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Structural and functional analysis of a novel O-antigen-modifying enzyme, phosphoethanolamine transferase, of Shigella flexneri

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Background: Shigellosis, an unsolved global health problem, is a gastrointestinal disease that is primarily caused by *Shigella flexneri*. If ingested, *S. flexneri* initiates a serotype-specific immune response that targets the O-antigen – a part of the pathogens' lipopolysaccharide. The O-antigens play a significant role in *Shigella* pathogenesis and can undergo specific modifications through the addition of various chemical residues. This modification process gives rise to numerous serotypes of *S. flexneri*. A recently identified novel O-antigen phosphoethanolamine transferase (encoded by *opt*) is responsible for the O-antigen modification via the addition of phosphoethanolamine (PEtN) residues. The presence of this PEtN modification is responsible for the novel serotypes Xv, 4av and Yv that were derived from traditional *S. flexneri* serotypes X, 4a and Y, respectively. Serotype Xv has recently become resistant to multiple antibiotics and is the most prevalent *S. flexneri* serotype in China. However, the topology or the mechanism of action of this protein is not yet known.

Material/methods: The topology of Opt was predicted using a variety of membrane topology prediction programs such as DAS, HMMTOP, TMHMM, TopPred, Predict Protein, PSORTb, SOSUI and TopPredII. The validity of this topology model was verified using the substituted cysteine accessibility method (SCAM). Identification of critical domains and residues was carried out using site-directed mutagenesis and functionality of the protein was determined by using specific antibodies to PEtN modification.

Results: A computer-generated Opt topology model predicted Opt to have four transmembrane segments, five loop segments, a cytoplasmic N-terminus and a C-terminus at the end of a large cytoplasmic tail. This topology model was verified using SCAM, which rather suggested the presence of three transmembrane regions, four loop segments, a periplasmic C-terminus at the end of a long cytoplasmic tail. Bioinformatics analyses identified that Opt consists of an alkaline phosphatase

homologous region close to its C-terminus; within which lies a conserved domain. A number of amino acids which lie within this conserved region as well as in the homologous region were identified to be critical for the function of the protein. Based on these findings, a model for its mechanism of action was proposed.

Conclusions: The topology of a novel O-antigen modifying protein was successfully determined using an experimental approach. The importance of a number of amino acid residues within this conserved domain and the homologous region with regard to the function of Opt was also established. These amino acids may play a key role in forming the catalytic domain found within the alkaline phosphatase homologous region. The study contributes to our understanding of the structure and function of a novel bacterial membrane protein of a major bacterial pathogen. Such information is vital for the development of a multivalent vaccine against shigellosis in future.