Abstract

Entamoeba histolytica is an amitochondriate protozoan parasite causing amoebiasis in tropical and subtropical countries where sanitary condition is poor. Metronidazole (Mtz) the best recommended and potent drug against amoebiasis needs activation inside the cell. Different enzymes like Pyruvate ferridoxin oxidoreductase (PFOR), nitroreductase and thioredoxin reductase have been found to be involved in the activation of the drug in *E. histolytica*. Malic enzyme (ME), which has been reported to activate Mtz in *T. vaginalis* is also present in *Entamoeba.* To investigate the role of malic enzyme in *E. histolytica,* the EhME gene has been PCR amplified using genomic DNA of *Entamoeba*, cloned and expressed in bacterial system as His-tag fusion protein. Enzyme activity of recombinant EhME (rEhME) was measured using NADP and L-malate as substrate. The K_m and V_{max} for L-malate was found to be 4.2mM and 0.41 $mmolmin^{-1}$ and K_m and V_{max} for NADP was found to be 69.6µM and 0.38$mmolmin^{-1}$ respectively. Ovreexpressed rEhME in *E.coli* (strain JM109) has been able to increase the susceptibility 5-6 times more than that of control cell (JM109-empty vector) against Mtz. RT-PCR and western blot using anti EhME rabbit polyclonal antibody reveal that upon exposure to Mtz, the expression of ME increases in *E. histolytica*. ME which is secreted to the extracellular environment was also detected by western blot. The released protein has the capacity to bind in both colonic cells and *Entamoeba* itself. Binding of native and recombinant EhME (labeled with FITC) to membrane of colonic cells were confirmed by confocal microscope.

To analyze the structural aspect of EhME, two step purification of expressed protein was performed and crystallization was done by sitting drop method using 1.5 M $(NH_4)_2SO_4$, 0.1 M CH_3COONa pH 5.0, 12% (v/v) glycerol. A complete diffraction data was collected to 2.2Å resolution using the Rigaku Micromax007 CuK_α X-ray generator and Rigaku Raxis IV^{++} detector. The structure has been solved by single isomorphous replacement with anomalous scattering (SIRAS), as molecular replacement theory failed to determine the structure. Structural analysis shows that, EhME is a dimeric protein with five domains in each monomer. Rossamman's folds are present within the domain C and domain D which bears the active site and cofactor binding sites. The structure also favours strong specificity for NADP. This is the first reported structure of malic enzyme among the protozoan parasite.

Keywords: *Entamoeba histolytica*, Malic enzyme, Drug activation, Pathogenesis, Crystal structure.