TatAc, the Third TatA Subunit of *Bacillus subtilis*, Can Form Active Twin-Arginine Translocases with the TatCd and TatCy Subunits

Carmine G. Monteferrante,* Jacopo Baglieri, Colin Robinson, and Jan Maarten van Dijl*

Department of Medical Microbiology, University of Groningen and University Medical Center Groningen, Groningen, the Netherlands,* and School of Life Sciences, University of Warwick, Coventry, United Kingdom

Two independent twin-arginine translocases (Tat) for protein secretion were previously identified in the Gram-positive bacterium *Bacillus subtilis*. These consist of the TatAd-TatCd and TatAy-TatCy subunits. The function of a third TatA subunit named TatAc was unknown. Here, we show that TatAc can form active protein translocases with TatCd and TatCy.

Protein transport from the cytoplasm to different bacterial compartments or the external milieu is facilitated by dedicated molecular machines (6). Among these protein translocases, the twin-arginine translocases (Tat) stand out because they permit the passage of tightly folded proteins across the cytoplasmic membrane. The proteins translocated by Tat are synthesized with signal peptides that contain a well-conserved twin-arginine (RR) motif for specific targeting to a membrane-embedded Tat translocase (13, 17, 23). The Tat translocases of Gram-negative bacteria, such as *Escherichia coli*, are composed of three subunits named TatA, TatB, and TatC (4, 18). The formation of an active protein-conducting channel is believed to require the formation of a supercomplex composed of a TatABC heterotrimeric complex and homo-oligomeric TatA complexes (1, 8). In contrast, most Gram-positive bacteria possess minimized Tat translocases that contain only TatA and TatC subunits. Nevertheless, various studies indicate that these TatAC translocases employ a mechanism similar to that of the TatABC translocases of Gram-negative bacteria (10, 17).

The Gram-positive bacterium *Bacillus subtilis* is a well-known “cell factory” for secretory protein production (20, 21). In this organism, two Tat translocases are known to operate in parallel. The TatAdCcd translocase consists of the TatAd and TatCd subunits, and the TatAyCy translocase consists of the TatAy and TatCy subunits (11, 12, 15). While the TatAdCcd translocase is produced mainly under conditions of phosphate starvation (12, 14, 15), the TatAyCy translocase is expressed under all conditions tested (12, 14). Interestingly, *B. subtilis* produces a third TatA subunit named TatAc (12). The function of TatAc has remained enigmatic due to the fact that no phenotype was so far detectable for tatAc mutant *B. subtilis* cells (11, 12, 20). Therefore, the present studies were aimed at determining whether TatAc can actually form active translocases in combination with TatCd or TatCy. This possibility was tested by expressing the respective *tat* genes in *E. coli*, because the activity and assembly of *Bacillus* Tat translo
cases can be assayed more readily in this organism than in *B. subtilis* (2). For this purpose, the tatAc gene was amplified from the *B. subtilis* genome (GenBank/EMBL/DDBJ accession number AL009126) and cloned into plasmid pBAD24, resulting in pBAD-Ac. Next, the tatCd and tatCy genes were PCR amplified such that the respective proteins contain a C-terminal StreptII tag. The amplified *tatCd-StreptII* and *tatCy-StreptII* genes were cloned into pBAD-Ac, resulting in pBAD-AcCd-Strep and pBAD-AcCy-Strep, respectively. These vectors were subsequently used to trans-
lowed by immunoblotting with antibodies against the StrepII tag. As shown in Fig. 3, TatCd-StrepII and TatCy-StrepII alone formed bands of ~66 kDa. In addition, TatCd-StrepII formed a minor band of ~100 kDa. TatAc-StrepII expressed by itself formed a small homogeneous complex of ~100 kDa. Importantly, when TatAc (nontagged) was coexpressed with either TatCd-StrepII or TatCy-StrepII, bands of ~230 kDa or ~200 kDa, respectively, were observed. This showed that TatAc does indeed form membrane-embedded complexes with TatCd and TatCy.

In conclusion, our present studies document for the first time that the hitherto enigmatic third TatA subunit of *B. subtilis* known as TatAc can engage in the formation of active TatAC-type trans-

FIG 1  *B. subtilis* TatAcCd and TatAcCy facilitate TorA export in *E. coli*. Cells of *E. coli* ΔtatABCDE were subjected to subcellular fractionation. Proteins in the periplasmic (P), membrane (M), and cytoplasmic (C) fractions obtained were separated by native PAGE, and the gels were subsequently analyzed for TMAO reductase (TorA) activity. Strains used in this analysis were *E. coli* MC4100 (WT), *E. coli* ΔtatABCDE (Δtat), or *E. coli* ΔtatABCDE expressing *B. subtilis* TatAc from plasmid pBAD-Ac-Strep (Δtat + TatAc), *B. subtilis* TatCd from plasmid pBAD-Cd-Strep (Δtat + TatCd), *B. subtilis* TatCy from plasmid pBAD-Cy-Strep (Δtat + TatCy), *B. subtilis* TatAcCd from plasmid pBAD-AcCd-Strep (Δtat + TatAcCd), or *B. subtilis* TatAcCy from plasmid pBAD-AcCy-Strep (Δtat + TatAcCy). The position of active full-length TorA is indicated. TorA* indicates a faster-migrating form of TorA (22).

FIG 2  *B. subtilis* TatAcCd and TatAcCy facilitate AmiA and AmiC export in *E. coli*. The export of AmiA and AmiC in *E. coli* was assayed indirectly by assessing the chain length of exponentially growing cells. (A) *E. coli* MC4100 (WT); (B) *E. coli* ΔtatABCDE forms long chains due to the mislocalization of AmiA and AmiC (9); (C) *E. coli* ΔtatABCDE producing TatAcCd from plasmid pBAD-AcCd-Strep; (D) *E. coli* ΔtatABCDE producing TatAcCy from plasmid pBAD-AcCy-Strep. As evidenced by the significantly reduced chain length, the export of AmiA and AmiC in *E. coli* ΔtatABCDE is at least partially restored by the production of TatAcCd or TatAcCy.