Poster Presentation

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A preliminary study of Dihydroorotase from Methanococcus jannaschii

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Dihydroorotase (DHOase) catalyzes the reversible cyclization of N-carbamoyl-L-aspartate to form L-dihydroorotate in the third step of de novo pyrimidine biosynthesis. It is a Zinc metalloenzyme and a member of the aminohydrolase superfamily. There are two classes of the enzyme. Class I, typically ~45 kDa, is found in higher organisms, bacteria and yeast. Class II, typically ~38 kDa, is found in bacteria and fungi. Some organisms have multiple DHOase sequences.

The M. jannaschii pyrC gene coding for DHOase was subcloned and expressed in E. coli. Protein purification consisted of ammonium sulfate precipitation, heat treatment at 85° C, and phenyl-sepharose hydrophobic interaction chromatography. The protein was confirmed in the SDS gel using Liquid Chromatography-Mass Spectrometry (Proteomics Laboratory, Lerner Research Institute, Cleveland, OH). Size Exclusion Chromatography-Laser Light Scattering (Keck Biotechnology Laboratory, Yale University, New Haven, CT) indicated that the protein is a monomer in solution with a molecular weight of 47 kDa. A model constructed with the I-TASSER server (Zhang, 2008) suggested that the binding site contains only one Zn ion per monomer coordinated by the conserved His56, His58 and Asp302. Asp146 is further away and does not coordinate with the Zn ion. According to the mass spectrometry analysis, the protein does not contain a carboxylated lysine. Our progress on this study will be presented.

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