Chaperones involved in the Assembly and Export of Tat-dependent Proteins

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Abstract

The twin-arginine translocation (Tat) system is a protein-targeting pathway found in the membranes of many prokaryotes and in plant chloroplasts and mitochondria. Proteins transported on the Tat pathway are all synthesized as precursors with distinctive N-terminal signal peptides bearing a conserved SRRxFLK 'twin-arginine' amino acid motif. Furthermore, all Tat-dependent substrate proteins require to be fully folded prior to translocation. In this work, the paradigm Tat pathway of the model bacterium *Escherichia coli* has been studied. The majority of *E. coli* Tat substrates are complex metalloproteins associated with respiratory electron transport chains and such enzyme systems are often multi-subunit and contain numerous redox cofactors. It is important that Tat dependent redox enzymes are fully assembled before transport and it is likely, therefore, that cellular mechanisms exist to prevent premature export of immature substrates. This quality control mechanism is commonly referred to as 'proofreading'.

The most direct route to prevent premature export of a Tat substrate would be to mask the twin-arginine signal peptide from the membrane-bound Tat translocase until all assembly processes were complete. Using the *E. coli* Tat-dependent [NiFe] hydrogenases as models, a bacterial two-hybrid assay was employed to identify precursor-binding proteins. These experiments identified the previously uncharacterised HyaE protein as a specific chaperone for the Tat signal-bearing subunit (HyaA) of the Hydrogenase-1 respiratory complex. Subsequent genetic deletion of the *hyaE* gene demonstrated that HyaE protein was required to achieve maximal cellular hydrogen uptake activity, but that this gene product was not an obligate Tat targeting factor.

Biochemical characterization of purified HyaE demonstrated that the protein was indeed a Tat proofreading chaperone. The HyaE protein was shown to interact with the HyaA signal peptide *in vitro* with a dissociation constant of ~11 μM. HyaE was also shown to specifically bind GTP with a K_D of ~ 85 μM and the presence of excess Tat signal peptide *in vitro* induced a slow GTPase activity. It is likely, therefore, that HyaE acts as a *bona fide* Tat proofreading chaperone masking the HyaA twin-arginine signal peptide until GTP-hydrolysis releases the chaperone and exposes the now-active signal peptide to the translocase.

Finally, in attempt to assess the generality of mechanism of Tat proofreading chaperones, the roles of TorD and DmsD in assembly and export of Tat-dependent molybdenum-containing redox enzymes were explored. Like HyaE, TorD and DmsD are involved in the Tat proofreading process and, although not related in sequence to HyaE, share a commonality in function since each binds specific twin-arginine signal peptides and GTP. It is tempting to speculate, therefore, that there has been an evolutionary convergence towards a common Tat proofreading mechanism for complex bacterial respiratory enzymes.