Membrane interactions and self-association of the TatA and TatB components of the twin-arginine translocation pathway

Erik De Leeuw[a,1], Ida Porcelli[a,1], Frank Sargent[a], Tracy Palmer[a,b], Ben C. Berks[a,2,*]

[a] *Centre for Metalloprotein Spectroscopy and Biology, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK*
[b] *Department of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH, UK*

Received 25 July 2001; revised 10 September 2001; accepted 10 September 2001

First published online 19 September 2001

Edited by Gunnar von Heijne

Abstract The *Escherichia coli* Tat system mediates Sec-independent export of protein precursors bearing twin-arginine signal peptides. The essential Tat pathway components TatA, TatB and TatC are shown to be integral membrane proteins. Upon removal of the predicted N-terminal transmembrane helix TatA becomes a water-soluble protein. In contrast the homologous TatB protein retains weak peripheral interactions with the cytoplasmic membrane when the analogous helix is deleted. Chemical crosslinking studies indicate that TatA forms at least homotrimers, and TatB minimally homodimers, in the native membrane environment. The presence of such homo-oligomeric interactions is supported by size exclusion chromatography.

Key words: Tat protein translocase; Chemical crosslinking; Protein transport; *Escherichia coli*

1. Introduction

The majority of bacterial periplasmic proteins are exported across the cytoplasmic membrane by the Sec pathway [1,2]. However, a subset of periplasmic proteins, including many that bind redox-active cofactors, are translocated by a distinct Sec-independent mechanism. Such proteins are synthesised with N-terminal signal sequences containing a consensus S-R-R-x-F-L-K motif in which the arginine residues are (almost) invariant [3]. These 'twin-arginine' signal peptides target the precursor protein to the Tat (twin-arginine translocation) protein export system [4] which is mechanistically and structurally related to the ΔpH-dependent thylakoid import pathway of chloroplasts [5]. 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 TatA, TatB, TatC and TatE proteins have been shown to be components of the Tat pathway [6–10]. TatA, TatB, and TatE are sequence-related proteins that are each predicted to comprise a transmembrane N-terminal α-helix followed by an amphipathic α-helix at the cytoplasmic side of the membrane [7,11]. 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 [7,9]. Expression studies suggest that *tatE* may be a cryptic gene duplication of *tatA* [12]. 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 [13].

The TatA:TatB:TatC molar ratio in the *E. coli* membrane has been estimated to be approximately 40:2:1 [12,14]. A TatAB complex, containing a large excess of the TatA subunit and having an apparent molecular mass of 600 kDa, has been isolated in detergent solution and the structure of the complex has been visualised at low resolution by negative stain electron microscopy [14]. A TatABC complex has been purified using alternative methodology. This complex also has an apparent molecular mass of approximately 600 kDa but the three subunits are present at an approximately equimolar ratio [15]. Since both the TatAB and TatABC complexes are very much larger than the molecular masses of the individual Tat subunits (TatA, 9.6 kDa; TatB, 18.4 kDa; TatC, 28.9 kDa) these observations raise the possibility that the Tat proteins have homo-oligomeric interactions. In particular the isolation of a predominantly TatA-containing TatAB complex points to the likelihood of protein–protein interactions between TatA protomers.

In this study we show that homo-oligomeric contacts do indeed represent major interprotein interactions for TatA and TatB. In addition we have examined the nature of the interaction between these proteins and the cytoplasmic membrane.

2. Materials and methods

The *E. coli* strains used were M15 (F^-, *lac*, *ara*, *gal*, *mtl*) harbouring pREP4 (Kan^R, $lacI^+$, Roche Molecular Biochemicals) and DADE (MC4100 Δ*tatABCD*Δ*tatE*) [13]. Strains were routinely cultured aerobically at 37°C in LB medium [16] supplemented with appropriate antibiotics. General recombinant DNA techniques were carried out as described [16]. Plasmids pFAT584 (*tatA*; Amp^R) and pFAT586 (*tatB*; Amp^R) were constructed as follows. The complete *tatA* coding sequence (minus stop codon) was amplified by PCR using primers: 5′-GCGCCCATGGGTGGTATCAGTATTTGGCAG-3′ and 5′-GCGCAGATCTCACCTGCTCTTTATCGTGGCGC-3′, digested with

*Corresponding author. Fax: (44)-1603-592250.
E-mail address: b.berks@uea.ac.uk (B.C. Berks).

[1] These authors contributed equally to this work.
[2] Address from 1st October 2001: Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; DSS, disuccinimidyl suberate; IMVs, inner membrane vesicles

0014-5793/01/$20.00
PII: S0014-5793(01)02904-0

*Nco*I–*Bgl*II and cloned into the expression vector pQE60 (Qiagen) to give pFAT584. Similarly, *tatB* (minus stop codon) was amplified by PCR using primers: 5′-GCGCCCATGGTGTTTGATATCGGTTT-TAGCGAAC-3′ and 5′-GCGCAGATCTCGGTTTATCACTCGAC-GAAGGGG-3′, digested with *Nco*I–*Bgl*II and cloned into pQE60 to give pFAT586. Each construct would incorporate a C-terminal hexahistidine tag onto the expressed protein. pFAT584 and pFAT586 were shown to complement strains JARV16 (Δ*tatA*Δ*tatE*) and BØD-P (Δ*tatB pcnB1*) respectively for growth with trimethylamine *N*-oxide as terminal electron acceptor [9] indicating that these plasmids express functional proteins. Plasmids pFAT587 (ΔTMS-*tatA*; Amp^R) and pFAT594 (ΔTMS-*tatB*; Amp^R) encoding the C-terminal domains of TatA and TatB were constructed as follows. The *tatA* sequence for codons 20–89 was amplified by PCR using primers 5′-GCGCGGATCCCTTTTTGGCACCAAAAAGCTCGGC-3′ and 5′-GCGCAAGCTTCGCTAAAACCGATATCAAACACGG-3′ digested with *Bam*HI plus *Hin*dIII and cloned into the expression vector pQE30 (Qiagen) to give pFAT587. Similarly, truncated *tatB* (codons 20–171 inclusive) was amplified by PCR using primers 5′-GCGC-GGATCCCTGGGGCCGCAACGACTGCCTGTG-3′ and 5′-GCG-CAAGCTTGCGGTTGAGTATCTTCTACAGAC-3′ digested with *Bam*HI plus *Hin*dIII and cloned into pQE30 to give pFAT594. Each construct substitutes an N-terminal hexahistidine tag for the predicted N-terminal transmembrane helix. All clones constructed by PCR were verified by DNA sequencing.

Crude membrane preparations and inner membrane vesicles (IMVs) were prepared as described previously [7,17]. sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting analysis were as described [18,19]. Samples were routinely incubated at 100°C for 3 min prior to electrophoresis. Anti-TatA and anti-TatB antibodies were raised and purified as described [14]. The TatC antibody was a gift from M. Alami and M. Müller, University of Freiburg, Germany.

3. Results

3.1. Investigating the nature of the membrane association of Tat subunits

The TatA, TatB and TatC proteins have previously been shown to co-localise to the *E. coli* cytoplasmic membrane [14]. To examine the nature of the membrane association of these proteins in more detail IMVs were purified from the Tat wild-type *E. coli* strain M15 by sucrose gradient centrifugation and then treated with various agents to discriminate between peripheral and integral interactions with the membrane (Fig. 1). TatA, TatB and TatC were all found to be resistant to extraction by salt, urea or alkali indicating that all three Tat subunits are integral membrane proteins.

While TatC is predicted to contain six predicted transmembrane helices, TatA and TatB have much more unusual, and less hydrophobic, predicted structures in which only the extreme N-terminus has the potential to form a classic hydrophobic transmembrane helix. However, both TatA and TatB contain extensive regions that are predicted to adopt amphipathic α-helical structures and which could also be involved in the membrane association of these proteins. We sought to investigate the relative importance of the predicted transmembrane and amphipathic helical regions in the membrane association of TatA and TatB. Plasmids were constructed that express engineered forms of TatA and TatB in which the predicted transmembrane domain is replaced by a hexahistidine affinity tag. Cells expressing the truncated TatA or TatB proteins, ΔTMS-TatA and ΔTMS-TatB respectively, were lysed and then subjected to differential centrifugation to examine the subcellular localisation of the shortened proteins (Fig. 2). Upon overexpression the ΔTMS-TatA protein (9142 Da) could be detected as a protein of apparent molecular mass of 16 kDa under SDS–PAGE. Anomalous electrophoretic mobility has previously been reported for the native TatA molecule [7,14]. The overexpressed ΔTMS-TatA protein was found exclusively in the high speed supernatant following subcellular fractionation (Fig. 2A). Indeed, even at uninduced levels of expression the protein was located in the water-soluble fraction as determined by immunoblotting (not shown). The truncated TatA protein does not, therefore, associate with the cytoplasmic membrane. In contrast, ΔTMS-TatB was evenly distributed over the high speed supernatant and high speed pellet fractions indicating a possible membrane interaction of this domain (Fig. 2B). To exclude the possibility that the truncated TatB protein forms micro-aggregates that cosediment with the crude membrane pellet IMVs were purified by sucrose gradient centrifugation from cells expressing ΔTMS-TatB at either low (uninduced) or high (induced) levels. In both cases ΔTMS-TatB co-localised with the IMVs (Fig. 2C) indicating that the ΔTMS-TatB domain is indeed associating with the inner membrane. To probe the nature of this interaction the IMVs from the induced culture were treated with high salt, urea or alkali (Fig. 2D). A substantial proportion of ΔTMS-TatB was removed from the membranes by all treatments including the control wash with buffer containing 50 mM NaCl. Thus, the ΔTMS-TatB domain has a weak and peripheral association with the cytoplasmic membrane.

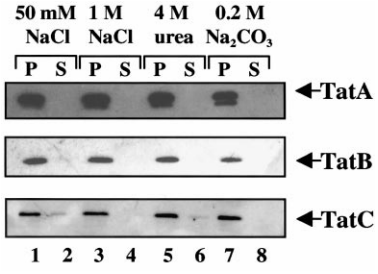

Fig. 1. TatA, TatB and TatC are integral inner membrane proteins. IMVs of M15 (pREP4) were extracted with 50 mM Tris–HCl, pH 7.5 containing 50 mM NaCl (lanes 1 and 2), 1 M NaCl (lanes 3 and 4) or 4 M urea (lanes 5 and 6). Alternatively the IMVs were extracted with 0.2 M Na_2CO_3 (lanes 7 and 8). Samples were separated by ultracentrifugation into pellet (P; lanes 1, 3, 5 and 7) and soluble (S; lanes 2, 4, 6 and 8) fractions and analysed by immunoblotting using antiserum against TatA (upper panel), TatB (middle panel) or TatC (lower panel). The lower band in the TatA blot is a non-specific cross-reaction.

The plasmid expressing the ΔTMS-TatA protein did not complement a Δ*tatA*Δ*tatE* mutant to a Tat^+ phenotype. It was also found that the plasmid expressing the ΔTMS-TatB protein was unable to complement the Tat^- phenotype of a Δ*tatB* mutant even though ΔTMS-TatB can be found in the membrane fraction. The observed membrane association of ΔTMS-TatB is therefore either non-functional, or the transmembrane helix of TatB has a role beyond that of a membrane anchor.

3.2. Homo-oligomeric interactions of the TatA and TatB proteins

To test the possibility that the Tat proteins participate in homo-oligomeric interactions each protein was individually expressed in the complete *tat* deletion strain DADE (Δ*tatABCD*Δ*tatE*). TatA and TatB were found to be localised to the membrane fraction under these conditions indicating that membrane targeting of these proteins does not depend on

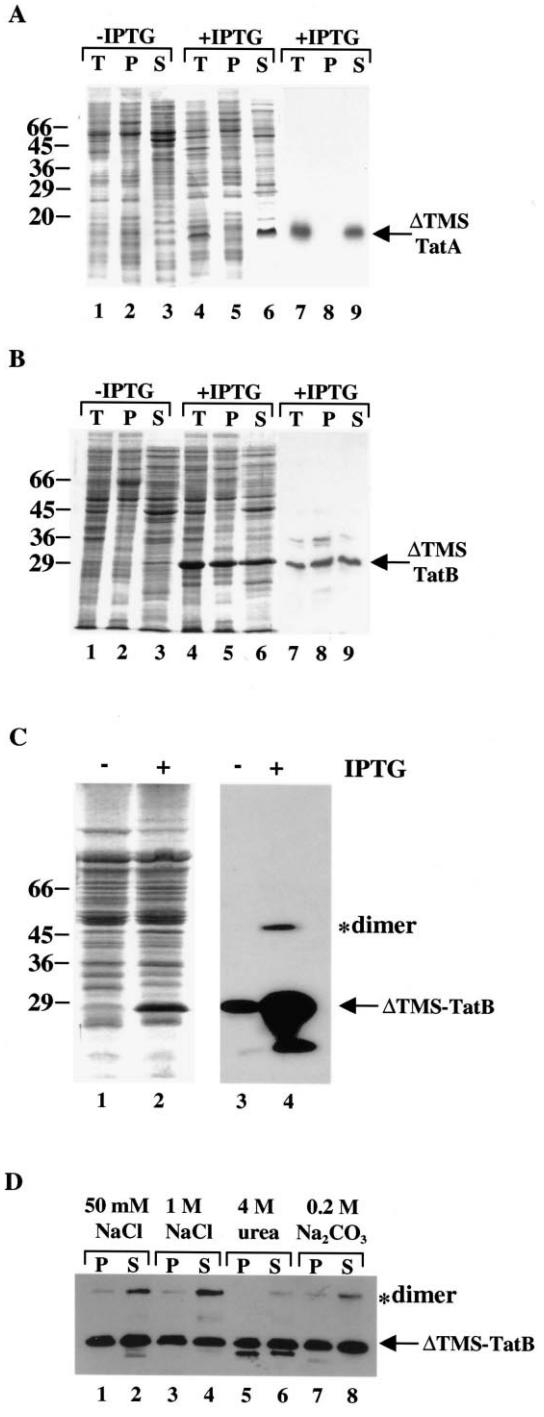

Fig. 2. The interaction of TatA and TatB with the membrane is perturbed by removing their predicted transmembrane helices. Subcellular fractionation of M15 (pREP4) cells expressing either (A) ΔTMS-TatA or (B) ΔTMS-TatB. Cells were grown to an OD_{660nm} of 0.3 and expression was left uninduced (lanes 1–3) or induced with 2 mM IPTG (lanes 4–9). Samples were collected 2 h after induction and then fractionated. Fractions corresponding to 10 μg of protein were analysed by SDS–PAGE and Coomassie brilliant blue staining (lanes 1–6) or immunoblotting (lanes 7–9) using anti-TatA (A) or anti-TatB (B) antisera. Lanes 1, 4 and 7 are the total cell lysates (T), lanes 2, 5 and 8 the crude membrane fraction (P) and lanes 3, 6 and 9 the water-soluble fraction (S). C: IMVs of M15 (pREP4) expressing ΔTMS-TatB at uninduced (−IPTG; lanes 1 and 3) or induced (+IPTG; lanes 2 and 4) levels were purified by sucrose gradient centrifugation and analysed by SDS–PAGE followed by staining with Coomassie brilliant blue (lanes 1 and 2) or immunoblotting with anti-TatB antiserum (lanes 3 and 4). D: Purified IMVs of M15 (pREP4) induced for expression of ΔTMS-TatB were extracted with 50 mM Tris–HCl, pH 7.5 containing 50 mM NaCl (lanes 1 and 2), 1 M NaCl (lanes 3 and 4) or 4 M urea (lanes 5 and 6). Alternatively the IMVs were extracted with 0.2 M Na_2CO_3 (lanes 7 and 8). Following ultracentrifugation the pellet (P; lanes 1, 3, 5 and 7) and supernatant (S; lanes 2, 4, 6 and 8) fractions were analysed by immunoblotting using antiserum directed against TatB. For A–C the molecular masses in kDa of marker proteins are given to the side of the gels.
←

