Poster Presentation

MS009.P40

Characterization of dihydroorotase from M. jannaschii

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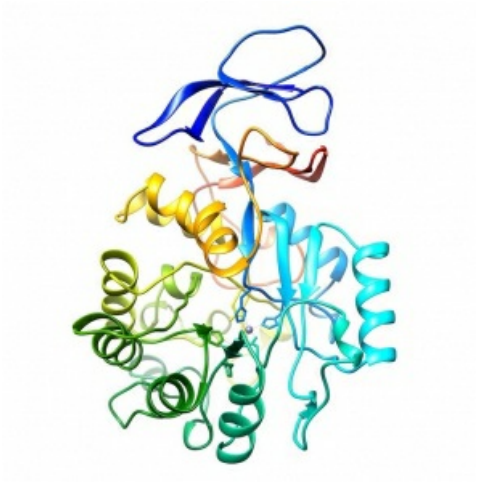
The gene that codes for the putative dihydroorotase in the hyperthermophilic archaeon Methanococcus jannaschii was subcloned in pET-21a and expressed in Escherichia coli. A purification protocol was devised. The purity of the protein was evaluated by SDS-PAGE and the protein was confirmed by sequencing using LC-MS. The calculated molecular mass is 48104 Da. SEC-LS suggested that the protein is a monomer in solution. ICP-MS showed that there are two Zn ions per monomer. Kinetic analysis of the recombinant protein gave hyperbolic kinetics with Vmax = 12.2 µmol min-1 mg-1 and Km = 0.14 mM at 25 °C. Furthermore the activity of the protein increased with temperature consistent with the hyperthermophilic nature of the organism. A homology model was constructed using the mesophilic Bacillus anthracis protein as the template. Residues known to be critical for Zn and substrate binding were conserved. The activity of the enzyme at 85 °C and 90 °C was found to be relatively constant over 160 min and this correlates with the temperature of optimal growth of the organism of 85 °C. The amino acid sequences and structures of the two proteins were compared and this gave insight into some of the factors that may confer thermostability – more Lys and Ile, fewer Ala, Thr and Gly residues, and shorter N- and C-termini. Additional and better insight into the thermostabilization strategies adopted by this enzyme will be provided when its crystal structure is determined.



Keywords: Pyrimidine Biosynthesis, Thermostability, Enzyme Kinetics