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MOLECULAR MODELING OF POLYPEPTIDE DEFORMYLASE FROM ACIDITHIOBACILLUS FERROOXIDANS

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Short Abstract: We report the molecular modeling of the polypeptide deformylase from A. ferrooxidans. The model presents all the conserved residues that characterize this family of protein including, the HEXXH domain and the Gln50, Cys90, Leu91, Ser92 residues. Interactions among many residues showed some important differences when compared with the reference structure.

Long Abstract:
Acidithiobacillus ferrooxidans is a gram-negative, mesophilic, acidophilic, chemolithoautotrophic bacterium that is able to derive energy for growth from the oxidation of ferrous ion, elemental sulfur and reduced inorganic sulfur compounds. It is one of the most important microorganisms involved in the bioleaching of sulfide ores since this bacterium can oxidize metal sulfides to acid soluble metal sulfates. A. ferrooxidans strain LR was grown in the presence of ferrous ion until 80% of oxidation and then, it was kept in contact with 2.5% of covelite for 24 hours. Using RNA arbitrarily primed PCR a cDNA of 238 bp with a high expression pattern in the presence of ferrous ion was isolated and sequenced. The complete sequence of this cDNA was obtained from the A. ferrooxidans ATCC23270 genome (Tigr – Unfinished Microbial Genome – http://www.tigr.org/). The obtained sequence was compared with GenBank sequences using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/). A similarity (E-value 4e-47, identity 58% and similarity 74%) with the def gene from Thiobacillus denitrificans, which encodes for the enzyme polypeptide deformylase (PDF), was found. This metalloenzyme utilizes ferrous ion as the catalytic metal and is involved in the removal of the N-terminal formyl group in a growing polypeptide chain following translation initiation during protein synthesis. This protein is present in all Eubacteria since its activity is essential. The def gene encodes for 212 residues that corresponds to the PDF protein. As in E. coli, the A. ferrooxidans def gene is part of an operon, which includes the fmt gene that encodes for metionil-tRNA formyltransferase. An analysis of the deduced amino acid sequence from A. ferrooxidans PDF on PSORTb v2.0.4 (http://www.psort.org/psortb/) showed that the protein localization is in the cytoplasm. Using the Protparam Tool (http://www.expasy.org/tools/protparam.html) it was possible to determine the protein molecular weight (23kDa) and the theoretical pI (6.97). The higher expression pattern of the def gene in the presence of ferrous ion may be explained by the fact that the bacteria were on the exponential growth phase. In this phase, the protein synthesis is high, which could justify the increase in the amount of this enzyme. This hypothesis was confirmed

by the determination of the gene def relative expression using Real Time PCR. For this, A. ferrooxidans LR were cultivated in the presence of ferrous ion and cells were collected after 50, 75 and 100% of ferrous ion oxidation. The relative expression value of the def gene in 75% of ferrous ion oxidation was 86-fold higher than in 50% of oxidation. A decrease in expression was observed when the bacteria were maintained in contact with covelite, probably as result of stress. In order to analyze the structure of the A. ferrooxidans PDF, a search was performed on the Protein Data Bank (PDB) using the BlastP (http://www.ncbi.nlm.nih.gov/BLAST/). A reference structure from E. coli (PDB code 1DFF – resolution 2.9Å; E-value 3e-47, identity 56% and similarity 74%) was obtained and used to generate the PDF three-dimensional structure (MODELLER software version 8v0). The structural analysis of this model was made using the Diamond STING software (http://sms.cbi.cnptia.embrapa.br/SMS/). The model obtained for the A. ferrooxidans polypeptide deformylase presents all the conserved residues that characterize this family of protein including, the HEXXH domain and the Gln50, Cys90, Leu91, Ser92 residues. Interactions among many residues, the metal and the substrate showed some important differences. The Leu91 residue presents two new hydrophobic interactions that move the lateral chain slightly. This movement may be responsible for an increase in the accessibility of the catalytic site. The residues Cys90, His132 and His136, involved in the fixation of the metal by the protein, also showed different interaction patterns when compared with the reference structure. Further analysis of the PDF model may contribute to a better understanding of the role of this protein when A. ferrooxidans is used in the bioleaching of covelite.