Common principles in the biosynthesis of diverse enzymes

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Abstract

A subset of bacterial periplasmic enzymes are transported from the cytoplasm by the twin-arginine transport apparatus. Such proteins contain distinctive N-terminal signal peptides containing a conserved SRRXFLK ‘twin-arginine’ amino acid motif and often bind complex cofactors before the transport event. It is important that assembly of complex cofactor-containing, and often multi-subunit, enzymes is complete before export. Studies of the unrelated [NiFe] hydrogenase, DMSO reductase and trimethylamine N-oxide reductase systems from Escherichia coli have enabled us to define a chaperone-mediated ‘proofreading’ mechanism involved in co-ordinating assembly and export of twin-arginine transport-dependent enzymes.

Introduction

Escherichia coli displays a remarkable flexibility in its respiratory electron transport processes. As an obligate anaerobe, E. coli can utilize nitrate, nitrite, fumarate, trimethylamine N-oxide and DMSO as terminal electron acceptors as alternatives to oxygen [1]. E. coli can also perform a mixed-acid fermentation in the absence of any exogenous electron acceptors. In addition, NADH, formate, glycerol 3-phosphate, succinate, lactate, pyruvate and hydrogen can all be utilized as respiratory electron donors [1]. All of these redox reactions are performed by complex cofactor-containing enzymes localized within, or close to, the cytoplasmic membrane. The cell has developed intricate mechanisms, therefore, to facilitate the biosynthesis of complex membrane-bound and extracellular proteins.

In E. coli, respiratory hydrogen oxidation (‘uptake’) linked to quinone reduction is performed by two hydrogenase iso-enzymes, hydrogenase-1 and hydrogenase-2 [2]. Each is a multisubunit, membrane-bound, nickel-containing Fe-S protein, and the bulk of such proteins, including the subunits binding the Ni-Fe active sites, are exposed to the periplasmic space. The [NiFe] hydrogenase-1 isoenzyme is encoded by an operon of six genes, hyaABCDEF. Minimally, the core enzyme consists of a heterodimer of an Fe-S cluster-binding β-subunit (HyαA), together with an α-subunit that binds the Ni-Fe active site cofactor (HybA). The [NiFe] hydrogenase-2 isoenzyme is encoded by an operon of eight open reading frames, hybOABCDEFG. The core catalytic αβ2-dimer consists of the HybOC complex in which HybC is the α-subunit and HybO is the β-subunit. Both uptake hydrogenases are synthesized as precursors with N-terminal ‘twin-arginine’ signal peptides that direct the preassembled complexes across the cytoplasmic membrane [3].

The Tat (twin-arginine transport) protein transport system

Twin-arginine signal peptides are found on a subset of exported proteins, including all extracellular [NiFe] and [Fe] hydrogenases so far identified [2], and contain a distinctive conserved SRRXFLK ‘twin-arginine’ amino acid sequence motif [3]. Precursor proteins bearing twin-arginine signal peptides are recognized and transported post-translationally across the cytoplasmic membrane by the Tat system [4]. Most importantly, Tat substrates must be fully folded before transport can be competed. In E. coli, the integral membrane proteins TatA, TatB and TatC form the core components of the Tat translocation apparatus (or ‘translocon’). TatA and TatB are small sequence-related proteins each comprising an N-terminal transmembrane helix followed by a cytoplasmic C-terminal domain. TatC is a polytopic integral membrane protein with six transmembrane segments and both termini at the cytoplasmic face of the bilayer. In E. coli, TatB and TatC form a stable unit (the ‘signal-binding module’) containing the twin-arginine signal peptide recognition site. The E. coli TatA protein forms a separate large oligomeric ring-structure supposed to be the protein-conducting channel (the ‘channel module’) [4]. It is thought that binding of a Tat substrate to the signal-binding module (TatBC) allows the channel module (TatA) to dock on to it and ultimately facilitate transport of the substrate protein [4]. A detailed mechanistic map of this process is not yet available; however, it is known that a transmembrane electrochemical gradient is absolutely essential for Tat export and there is some evidence to suggest that the Tat system does indeed act as a protein-proton antiporter [5].
‘Proofreading’ versus ‘quality control’

Assembly of the core hydrogenase αβ-unit requires careful co-ordination of cofactor biosynthesis and insertion, subunit recruitment and protein targeting processes. It is very important that Tat-mediated transport does not take place before the cofactor insertion into the α- and β-subunits is complete, and before the signalless α-subunit has docked with its β-subunit partner. Cellular mechanisms must exist, therefore, to prevent premature targeting of the signal-containing β-subunit until all of these processes are completed. Indeed, such a system of ‘proofreading’ has been postulated ever since the Tat translocon was first conceived [6]. Note that the phrase ‘proofreading’ in the context of Tat is taken to mean the monitoring or checking of the assembly state of a Tat substrate before export. The alternative phrase ‘quality control’ would have been the preferred and more accurate description of this process. However, this has recently been commandeered to describe a slightly different process by which the TatABC translocon accepts or rejects substrates based on their fundamental folded state [7]. Thus, while the Tat ‘quality control’ system will be in operation for all traffic on the Tat pathway, a ‘proofreading’ system, as we have defined it, is an added-extra in the biosynthesis of complex cofactor-containing (often multisubunit) extracellular proteins.

