Ghazaleh Gharib, J Proteomics Bioinform 2018, Volume 11
DOI: 10.4172/0974-276X-C3-118

Cloning, sequencing, purification and characterization of highly thermostable aspartate aminotransferase from *Geobacillus thermopakistaniensis*

Ghazaleh Gharib
Sabanci University Nanotechnology Research and Application Center, Turkey

Aspartate aminotransferase gene consists of 1,182 bp nucleotide encodes for 393 amino acids was sequenced, cloned, expressed and purified to homogeneity. Enzyme exhibited maximum activity at pH 7 at 65 °C. Mass spectrometry determined molecular mass of 42,562 Da and gel filtration indicated the protein exist as a dimeric form. Thermo stability experiment showed 100% stability of a protein at 65 °C for 16 hours and half-life of 15 mins at 75 °C. The thermal denaturation studies by CD spectroscopy showed no significant change in ellipticity of the helical structure of the protein below 70 °C. Km and Vmax values towards aspartate were 1.61 mM and 97 μmol min^{-1} mg^{-1} and towards α-ketoglutarate were 2.5 mM and 50 μmol min^{-1} mg^{-1}, respectively. Substrate specificity experiment indicated maximum activity with aspartate and its respective keto acid (α-ketoglutarate) while exhibits 27% of activity with Tyr and 16% of its activity with Pro and Cys and no activity with Glu how ever in reverse reaction of Glutamate with its keto acid, oxaloacetate, 70% of activity was observed. Pyridoxal phosphate quantification exhibited 0.1 mole of PLP per mole of enzyme. Amino acid analysis showed high contents of charged and especially acidic residues of Aspartate and glutamate in structure of ASTSBS enzyme. Homology modeling determined the Dimeric structure of ASTSBS which contained high number of proline on a surface of each sub-units as compare to its mesophilic counter parts that is a reason for stability of a protein at high temperature.

Biography

Ghazaleh Gharib received her Bachelor's degree in General Biology from Ferdowsi University of Mashhad, Iran in 2004. She completed her MSc in Biotechnology from Institute of Biochemistry and Biotechnology University of the Punjab in 2008 and then completed her PhD in 2016 from School of Biological Sciences, University of the Punjab under HEC scholarship. Her work is majorly focused on study of hyperthermophiles and archaeal enzymes. She is currently pursuing her Postdoctoral research studies on a project approved by TUBITAK International fellowship in Sabanci University Nanotechnology Research and Application Center, Istanbul, Turkey.

ghazalehgharib@sabanciuniv.edu

Notes: