Structure based inhibition of polyamine biosynthetic pathway enzyme arginase from Entamoeba histolytica

Malik, A.[1], Dalal, V.[1], Singh, H.[1], Kumar, P.[1] and Tomar, S.[1]

[1] Indian Institute of Technology, Roorkee, India

Entamoeba histolytica is an anaerobic unicellular parasitic protozoan, which infects the gastrointestinal tract of humans and cause amoebic colitis and amoebic liver abscession. Amoebiasis caused by *E. histolytica* results in approximately 100,000 deaths per year, making it second leading cause of death due to protozoan infection. Presently, there is no efficient drug/vaccine available against *E. histolytica,* as antiamoebic drugs are losing their efficacy due to the rapid development and spread of the drug resistant parasites. Parasitic protozoans depend on polyamines for their growth and survival, which makes enzymes of polyamine biosynthetic pathway suitable target to develop anti-amoebic drug therapy and inhibitors.

The binuclear manganese dependent metalloenzyme arginase (EhArg) is the first rate limiting enzyme of the polyamine biosynthetic pathway and thus represent an attractive drug target for treatment of amoebiasis. In this study we have elucidated the crystal structure of arginase from *E. histolytica*. EhArg is a metal dependent hydrolytic enzyme involved in hydrolysis of L-arginine to yield urea and L-ornithine, which further enters into a cascade of reactions to generate polyamines like spermidine and spermine. EhArg has been cloned, expressed and purified using Ni-NTA chromatography to ~95% homogeneity. Preliminary solution studies using gel filtration chromatography revealed that the protein exist in both monomeric and dimeric state in the solution, which suggest that it exhibits a different oligomerization pattern than that of the previously characterized eukaryotic arginases. Binding studies of EhArg was performed with the substrate analogue inhibitors using isothermal titration calorimetery, and it has further been crystallized in complex with the characterized inhibitors. Crystal structure of arginase has been solved and structural analysis of the metal binding site and active site has been elucidated.

The overall structure shows EhArg is a homodimer possessing thirteen Φ#177;- helices and seven β-sheets in each monomer. The active site cavity of enzyme is formed primarily by two long loops (residues 124-145 and 229-246) and a short helix (residues 226-228). Multiple sequence alignment of EhArg with other structurally characterized eukaryotic arginases shows the absence of a 14- residues long oligomerization motif at C-terminal of EhArg, which was reported to be essential for the oligomerization. Therefore, EhArg is believed to acquire a different oligomerization pattern than that is of other eukaryotic arginases. The structure of EhArg reveals the presence of two manganese atoms which are separated by 3.3 Å, placed asymmetrically in the active site. One of these Mn is embedded deep in the active site and the other one is surface exposed. Both the manganese ion are connected by a bridging water molecule, located at a distance to the substrate analogue at which it can make nucleophillic attack on the guanidium carbon of the substrate. The crystal structure of arginase from *E. histolytica* will further pave the way to develop strategies for structure based drug designing against the parasite.