TorD is a proofreading chaperone on the Tat pathway

Among the most remarkable features of twin-arginine signal peptides is their sequence conservation. Closely related enzymes such as uptake hydrogenases, for example, not only share sequence identity within the actual enzyme subunits but also within the signal peptides [3]. Such selective pressure has never been seen for signal peptides of the alternative general secretory (Sec) system and suggests that Tat signal peptides may provide some special activities in the export of their cognate passenger proteins [3]. Recent ‘signal swapping’ experiments seem to bear-out this theory. Swapping of the native signal peptide of the E. coli DMSO reductase for that of the hydrogenase-1 αβ-subunit before docking of the hydrogenase-2 subunit appears to be essential for DMSO reductase assembly [12]. DmsD has been shown to be essential for DMSO reductase assembly [12], and therefore the experiment of swapping the DmsA signal peptide for that of TorA [8] would also have removed an essential biosynthetic protein from the assembly process.

Hydrogenase-specific proofreading chaperones

The functions of the accessory genes encoded by the E. coli hya and hyb operons have been characterized to variable degrees and most are involved in assembly of the nickel cofactor [2]. However, since no homologues of either HyaE or HybE are required for the biosynthesis of cytoplasmic hydrogenases, it was postulated that these proteins may have a specific role in maturation of Tat-dependent hydrogenases [13]. Subsequent two-hybrid work demonstrated that HyaE interacts specifically with the precursor form of HyaA, the hydrogenase-1 β-subunit, and that HybE interacts specifically with the β-subunit precursor of hydrogenase-2, HybO [14]. It is tempting to speculate that HyaE and HybE are signal peptide binding proteins, but there is as yet no direct evidence available. The phenotype of an E. coli hyaE mutant could not shed further light on its physiological role [13]; however, the phenotype of a hybE mutant was much more forthcoming [9]. A ΔhybE strain has very low hydrogenase-2 activity, apparently due to the premature targeting of the β-subunit before docking of the α-subunit [9]. In fact, this phenotype was very reminiscent of that observed for the signal swap experiment [9], again suggesting that removal of a native signal peptide also removes a chaperone from the biosynthetic process.

Despite the ability of the TorA signal/TorD couple to complete the maturation of an E. coli hydrogenase, HyaE and HybE are not related to TorD in sequence. The HyaE protein is a homologue of Ralstonia eutropha HoxO and Rhizobium leguminosarum HupG, and HybE is a homologue of R. eutropha HoxT and Rh. leguminosarum HupJ [11].
Interestingly, these proteins are not present in Wolinella succinogenes, however, it seems that a completely unrelated protein (HydE) performs as a proofreading chaperone for the Tat-dependent hydrogenase in that bacterium [15].

Concluding remarks
This essay highlights that some twin-arginine signals operate in tandem with specific binding chaperones in a proofreading process that prepares the complex Tat substrates for export. Direct binding of a twin-arginine signal peptide by a specific chaperone would ‘mask’ the signal from the Tat translocon until all other assembly processes were complete. In E. coli, TorA utilizes TorD as a proofreading chaperone, DmsA has DmsD, hydrogenase-2 has HybE and hydrogenase-1 may utilize HyaE for the same purpose. As a result, removing native signal peptides from complex enzymes may also have the knock-on effect of removing any proofreading chaperones from the assembly process. Indeed, moving Tat signal peptides on to heterologous reporter proteins may also introduce chaperone activity, which could add unnecessary layers to an already complicated export process.

Finally, the remarkable ability of the TorA signal/TorD couple to be actively transposed on to completely unrelated systems, such as hydrogenase, clearly suggests that the biochemical principles of this proofreading mechanism are functionally conserved across a broad spectrum of Tat-dependent enzymes. In principle, a hydrogenase-based signal-fusion assay could be used to identify signal peptide/chaperone pairs from any biological system.

R.I.J. was funded by BBSRC grant 88/P11832 and A.D. by a University of East Anglia postgraduate studentship. T.P. is an MRC Senior Non-Clinical Research Fellow and F.S. is a Royal Society University Research Fellow.

References

Received 30 September 2004