“Characterization of clostridial lytic enzymes with similarity to eukaryotic peptidoglycan recognition proteins”

mgr Agnieszka Morzywołek

The clostridia are Gram-positive, anaerobic, spore-forming bacilli capable of producing toxins, some of which are extremely potent. Phage-encoded enzymes and other lytic enzymes, which can lyse bacteria when exposed externally, have the potential as agents to combat pathogenic bacteria. Bioinformatics analysis revealed in the genomes of several *Clostridium* species genes encoding putative N-acetylmuramoyl-L-alanine amidases belonging to the AMI-2 domain family. Further studies showed their similarity to eukaryotic peptidoglycan recognition proteins with a distinctive Zn^{2+} binding motif.

In the frame of the present work, three enzymes have been characterized, and their anti-clostridial potential was evaluated. The first, designated as LysB (224-aa), from the prophage of *C. botulinum* E3 strain Alaska E43 is part of the *lysB-holB* lytic cassette. The recombinant 27,726 Da protein has been overproduced and purified from *E. coli* Tuner(DE3) with a yield of 37.5 mg per 1 liter of cell culture. Size exclusion chromatography and analytical ultracentrifugation experiments have shown that the protein is dimeric in solution. Bioinformatics data strengthened by analysis of the LysB molecular model and site-directed mutagenesis studies imply that five residues, H25, Y54, H126, S132, and C134, form the catalytic center of the enzyme. Residues involved in Zn^{2+} binding are underlined. Twelve other residues, M13, H43, N47, G48 W49, A50, L73, A75, H76, Q78, N81, and Y182, had been predicted to be involved in anchoring the protein to the lipotejchoic acid, a significant component of the Gram-positive bacterial cell wall. The LysB enzyme demonstrated lytic activity against bacteria belonging to the genera *Clostridium*, *Bacillus, Staphylococcus*, and *Deinococcus* but did not lyse Gram-negative bacteria. Optimal lytic activity of LysB occurred between pH 4.0 and 7.5 in the absence of NaCl. Two other proteins, LysP (222-aa; 25,690 Da) and LysPS (157-aa; 17,840 Da) are from *C. perfringens* NCTC 8239 and *C. perfringens* ATCC 13124, respectively. The proteins differ only with the 65-aa extension at the N-terminus present in LysP but not in the LysPS enzyme. It was found that LysPS protein, contrary to the LysP enzyme, is extremely thermoresistant. Site-directed mutagenesis provided direct proof that the Zn^{2+} binding motif is critical for each enzyme lytic activity (LysP: H95, H193, C201; LysPS: H128, C136). Lytic activity of LysP and LysPS enzymes was demonstrated against bacteria belonging to the genera *Clostridium, Bacillus* but not Gram-negative bacteria. In addition, it was found that the lytic spectrum of LysPS is broader than LysP and also obeys *S, aureus*, and *D.radiodurans*. Finally, it was proven that HolB protein of *C. botulinum* E3 strain Alaska E43 *lysB-holB* lytic cassette functions as a holin.