Abstract

Enterobacter cloacae IIT-BT08 is a Gram negative, facultative anaerobe, H_2-producing strain. The high H_2 producing enzyme, Fe-hydrogenase from *E. cloacae* is cytoplasmic, monomeric protein with molecular weight of 51 kDa and is extremely sensitive to oxygen inactivation. The four chromatographic steps of purification results in approximately 1285 fold purification of Fe-H_2ase with specific activity of 334.8 μmol H_2 $min^{-1}mg^{-1}$ of protein for H_2- evolution using reduced methyl viologen as an electron donor at 25°C. The internal amino acid sequence analysis of Fe-H_2ase by ESI MS/MS Q-ToF and BLAST search analysis of the selected peptide fragments with sequences GELFNMGHGK and EATELVAR from ESI MS/MS have confirmed 70% (7/10) and 87% (7/8) identity with Fe-H_2ase (**AAA23248, P29166, XP1330775**) from *Clostridium sp.* and *T. vaginalis* G3 respectively. The enzyme has isoelectric point at pH ~ 5.6 and contains ~11.46±0.54 gm-atom Fe/mole of the enzyme. The presence of multiple copies of [4Fe-4S] clusters has been affirmed from rhombic EPR signal at g = 2.1 owing to the paramagnetic nature of the clusters. The enzyme displayed maximum H_2 evolution at pH 7.0 and 37°C.

Cloning of Fe-H_2ase encoded gene (hydA) shows 40% similarity with the C-terminal region of other Fe-H_2ases. The secondary structure analysis of recombinant Fe-H_2ase using CD spectra has ascertained that the enzyme is composed of 36.5% α helix, 22.4 % β sheets and 31.1% of random coils. The recombinant enzyme exists as αβ protein. The cysteine residues present at the C-terminal end of the enzyme are conserved and may be crucial for the enzyme catalysis.

The maximum activity of Fe-H_2ase is observed using reduced methyl viologen as the substrate in comparison to other reduced artificial electron donors (such as benzyl viologen, methylene blue, NADH/ NADPH). Ferredoxin from *E. cloacae* is of 8 kDa in size and displays characteristic absorbance maxima at 424 nm corroborating the presence of redox active Fe-S clusters. The low K_m value of ferredoxin (0.72±0.04 μM) than methyl viologen (0.56±0.03 mM) suggests its greater affinity towards the enzyme. Therefore, ferredoxin may be the probable physiological electron donor of Fe-H_2ase from *E. cloacae* IIT-BT08.

Keywords: Fe-hydrogenase; hydrogen evolution; Fe-hydrogenase encoded gene, hydA; Fe-S protein, redox partner, ferredoxin, *Enterobacter cloacae* IIT-BT08.