

Chemistry Department

Antibacterial proteins Folding and Regulation

REQUIMTE / Structural Biology NMR – NMR@REQ



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2007-2011 Post doctoral fellow – MCW, USA
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Objectives

Endolysins are peptidoglycan (PPG) hydrolases that possess antibacterial activity (Fig. 1) and are classified as enzybiotics with pharmacological interest. The modular nature of endolysins is responsible for the specificity and narrow spectrum of activity.



- Structural characterization of the autoinhibitory mechanism of endolysins
- Identification of the cell wall structures (epitopes) responsible for endolysin recognition
- Characterization of the conformational changes associated with epitope binding
- Engineering of constitutively active enzymes

Methodology

- Structural determination of the endolysins functional domains by NMR (Figs. 2 and 3)
- Interdomain/inhibitory interactions mapping by NMR and activity assay (Fig. 4)
- Epitope identification and binding by UV-visible, fluorescence and FP spectroscopies
- Competition assays
- Molecular modeling

Expected Results

- Structural determination of domains with no known homologs
- Identification of unique epitopes for endolysins in the bacterial cell wall
- Structural elucidation of autoinhibitory mechanisms
- Folding / unfolding equilibria in enzyme activity and allosteric regulation

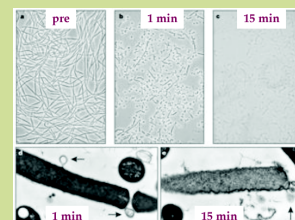


Figure 1 – *in vitro* lytic activity of PlyG against *B. anthracis*

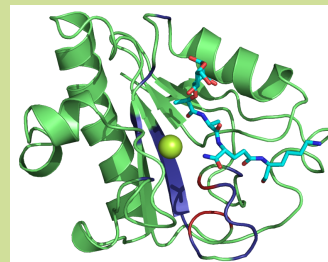


Figure 2 – Model of the NMR solution structure of the catalytic domain complexed with a PPG mimetic

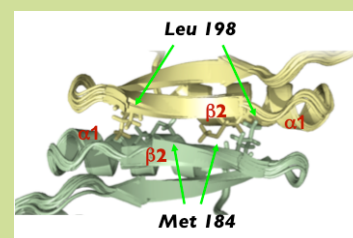


Figure 3 – NMR solution structure of the cell wall binding domain (regulatory)

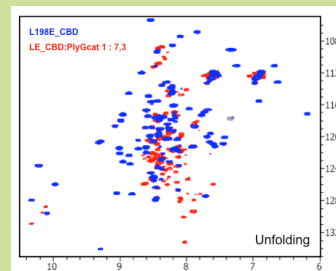


Figure 4 – ^{15}N - ^1H HSQC spectra of the monomeric cell wall showing that the inhibitory complex involves domain unfolding