other Tat components (Fig. 3A). TatC could not be detected when expressed alone probably because, as previously reported [9], this protein is unstable in the absence of TatB. The membranes containing TatA or TatB alone were solubilised in the detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and the resulting soluble extract fractionated by size exclusion chromatography. Fractions containing the Tat proteins were identified by immunoblotting and the apparent native molecular mass of the eluted material estimated by reference to water-soluble standards (Fig. 3B). The TatA protein eluted across a very broad range of fractions but was enriched in fractions corresponding to apparent molecular masses of between 100 and 200 kDa (Fig. 3B; upper panel). TatB eluted as a distinct peak of molecular mass of approximately 150–200 kDa (Fig. 3B; lower panel). The apparent molecular mass of TatA and TatB determined by gel permeation chromatography will differ from the true protein molecular mass due to both hydrodynamic factors and bound detergent. Nonetheless, given the very low molecular masses of the individual TatA and TatB polypeptides, the results of the gel permeation experiment suggest that these polypeptides are present in detergent extract as homo-oligomeric complexes. Gel permeation chromatography of ΔTMS-TatA in crude soluble extracts gave an apparent molecular mass for this protein of below 30 kDa (data not shown). This observation suggests that the truncated protein is either monomeric or forms smaller oligomers than the native TatA protein.

To investigate whether TatA and TatB also exhibit homo-oligomeric interactions in the membrane environment chemical crosslinking studies were carried out. Membrane preparations from DADE cells individually expressing either TatA or TatB were treated with the membrane-permeable, primary amine-specific, crosslinking agent disuccinimidyl suberate (DSS). Following DSS treatment of the TatA-containing membranes and immunoblotting with anti-TatA antiserum, two TatA-containing crosslinked products were detected with apparent molecular masses of 25 kDa and 35 kDa (Fig. 4A, compare lanes 1 and 2). Since TatA exhibits highly anomalous migration under SDS–PAGE it is difficult to infer the true mass of the proteins that are crosslinked to TatA (9.6 kDa) in these products. Nevertheless, given that TatA has only ever been reported to co-purify with TatB or TatC [14,15], and that neither TatB or TatC is present in the membranes used in this experiment, it is most likely that the 25 kDa and 35 kDa crosslinked species represent TatA homodimers and homotrimers respectively. A similar experiment was performed using membranes expressing only TatB. In this case a single TatB-containing crosslinked product of apparent molecular mass 45 kDa was detected (Fig. 4B, lanes 1

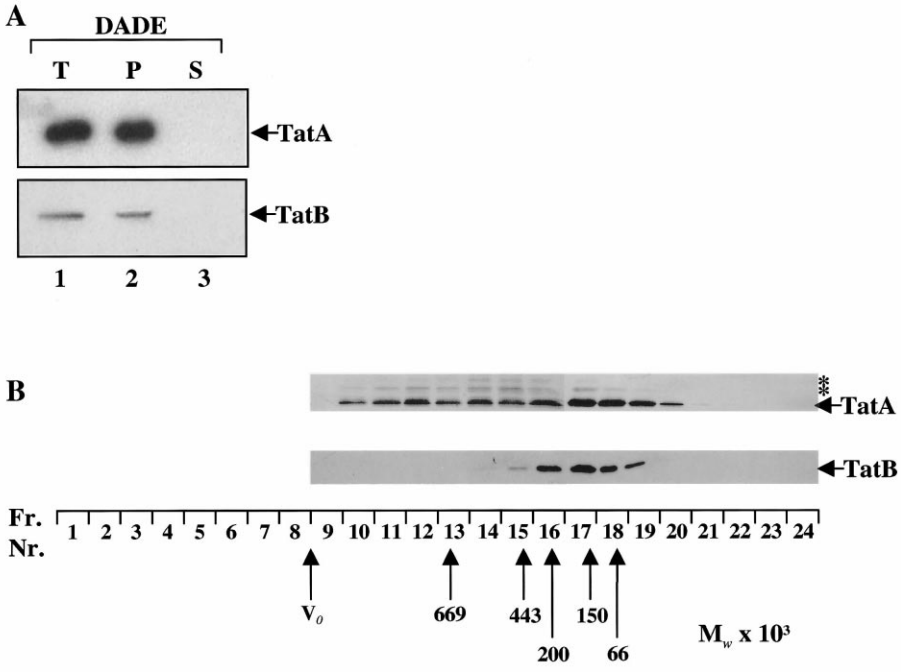

Fig. 3. TatA and TatB form homo-oligomeric complexes in detergent solution. A: Whole DADE (Δ*tatABCD*Δ*tatE*) cells (T; lanes 1) expressing TatA (top panel) or TatB (bottom panel) were disrupted by sonication followed by differential centrifugation to separate pellet (P; lanes 2) and soluble (S; lanes 3) fractions. TatA (top panel) and TatB (bottom panel) were detected by immunoblotting using anti-TatA and anti-TatB antiserum respectively. B: Crude membranes prepared from DADE (Δ*tatABCD*Δ*tatE*) cells expressing TatA or TatB were solubilised in separate experiments in 20 mM MOPS pH 7.2, 50 mM NaCl, 5% glycerol, 50 mM CHAPS. Insoluble material was removed by ultracentrifugation and the supernatant fraction loaded on a Superose-6 HR10/30 column (Amersham Pharmacia Biotech) equilibrated with 20 mM Na–MOPS pH 7.2, 50 mM NaCl, 5% glycerol, 5 mM CHAPS. Fractions eluting from the column were subjected to TCA precipitation and analysed by SDS–PAGE and immunoblotting using anti-TatA antibodies for the TatA extract (upper panel) or anti-TatB antibodies for the TatB extract (lower panel). The elution positions of water-soluble marker proteins and of the void volume of the column (V_o) are indicated at the bottom of the figure. The marker proteins used were thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa) and bovine serum albumin (66 kDa).

and 2). Using analogous reasoning to the TatA experiment this crosslinked product is best interpreted as a TatB homodimer. It is interesting to note at this juncture that a proportion of the ΔTMS-TatB protein in cell extracts runs as an apparent dimer under SDS–PAGE (Fig. 2C and D).

DSS crosslinking experiments were also carried out on membranes from cells with a wild-type *tat* genotype. Immunoblotting analysis demonstrates that the crosslinked TatA and TatB products formed in this experiment are the same as those detected with the membranes containing only TatA or TatB (Fig. 4). This shows that the homo-oligomeric contacts observed are physiologically relevant since they are also seen in membranes expressing a wild-type Tat system. In addition these experiments demonstrate that the self–self interactions detected are not influenced by the presence of other Tat components.

For comparative purposes crosslinking experiments were carried out with a second type of primary amine-specific reagent, formaldehyde. Membrane preparations from cells expressing only TatA or TatB were treated with formaldehyde and the TatA- or TatB-containing products analysed by immunoblotting (Fig. 5). The crosslinked products corresponding to the TatB homodimers and the TatA homodimers and homotrimers that were observed upon reaction with DSS were also detected in the formaldehyde-treated membranes. In addition a putative TatA homotetramer with an apparent molecular mass of 45 kDa was evident in TatA-containing membranes treated with 1% formaldehyde (Fig. 5A). As expected, the formaldehyde crosslinking could be partially reversed by heating the samples (Fig. 5, lanes 3 and 5).

No crosslinks were detected for the ΔTMS-TatA protein in soluble extracts treated with either DSS or formaldehyde (data not shown). This observation supports the view that ΔTMS-TatA is a monomer.

4. Discussion

Here we show that all three of the essential Tat components TatA, TatB and TatC are fully integral membrane proteins. For the homologous TatA and TatB proteins the membrane interaction is independent of the presence of other Tat components while independently expressed TatC proved to be too unstable to study. Both TatA and TatB contain a proposed N-terminal transmembrane helix together with substantial polypeptide regions predicted to form amphipathic helical structures [11]. In the absence of the predicted transmembrane helix TatA failed to interact with the cytoplasmic membrane. For the TatB protein removal of the predicted transmembrane helix did not abolish peripheral interactions with the cytoplasmic membrane. This residual interaction might be mediated by the more extended amphipathic region present in TatB relative to that found in TatA. However, for both TatA and TatB the transmembrane helix was shown to be required for Tat pathway function.

Homo-oligomeric interactions between Tat protomers can be predicted both from theoretical considerations [4] and from

