Protein Homology Models of Putative Carboxypeptidases in *Borrelia burgdorferi*

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Borrelia burgdorferi is a spirochete that causes Lyme disease, the most common tick-borne disease in the United States. This bacterium is susceptible to beta-Lactam antibiotics (the Penicillins) *in vitro*, but becomes resistant to penicillin in the host. *B. burgdoferi* is also known to facilitate protein degradation during the heat shock associated with infection in a warm blooded animals. An important class of proteins believed to be involved in the heat shock response and penicillin resistance are carboxypeptidases. These enzymes hydrolyze peptide bonds at the section of the protein nearest to the negatively charged carboxy terminus and also sometimes function as penicillin binding proteins that can hydrolyze the beta-lactam structure of penicillin. The substrates that these enzymes act upon, as well as their beta-lactamase activity are determined by their unique tertiary structures. For these reasons the structure of carboxypeptidase is important to understanding virulence in *B. burgdorferi*. In this study, we focus mainly on two putative genes for carboxypeptidases of B. burgdorferi given by GenBank accession numbers -- AAC66969.1 and AAC66941.1. Sequence homology indicates that AAC66969.1 is a serine-type D-Ala-D-Ala carboxypeptidase (dacA). BLASTP searches have turned up three crystal structures (1jn4, 1skf, and 1esi) in bacteria that show 31% identical primary sequences and 50% similar amino acid sequences with dacA. These crystal structures indicate that the active site of these serine proteases are associated with a conserved- SXXK, SXN, and KTG sequence motif. These crystal structures and conserved motifs were used to construct initial threaded homology models of carboxypeptidase using the SWISS-MODEL server. The resulting structures were refined using energy minimization and simulated annealing based on AMBER protein dynamics calculations. The AMBER calculations were constrained to have the same secondary structure as predicted by PSIPRED neural network. Secondary structure constraints were imposed using AMBER's NMR torsion constraints. Our methods will be tested against CASP (Critical Assessment of Structure Prediction) in order to determine their effectiveness. The structures of these two putative carboxypeptidases, once predicted, will be useful in determining similarities between the heat shock response and beta-lactamase activity of Borrelia and other bacteria whose carboxypeptidases are known.