HETERONUCLEAR NMR INVESTIGATIONS OF DDAH ENZYME
STRUCTURE AND DYNAMICS.

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ABSTRACT

Dimethylarginine dimethylaminohydrolase (DDAH) is an enzyme found in all mammalian cells. It is involved in the degradation of methylarginines, specifically asymmetric dimethylarginine (ADMA) and NG-monomethyl-L-arginine (MMA). The methylarginines ADMA and MMA inhibit the production of nitric oxide synthase. Inhibition of DDAH activity causes methylarginines to accumulate, which results in the blockage of nitric oxide (NO) synthesis. The dysfunction of DDAH activity results in the elevation of plasma ADMA level and impairment of vascular relaxation observed in humans with increased cardiovascular disease or risk factors (such as hypercholesterolemia, diabetes mellitus, and insulin resistance). The two isoforms of DDAH (1 & 2) are found in mammals. The DDAH from bacterium source, Pseudomonas aeruginosa (PaDDAH) was studied by Beatriz Magalhaes (2006) at the laboratory of Biochemistry and Molecular Biology (UCL) is so far the only structurally tractable homologue of mammalian DDAH isoforms. For the study of interaction between enzyme with substrate and inhibitor bound ligands, she obtained PaDDAH in its monomeric form by substitution of several interface residues which resulted in the shifting of equilibrium position towards the monomer, which allowed her to design a double mutant variant (Agr40ÆGlu, Arg98ÆHis) that behaved exclusively as monomer and retained 95% catalytic activity more than wild type (WT). The theme of the present research is linked to the research previously done by the above mentioned scientist. The first task is to recapitulate the methods for protein expression, purification, isotope labeling of monomeric WT and C249S PaDDAH for NMR studies. The polypeptide chain of PaDDAH in apo and inhibitor bound states will be observed and study of dynamics of C249S PaDDAH in substrate and product bound states will be performed. The emphasis of research will be more towards the high expression of human DDAH isoforms (1 & 2), optimal for isotope labeling and thus can be utilized for the examining the structural design by using NMR technique. Our approach would be to assess the expression level for ‘synthetic genes’ that have optimal codon usage for Escherichia coli expression where consideration of mRNA secondary structure has been applied. The exploration of comparative NMR characteristics of Mycobacterium tuberculosis DDAH will be done, in order to express the MtDDAH in sufficient yield and form that is tractable for NMR studies. The analysis of data will be performed using CCPN (Collaborative Computing Project) for NMR and the previous available data files will also be converted into CCPN format. The comparative screening of PaDDAH, MtDDAH and human DDAH isoforms (1&2) will help us to differentiate the role of DDAH in bacterium and human which can be interacted with a chemical library as model systems for early stage drug discovery efforts.