the large size of the purified Tat complexes relative to the individual Tat components [14,15]. Here we provide direct experimental support for this conjecture from chemical crosslinking studies and from size exclusion chromatography of detergent solubilised membranes containing single types of Tat subunit. Remarkably the crosslinking experiments suggest that for both TatA and TatB the most intimate protein–protein interactions in wild-type membranes are those between chemically identical subunits. These TatA and TatB self–self interactions are retained in the absence of other Tat components suggesting that higher order structures may be assembled from homo-oligomeric units. Experiments using DSS as the crosslinker suggest that TatA can form homotrimers and TatB homodimers. However, these are minimum estimates of the possible size of the oligomers since it cannot be excluded that higher oligomers are present but not detected due to inefficient crosslink formation or a lack of closely juxtaposed primary amine groups at the interface region. Indeed, a possible TatA tetramer was detected by formaldehyde crosslinking. Further, the size exclusion experiments detected a portion of the TatA proteins at very high molecular masses. It is possible that these higher molecular mass species approximate to the predominantly TatA-containing TatAB complexes purified by Sargent and co-workers [14] but that they are destabilised by the absence of TatB. Both TatA and TatB are unusually rich in lysine residues (13.5% and 7.6% respectively). However, these residues are not highly clustered in either sequence and as a consequence it is not possible to identify the regions of each polypeptide that are involved in

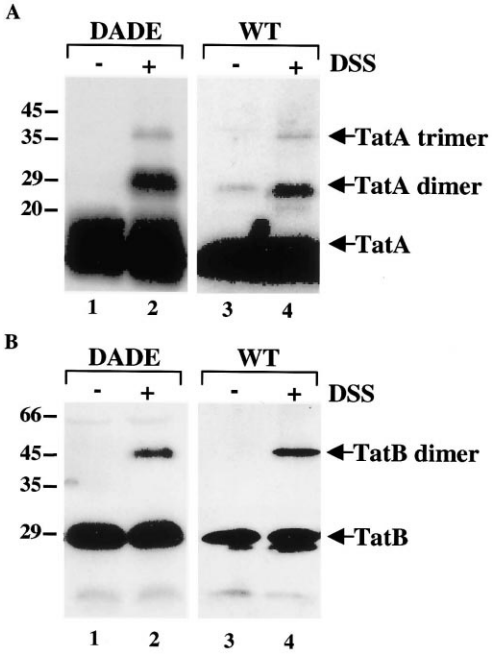

Fig. 4. Detection of TatA and TatB homo-oligomers by DSS crosslinking. Crude membranes prepared from DADE (Δ*tatABCD*Δ*tatE*) cells expressing TatA (A, lanes 1 and 2), DADE cells expressing TatB (B, lanes 1 and 2) or M15 (pREP4) cells (A and B, lanes 3 and 4) were suspended in 50 mM K–HEPES pH 7.5, 50 mM KCl and either left untreated (lanes 1 and 3), or incubated with 2 mM DSS (lanes 2 and 4), on ice for 1 h. The crosslinking reactions were quenched by the addition of 0.1 volumes of 1 M Tris–HCl pH 8.0 and the samples were analysed by immunoblotting using (A) anti-TatA antibodies or (B) anti-TatB antibodies. The molecular masses in kDa of marker proteins are given to the side of the gels.

