ABSTRACT

Present work is concerned with the kinetic and thermodynamic studies of thermostable recombinant α-amylase cloned from *Thermotoga petrophila*. The protein was purified by heat treatment and anion exchange chromatography and a single band corresponding to molecular mass of 52 kDa was obtained when purified protein fraction was run on SDS-PAGE. V_{max} and K_m of the cloned enzyme for soluble starch were calculated at different temperatures (80-95°C) from Lineweaver-Burk plot as well as Eadie-Hofstee plot. The maximum V_{max} (0.064 mg/min) was obtained at 90°C with 1.109 mg K_m. The maximum specificity constant (19.83 mg^{-1} min^{-1}) and k_{cat} were also obtained at 90°C. Arrhenius plot was used to calculate activation energy i.e. 64.93 kJ mol^{-1}. Thermodynamic parameter i.e. ΔG, ΔS and ΔH for α-amylase at 90°C were found to be 92.59 kJ mol^{-1}, -84.48 J mol^{-1} K^{-1} and 61.91 kJ mol^{-1}, respectively at optimum temperature (90°C). Whereas, $\Delta G_{E\text{-}S}$ (free energy of substrate binding) and $\Delta G_{E\text{-}T}$ (free energy of transition state binding) were 0.316 and 3.331 kJ mol^{-1}, respectively. Desizing ability of recombinant α-amylase was also tested by performing experiment with sized fabric. The maximum % loss was observed after 4 h treatment and it was comparable to the rating 8 of TEGEWA Violet Scale.