. Multivariate statistical analysis (MSA) of the untilted particles confirmed the presence of size variations. Using MSA, we sorted the particles into 10 size groups using the strategy described by White *et al.* (28). The class averages are shown in Fig. 2A. Their members (86–249 images per class) were aligned by using a reference-free approach (29) to determine the in-plane orientation of each particle (Fig. 2B). For each class, a 3D density map was reconstructed by using the corresponding data from the tilted specimen. The maps showed a cylindrical ring with additional protein density forming a lid covering the central channel and less density on the opposite surface. Separate analysis of an independent sample (data not shown) confirmed the appearance of a cylindrical structure with a lid at one end only. The asymmetric exposure of protein at the two surfaces is also consistent with the results of protease accessibility studies of the TatA complex in its native membrane environment (14).

After refinement by projection matching, the series of conical tilt reconstructions obtained from the set of different size classes showed a corresponding range from small to large TatA com-

Fig. 4. The 3D architecture of assembled TatA complexes. Shown are four TatA complexes with increasing diameter. The 3D maps are filtered between 150 Å and 25 Å and contoured at $\approx 4 \sigma$ (standard deviations above the mean density). (A) TatA complexes viewed from the closed end of the channel, proposed to be at the cytoplasmic side of the membrane (C-face; see text). Density forming the lid domain can be clearly seen. (B) TatA complexes viewed from the open end of the channel, proposed to be at the periplasmic side of the membrane (P-face). (C) Side views of TatA. The front half of each molecule has been cut away to reveal internal features. (D) Views of TatA parallel to the membrane plane. The proposed position of the lipid bilayer is indicated in blue. (Scale bar, 100 Å.) The figure was prepared with Pymol (www.pymol.org).

	Class 1	Class 4	Class 8	Class 10
No. of particles	147	206	235	86
Outer diameter, Å	85–90	90–95	100–110	125–130
Channel diameter, Å	30–35	40–45	55–60	65–70
Total height, Å	55–60	65–70	75–80	80–85
Ring height, Å	45–50	45–50	50–55	50–55
Ring width, Å	25–30	25–30	25–30	25–30
Molecular mass, kDa	130–150	160–180	230–280	330–390
Subunit number, volume	12–14	15–17	21–25	30–35
Subunit number, circumference	19	21	26	31

plexes. For the following interpretation, we have chosen four classes representing the smallest and the largest complex as well as two intermediate sizes (Fig. 3A). The selected class averages are compared with the corresponding reprojections of the final 3D density maps in Fig. 3B. For the tilted views, the orientation of each is defined by the tilt geometry, so they cannot be grouped into class averages. In this case, individual tilted particles representing the size range (Fig. 3C) are compared with reprojections at the same orientations (Fig. 3D).

Because the TatA subunits must all be inserted into the membrane in equivalent environments, the plane of the ring must lie in the plane of the membrane. The 50-Å height of the ring is comparable with the thickness of a lipid bilayer. Therefore, the structure is suitable to act as a channel for the translocation of folded proteins across the membrane, with the lid domain controlling entry to this channel, as shown in Fig. 4D. We propose that the lid domain is on the cytoplasmic side on the basis of previous protease accessibility studies (14). TatA is not accessible to digestion by proteinase K from the periplasmic side of the membrane, whereas it is readily degraded from the cytoplasmic side. Provided that TatA has the same conformation

The transmembrane portion of the TatA complex forms a ring that is $\approx 45\text{--}55$ Å high and $\approx 25\text{--}30$ Å wide (Fig. 4C and Table 1). Similar dimensions are found in the c_{10} ring of yeast ATP synthase, in which the protein ring is $23\text{--}28$ Å wide and $47\text{--}58$ Å high and is formed by two transmembrane α -helices linked by a loop (31). Depending on the contribution of the detergent molecules to the density, this comparison suggests that the TatA ring is one or two transmembrane helices wide. The number of TatA subunits forming the ring can be estimated from the circumference, assuming either a single ring of transmembrane helices or two concentric rings of helices, with one or two helices, respectively, being contributed by each subunit. For a TatA average diameter of $60\text{--}100$ Å and a helix center-to-center distance of 10 Å, this would give an estimate of $19\text{--}31$ subunits (Table 1).

TatA proteins are predicted to have a secondary structure in which an N-terminal hydrophobic α -helix is followed by an amphipathic α -helix and then a charged and largely unstructured C-terminal tail (9, 32). This model for the secondary structure content is consistent with circular dichroism spectroscopy of TatA (14). Protein engineering experiments have demonstrated that the N-terminal hydrophobic region up to residue 20 is essential for both the membrane association and oligomerization of TatA (14), compatible with the proposal that the N-terminal region forms a transmembrane helix. The TatA ring could be formed by the N-terminal helices from each subunit or by a ring of helical hairpins in which a second, amphipathic helix from each subunit lines the channel, surrounded by an outer ring of hydrophobic helices. The range of molecular masses of the TatA complexes estimated from the contoured volume of the density maps is 130–390 kDa (Table 1). Because the molecular mass of the TatA protomer is 10,730 Da, the volume estimates suggest $\approx$ 12–35 monomers per complex (Table 1), in good agreement with the results inferred from fitting helices into the transmembrane ring, favoring the model in which amphipathic helices line the channel.

In conclusion, the observed size variation in TatA shows a remarkable flexibility in its structural organization and supports the possibility that the Tat channel forms a close seal around the substrate protein during the transport process by adjusting the number of TatA protomers in the channel complex (21). The observed variation in the volume of the internal channel is sufficient to accommodate the variation in size of the Tat substrate proteins.

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