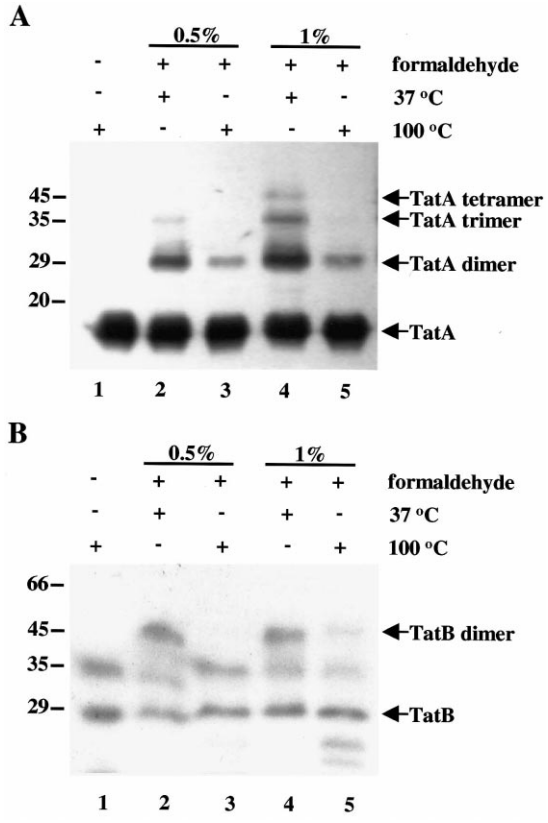

Fig. 5. Detection of TatA and TatB homo-oligomers by formaldehyde treatment of membranes. Crude membranes prepared from DADE (Δ*tatABCD*Δ*tatE*) cells expressing TatA (A) or TatB (B) were suspended in 50 mM K–HEPES pH 7.5, 50 mM KCl and either left untreated (lanes 1) or incubated with 0.5% (lanes 2 and 3) or 1% (lanes 4 and 5) formaldehyde for 20 min at 20°C. The samples in lanes 1, 3 and 5 were boiled for 5 min prior to loading while those in lanes 2 and 4 were incubated at 37°C for 5 min. Samples were analysed by immunoblotting using (A) anti-TatA antibodies or (B) anti-TatB antibodies. The band at approximately 35 kDa present in all lanes in B is a non-specific cross-reaction found in this batch of affinity-purified antiserum. The molecular masses in kDa of marker proteins are given to the side of the gels.

the homo-oligomeric crosslinks. Since no self–self crosslinks were observed for ΔTMS-TatA it appears that the TatA transmembrane helix is necessary for the formation of the TatA homo-oligomers. The generation of self–self crosslinks for both TatA and TatB with the one carbon reagent formaldehyde indicates that the protomers are in intimate contact.

Acknowledgements: We thank Verity Lyall for technical support and Meriem Alami and Matthias Müller for the gift of the anti-TatC serum. This work was supported by the Commission of the European Community through the programme 'ExporteRRs' and by the Biotechnology and Biological Sciences Research Council of the UK through project grant 88/P09634. I.P. is the recipient of a studentship from the School of Biological Sciences, University of East Anglia. F.S. and T.P. are Royal Society University Research Fellows. B.C.B. is RJP Williams Senior Research Fellow at Wadham College, Oxford.

References

[1] Pugsley, A.P. (1993) Microbiol. Rev. 57, 50–108.
[2] Manting, E.H. and Driessen, A.J.M. (2000) Mol. Microbiol. 37, 226–238.
[3] Berks, B.C. (1996) Mol. Microbiol. 22, 393–404.

[4] Berks, B.C., Sargent, F. and Palmer, T. (2000) Mol. Microbiol. 35, 260–274.
[5] Keegstra, K. and Cline, K. (1999) Plant Cell 11, 557–570.
[6] Weiner, J.H., Bilous, P.T., Shaw, G.M., Lubitz, S.P., Frost, L., Thomas, G.H., Cole, J.A. and Turner, R.J. (1998) Cell 93, 93–101.
[7] Sargent, F., Bogsch, E.G., Stanley, N.R., Wexler, M., Robinson, C., Berks, B.C. and Palmer, T. (1998) EMBO J. 17, 3640–3650.
[8] Bogsch, E.G., Sargent, F., Stanley, N.R., Berks, B.C., Robinson, C. and Palmer, T. (1998) J. Biol. Chem. 273, 18003–18006.
[9] Sargent, F., Stanley, N.R., Berks, B.C. and Palmer, T. (1999) J. Biol. Chem. 274, 36073–36082.
[10] Yahr, T.L. and Wickner, W.T. (2001) EMBO J. 20, 2472–2479.
[11] Settles, A.M., Yonetani, A., Baron, A., Bush, D.R., Cline, K. and Martienssen, R. (1997) Science 278, 1467–1470.
[12] Jack, R.L., Sargent, F., Berks, B.C., Sawers, G. and Palmer, T. (2001) J. Bacteriol. 183, 1801–1804.
[13] Wexler, M., Sargent, F., Jack, R.L., Stanley, N.R., Bogsch, E.G., Robinson, C., Berks, B.C. and Palmer, T. (2000) J. Biol. Chem. 275, 16717–16722.
[14] Sargent, F., Gohlke, U., de Leeuw, E., Stanley, N.R., Palmer, T., Saibil, H.R. and Berks, B.C. (2001) Eur. J. Biochem. 268, 3361–3367.
[15] Bolhius, A., Mather, J.E., Thomas, J.D., Barrett, C.M. and Robinson, C. (2001) J. Biol. Chem. 276, 20213–20219.
[16] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[17] de Vrije, T., Tommassen, J. and de Kruijff, B. (1987) Biochim. Biophys. Acta 900, 63–72.
[18] Lämmli, U.K. (1970) Nature 277, 680–685.
[19] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.