

Characterization of a prolyl-4-hydroxylase Like Protein from Bacillus Anthracis

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Background and Objective: Collagen prolyl-4-hydroxylase (P4H) is an essential enzyme in the post-translational modification of procollagen. P4H catalyzes the hydroxylation of proline residues at the Yaa positions of (Gly-Xaa-Yaa)_n repeats in collagen and other proteins containing collagen like domains. P4H is a member of the α -ketoglutarate (α -KG) dependent mononuclear non-heme iron oxygenase family. The genome of *Bacillus anthracis*, the causative agent of anthrax, contains a gene annotated as a P4H based on the predicted amino acid sequence. Using anthrax-P4H as a model, we can elucidate information about the catalytic properties of human-P4H, which are currently unknown.

Methods: We have successfully cloned, expressed, and purified anthrax-P4H from genomic DNA, and shown enzymatic activity through assays on a Clark style O₂ electrode. Amino acid analysis was used to determine whether anthrax produces Hyp.

Results: Amino acid analysis performed on *B. anthracis* shows Hyp production associated with spore formation. Recombinant anthrax-P4H is a homodimer of ~24 kDa subunits, and requires Fe(II), O₂, and α -KG for catalytic activity. Kinetic suggest that anthrax-P4H can bind an in vitro human-P4H substrate, (Gly-Pro-Pro)₁₀.

Discussion and conclusion: We have successfully characterized anthrax-P4H as an α -KG-dependant iron oxygenase that can bind the same substrate as human P4H. We have also shown that *B. anthracis* cells can produce Hyp associated with sporulation. We would like to thank NIH, AHA, and KUCR for funding.