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The impact of length variations in the L2 loop on the structure and thermal stability of non-specific porins: the case of OmpCs from the *Yersinia pseudotuberculosis* complex

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Abstract

Porins are integral proteins of the outer membranes of gram-negative bacteria. In membranes, they exist as homotrimers and the L2 loops contribute to their stability. Comparison of OmpC porins of the *Yersinia pseudotuberculosis* complex with other enterobacterial porins demonstrated L2 loop length diversity, which is caused by varying numbers of dipeptide/tripeptide repeats. The OmpC porins are highly homologous to each other, and they can be subdivided into five isoforms based on their L2 loop structure. Optical spectroscopy and SDS-PAGE experiments revealed that particularities of the L2 loops affected the structure and thermal stability of the porins. Thermal denaturation studies showed that porins with shorter loops, compared to porins with longer loops, had more stable tertiary and less stable secondary and quaternary structures. According to our comparative modeling results, the L2 loops differ in their structure by adopting different spatial positions and forming different polar bonds with a neighbor monomer. The replacement of asparagine with arginine at the C-terminus of the L2 loop shifts the loop upwards and causes the loss of contacts with the arginine clusters within the pores. The increase in the length of these loops ensures that they shift down towards the pore and restore their contacts with arginines on the channel wall, as is the case in classical nonspecific porins. Despite the fact that the surface charge density varies considerably among the OmpC porins, the L2 loops form a typical negatively charged region in the center of the trimer.

Keywords

Porin; OmpC isoforms; Yersinia; thermal stability; computer modeling; optical spectroscopy

1. Introduction

Porins are pore-forming proteins that are localized in the outer membranes (OM) of gram-negative bacteria, mitochondria and chloroplasts. They form water-filled channels that are permeable to hydrophilic substances with low molecular weights (<600 Da) through which the cell receives nutrients and removes metabolites [1]. The cell-surface exposed regions of porins serve as receptors for phages, colicins and CdiA effectors [2–4]. Porins play a fundamental role in the host-pathogen interaction, including diverse biological activities and immune responses [5]. They influence the emergence of antibiotic resistant strains of bacteria but also represent useful targets the development of new therapeutics.

Bacterial porins are divided into two groups: specific porins, which have narrow substrate specificities, and non-specific (general) porins that transport ions and small molecules without special selectivity. Classical non-specific porins—OmpC, OmpF and PhoE, which differ slightly from each other in structure and function—are the major proteins of the OM of *Escherichia coli*. The structures of several non-specific porins have been determined by X-ray crystallographic analysis [6,7]. The porin molecule is a β-barrel formed by 16 β-strands, connected by long extracellular (L1-L8) and short periplasmic loops. Within the membranes, the porins exist as highly stable homotrimers due to hydrophobic interactions between the monomers. The L2 loop plays a significant role in stabilization of the trimer: it connects one monomer to its neighbor [8]. This loop is directed towards the adjacent monomer, fills the gap between the loops L2* (*refers