ABSTRACT

A 1.3 kb hyperthermophilic *xyl-A* gene encoding xylose isomerase from eubacterium *Thermotoga naphthophila* RKU-10 (TnapXI) was cloned and over-expressed in *E. coli* (BL21(DE3) to produce enzyme in mesophilic conditions that work at high temperature. The complete nucleotide sequence of the *xyl-A* gene was determined. Comparison of the nucleotide sequences with other *xyl-A* genes in the database showed that the *xyl-A* gene has 97% homology with that of the *xyl-A* gene from *T. naphthophila* available at NCBI. The inferred amino acid sequence showed that the enzyme was from class II of xylose isomerases. The TnapXI was concentrated by lyophilization and purified by heat treatment, fractional precipitation and UNOsphere Q anion-exchange column chromatography to homogeneity level. It was an acidic protein with theoretical isoelectric point (pI) 5.4 and theoretical molecular weight was calculated as 50.84 kDa. The apparent molecular mass (*Mr*) was estimated by SDS-PAGE to be 49.5 kDa. The active enzyme showed a clear zone on native-PAGE when stained with 2, 3, 5-triphenyltetrazolium chloride. The optimum temperature and pH for D-glucose to D-fructose isomerization were 98°C and 7.0, respectively. Xylose isomerase retains 85% of its activity at 50°C ($t_{1/2}$ 1732 min) for 4 h and 32.5% at 90°C ($t_{1/2}$ 58 min) for 2 h. It retains 90-95% of its activity at pH 6.5 to 7.5 for 30 min. The enzyme was highly activated (350%) with the addition of 0.5 mM Co^{2+} and to a lesser extent about 180 and 80% with the addition of 5 and 10 mM Mn^{2+} and Mg^{2+}, respectively but it was inhibited (54-90%) in the presence of 0.5-10 mM Ca^{2+} with respect to apo-enzyme. $t_{1/2}$ of TnapXI increased significantly by the addition of 1 mM Co^{2+} from 39.13% to 1466.67% as compared to apo-enzyme at temperature range 80-100°C. The enzyme showed a half life ($t_{1/2}$) of 18 min for apo-enzyme (K_d 0.0385 min^{-1}) and 65 min for holo-enzyme (K_d 0.0106 min^{-1}) at 95°C.

The catalytic affinities (K_m) of the enzyme for xylose and glucose were 0.96 and 7.67 mM, respectively, while V_{max} were 384 and 90 μmol/mg.min^{-1}, respectively. The turn-over (k_{cat}) rate was 5245 min^{-1} for D-xylose and 1229 min^{-1} for D-glucose. Catalytic efficiencies (k_{cat}/K_m) of enzyme for xylose and glucose were 5,463 and 160.2 $min^{-1}mM^{-1}$, respectively. The ionizable group of active site involved in controlling V_{max} of the

enzyme, showed pK_{a1} and pK_{a2} as 6.0 and 7.6, respectively. The pK_{a1} and pK_{a2} were assigned to His-101 and His-271, respectively. Temperature quotient (Q_{10}) was 2.05 while activation energy (E_a) was 82.25 kJ/mol. Thermodynamic parameters for D-glucose isomerization were ΔH^* 79.19 kJmol^{-1}, ΔG^* -6.93×10^{-53} kJmol^{-1}, ΔS^* 215 Jmol^{-1}K^{-1}, ΔG^*_{E-S} -14.9 kJmol^{-1} and ΔG^*_{E-T} -35.1 kJmol^{-1}, at 368 K. The D values for apo and holo TnapXI were calculated as 1.776 and 2.336 min, respectively whereas the z values for apo and holo enzyme were calculated as 12.65 and 32.68°C, respectively at 95°C. The activation energy ($Ea_{(d)}$) of isothermal irreversible deactivation at 95°C for apo and holo TnapXI were calculated as 209.5 and 770.1 kJ mol^{-1}, respectively. The thermodynamic parameters i.e., $\Delta G^*_{(d)}$, $\Delta H^*_{(d)}$, and $\Delta S^*_{(d)}$ for deactivation of the apo-enzyme were 206.44 kJmol^{-1}, 93.579 kJmol^{-1} and 0.306 Jmol^{-1}K^{-1} and for the holoenzyme were 767.04 kJmol^{-1}, 104.56 kJmol^{-1} and 1.800 Jmol^{-1}K^{-1}, at 368 K. D-glucose isomerization product was also analyzed by thin layer chromatography (R_f 0.65). The enzyme was very stable at slightly acidic to neutral pH and have the greater tendency to resist the thermal unfolding at sufficiently high temperature and required only trace amount of Co^{2+} for its optimal activity and stability. Overall, 52.2% D-fructose was achieved by the isomerization of D-glucose using TnapXI. Thus, it has a great potential for industrial applications.