The CW_7 cell wall-binding motifs of the Cpl-7 endolysin target the peptidoglycan muropeptide: structural and functional characterization

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The Cpl-7 endolysin, a lysozyme encoded by Cp-7 bacteriophage, is a remarkable exception among lysins (cell wall lytic enzymes) produced by *Streptococcus pneumoniae* and its phages, as it degrades pneumococcal cell walls containing either choline or ethanolamine. Its range of susceptible bacteria is also expanded compared with choline-dependent pneumococcal lysins, and exerts its killing activity on streptococcal (*S. pneumoniae, Streptococcus pyogenes, Streptococcus mitis* and *Streptococcus dysgalactiae*, among others) and non-streptococcal pathogens (*Enterococcus faecalis*). Moreover, Gram-negative bacteria become susceptible to the lysis by Cpl-7 in the presence of outer-membrane destabilizing-agents (Díez-Martínez *et al.* 2013). This ability results from acquisition of a C-terminal domain (C-Cpl-7) made of three identical repeats (CW_7 motifs) of 42 amino acid each involved in Cpl-7 attachment to the cell wall (Bustamante *et al.*, 2010). Interestingly, a search of the Entrez Protein Database has revealed the presence of CW_7 homologous motifs in more than 350 sequences of putative cell wall hydrolases, with Firmicutes and Actinobacteria being the most represented phyla, though Gram-negative bacteria and Gram-positive infecting phages were also present. We have proved now, using STD-NMR spectroscopy, that the CW_7 repeats recognize and bind N-acetyl-D-glucosaminyl-(β1,4)-N-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP), a structural analogue of the peptidoglycan monomer that is shared by Gram-positive and -negative bacteria, and the contacts provided by the GMDP ligand have been identified. Moreover, the relevance of the number of repeats for activity has been examined, and the three-dimensional structure of a single CW_7 repeat and of the complete C-Cpl-7 domain have been solved. Also, a structural model of the C-Cpl-7:GMDP complex, based on the domain crystal and solution structures and consistent with STD-NMR mapping, is proposed. Our results shed light on the interactions established between key amino acids of CW_7 motifs and peptidoglycan building blocks and explain the ability of Cpl-7 to kill both Gram-positive and Gram-negative bacteria. However, the still limited lytic spectrum of Cpl-7 further substantiates the notion that, in addition to the specificity provided by the cell wall binding motifs, other factors (net charge of lysins domains, global lysin three-dimensional structure, specific features of cell wall structure, etc.) help to define the susceptibility of bacteria to a given lysis. Nonetheless, our data provide a first rational for the use of CW_7 comprising lysins as anti-infectives and the assembly of CW_7 like motifs into tailor-made chimeric lysins.

References:
