Amplified expression, purification and characterisation of antibiotic resistance transport proteins

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Introduction

Membrane transport proteins are involved in antibiotic resistance, nutrient capture, environmental sensing and other vital functions in bacteria (e.g. Figure 1). However, membrane proteins are notoriously difficult to study. Owing to their extreme hydrophobicity they are refractory to direct manipulation and can only be removed from the membrane, and their solubility maintained, in the presence of detergent. In addition they are usually only expressed at low levels and constitute less than 0.1% of total cell protein. Such difficulties help to explain why, although the structures of thousands of soluble proteins have been solved, to date less than thirty membrane protein structures have been resolved to atomic resolution.

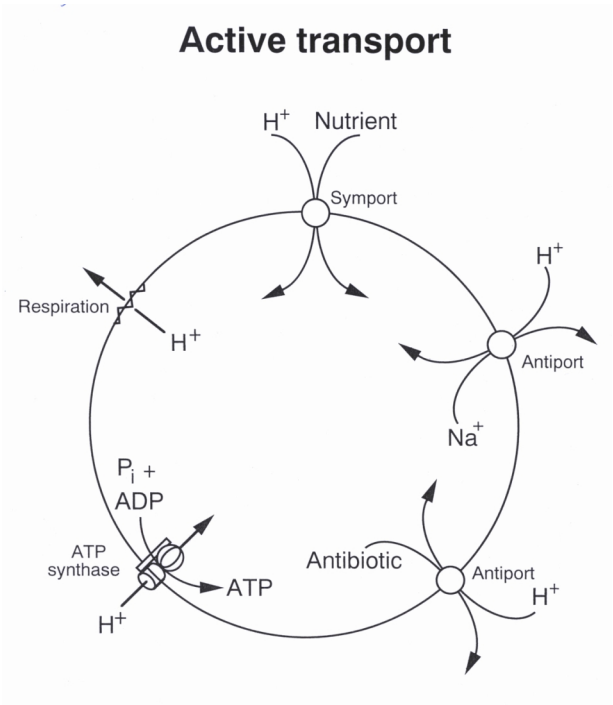

Figure 1. Secondary active transport systems in bacteria. The large circle represents the cytoplasmic membrane of the microorganism. A transmembrane electrochemical gradient of protons is generated by respiration, shown on the left. The gradient may be used to drive ATP synthesis and the proton-nutrient symport and proton-substrate antiport secondary active transport systems shown around the circumference. Each transporter is generally a single protein, usually of the 12-helix type.

Multidrug resistance proteins (Mdr's)

Analyses of the available bacterial genomes predict that transport proteins comprise 3-12% of the protein complement. Further examination of genomes from both prokaryote and eukaryote organisms reveals that many membrane transport proteins comprise the 'Major Facilitator Superfamily' (MFS) and may operate by facilitated diffusion, symport or antiport (Figure 1). MFS proteins are thought to be single polypeptides comprising 10-14 (usually 12) trans-membrane α–helices. In bacteria individual MFS proteins called Mdr's accomplish the active efflux of compounds like antibiotics, antibacterials, or toxins by a cation/substrate antiport mechanism (Figure 1), leading to resistance. Examples include the *Bacillus subtilis* transporter, Bmr, the *Staphylococcus aureus* norfloxacin transporter, NorA and the *Lactococcus lactis* transporter, LmrA. Curiously, the prokaryotic MFS multidrug efflux proteins are homologous to the vesicular monoamine transport proteins that function in neurotransmitter storage in nerve tissue. The overexpression and characterization of prokaryotic membrane transport proteins may, therefore, lead us to a greater understanding of

eukaryotic protein function. This avoids the problems associated with eukaryotic expression systems, such as incorrect post-translational modification and transience of expression.

Amplified expression and purification of multidrug resistance transport proteins

To allow the determination of structures of membrane transport proteins, a continuing supply of milligram quantities of protein is required. As native expression levels are usually less than 0.1% of total cell protein so genetical amplification of expression must be developed. Even if such amplification is successful a suitable detergent must then be found for purification.

In our laboratory a general strategy has been devised for the amplified expression, purification and characterisation of bacterial membrane transport proteins in *Escherichia coli*. Plasmid pTTQ18 is generally used as vector. Under optimised conditions for induction (by IPTG) and cell culture, amplified expression of 10-50X is achieved, with all the protein correctly folded in the inner membrane of the *E. coli* host strain. Inclusion bodies are not generally formed. So far the strategy has been successful for eighteen prokaryote transport proteins, including ones from *E. coli*, *B. subtilis*, *Brucella abortus*, *Staph. aureus*, *Methanococcus janaschii*, *Helicobacter pylori*, and *Rhodobacter sphaeroides*. By adding a $(His)_6$ tag to the C-terminus of each of the proteins, substantial purification of a protein is achieved using dodecyl-β-D-maltoside as detergent (Figure 2), with yields of between 1-5mg per litre of original culture. Reconstitution assays, and physical methods such as CD, FTIR, MS and fluorescence confirm the protein's integrity.

The amounts of these proteins obtained, and their purities, are now enough for crystallisation trials, both 2D and 3D, to be undertaken. These will be performed in Leeds, in Sheffield (Professor Per Bullough) and Imperial College London (Professor So Iwata and Dr Bernadette Byrne). Furthermore, the levels of expression of each protein in the inner membrane preparations is sufficient also for investigation of ligand binding by solid state NMR methods, which will be undertaken in Leeds (Dr Adrian Brough), Manchester (Dr David Middleton) and Oxford (Professor Tony Watts).

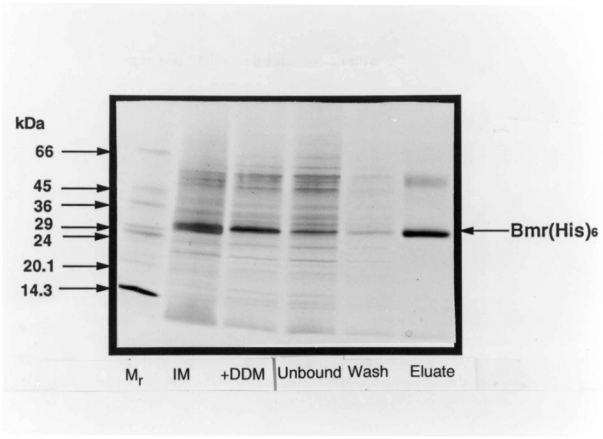

Figure 2. The purification of $Bmr(His)_6$ from inner membranes of *E. coli* using Ni-NTA affinity chromatography. The figure shows a silver stained 15% SDS-PAGE gel. Track 1, molecular weight markers ('M_r'). Track 2, inner membranes ('IM'). Track 3 (DDM), membrane proteins solubilised in 1% dodecyl-_-D-maltoside. Track 4 ('Unbound'), proteins remaining after treatment with Ni-NTA resin. Track 5 ('Wash'), proteins washed off the NiNTA resin containing bound $Bmr(His)_6$. Track 6 ('Eluate'), $Bmr(His)_6$ was eluted from the column with buffer containing 0.05% DDM and 200mM imidazole pH8, and collected in fractions; those fractions containing protein were concentrated. The tendency of some of the transport proteins to reveal an apparently higher M_r form (oligomer/conformer?) is illustrated in this case.

References

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