Antimicrobial resistance in staphylococci: Molecular architecture of a multidrug binding site

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A significant mechanism of bacterial resistance is the active export of antimicrobials from cells by broad specificity multidrug efflux systems. The export of antiseptics and disinfectants in staphylococci, mediated by the plasmid-encoded determinant *qacA*, is an example of such a multidrug efflux system (Brown & Skurray, 2001). QacA is a 514-amino acid membrane protein, containing 14 transmembrane segments (TMS), and is a member of the Major Facilitator Superfamily (MFS) of transport proteins. QacA confers resistance to more than 30 different monovalent and bivalent cationic, lipophilic antimicrobial compounds from 12 different chemical families *via* a proton motive force-dependent efflux mechanism. Transport and competition studies have indicated that QacA interacts with monovalent and bivalent compounds at two distinct substrate-binding sites (Mitchell *et al.*, 1999). Mutagenesis of the aspartic acid residue at position 323 in TMS 10 of the QacA protein has been shown to radically alter the substrate specificity of the transporter. Cysteine-scanning mutagenesis of 35 residues within and surrounding TMS 10 delineated the extents of the TMS and identified residues within the substrate-binding site; TMS 10 appears to play an integral role in the formation of the substrate-binding site of QacA (Xu *et al.*, 2006). Interestingly, the location of key negatively-charged residues in QacA has an influence on the subset of bivalent cations recognised (Hassan *et al.*, 2007).

To date there is no high resolution structure of a 14-TMS transport protein. However, since QacA possesses a number of amino acid sequence motifs conserved within the MFS protein family, is it practical to extrapolate the known structures of related 12-TMS transport proteins, such as GlpT and LacY? An alternative way of obtaining information on the structure of multidrug binding exporters, in particular their binding pockets, is to analyse other multidrug-binding proteins that may be more amenable to crystal structure analyses (Grkovic *et al.*, 2002). One such protein, the QacR transcriptional repressor, negatively regulates the expression of *qacA* and is induced by interaction with antimicrobials which are substrates of QacA; binding of these compounds conformationally modifies QacR such that it can not bind to the *qacA* DNA operator sequence (Schumacher *et al.*, 2002). Crystal structures of QacR complexed to a number of chemically- and structurally-different cationic compounds have shed light on the induction mechanism of QacR and also illustrate the versatility of the substrate-binding domain of this protein, revealing separate, but linked ligand-binding sites within a single protein (Schumacher *et al.*, 2001; Brooks *et al.*, 2007). Recent mutagenic studies have focused on the four glutamic acid residues, identified from these structures, that line and surround the QacR ligand binding pocket (Peters *et al.*, 2008). Biochemical analyses and examination of crystal structures have revealed that these acidic resides do not appear to play a role in charge neutralisation of cationic substrates but may be involved in substrate discrimination through affecting the positioning of the drugs within the binding pocket. This only serves to provide further evidence of the promiscuous nature of the binding pocket of multidrug binding proteins.

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