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Putative identification of an amphipathic, α-helical sequence in hemolysin of *Escherichia coli* (HlyA) involved in transmembrane pore formation

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Abstract

Escherichia coli hemolysin is a pore-forming protein belonging to the RTX toxin family. Cysteine scanning mutagenesis was performed to characterize the putative pore-forming domain of the molecule. A single cysteine residue was introduced at 48 positions within the sequence spanning residues 170–400 and labeled with the polarity-sensitive dye badan. Spectrofluorimetric analyses indicated that several amino acids in this domain are inserted into the lipid bilayer during pore formation. An amphipathic α-helix spanning residues 272–298 was identified that may line the aqueous pore. The importance of this sequence was highlighted by the introduction of two prolines at positions 284 and 287. Disruption of the helix structure did not affect binding properties, but totally abolished the hemolytic activity of the molecule.

Keywords: amphipathic α-helix-lined pore; fluorescence spectroscopy; protein-membrane interaction; RTX toxin.

Introduction

Hemolysin of *Escherichia coli* (HlyA) is a cytotoxic, pore-forming protein belonging to the family of RTX toxins (Menestrina et al., 1994; Welch, 2001). Structural domain prediction suggests that approximately the first half of the protein contains predominantly amphipathic α-helix structures; the C-terminal half contains the repeat units that are characteristic of RTX toxins (Coote, 1992) and are responsible for calcium-binding (Felmlee and Welch, 1988; Ludwig et al., 1988; Boehm et al., 1990; Cortajarena et al., 2002). Fatty acylation of lysine at position 690 is essential for pore formation (Stanley et al., 1994), and fatty acylation of a second lysine at position 594 enhances cytolytic activity. However, fatty acylation at neither site is required for the toxin to bind to target membranes (Moayeri and Welch, 1997; Hyland et al., 2001; Valeva et al., 2005b).

The mechanism of pore formation by HlyA is still not understood at a molecular level. Whether the toxin forms monomeric or oligomeric pores is a matter of debate (Bhakdi et al., 1986, 1988; Menestrina, 1988; Ludwig et al., 1993). One early analysis of the primary sequence led to the prediction that membrane pore formation is effected through insertion of several α-helices located within a long stretch of almost 200 amino acid residues (238–411, GenBank accession no. P08715). Unequivocal experimental data to support and refine this model have yet to be acquired. However, experimental evidence obtained with truncation mutants (Ludwig et al., 1987, 1991) indicated that this domain is required for pore formation and shows strong insertion-dependent labeling, with a photoactivatable probe incorporated into the lipid bilayer (Hyland et al., 2001). A model has been proposed in which the pore is formed by a series of transmembrane α-helices (Ludwig et al., 1991). If correct, this would place HlyA distinctly apart from other pore-forming bacterial cytolysins characterized to date, which have been shown to produce oligomeric pores lined by amphipathic β-sheets (Tilley and Saibil, 2006).

Mutant toxins with a single cysteine substitution that are site-specifically labeled with polarity-sensitive fluorescent dyes can be subjected to spectrofluorimetric analyses, allowing identification of sequences that insert into the lipid bilayer (Palmer et al., 1996; Valeva et al., 1996, 2005a; Shepard et al., 1998; Promdonkoy and Ellar, 2000; Nassi et al., 2002). A first study provided evidence that several amino-acid side chains located within the N-terminal sequence of HlyA spanning residues 177–380 inserted into the membrane and contacted lipids during pore formation (Schindel et al., 2001). The side chain of residue 288 in particular was definitively identified as contacting lipid in the pore.

This study reports the analysis of 48 cysteine substitution mutants labeled with badan in the putative pore-forming domain of the protein. A detailed investigation was performed within the sequence encompassing residues 272–301, which was found to form a putative amphipathic membrane-spanning α-helix (Ludwig et al., 1991). Replacement mutagenesis with proline demonstrated the importance of this α-helix in pore formation by HlyA.