“Molecular characterization of DNA polymerase of Thermus thermophilus MAT72 phage Tt72”

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In this work, I present a structural and functional analysis of the DNA polymerase of thermophilic *Thermus thermophilus* MAT72 phage vB_Tt72. The enzyme shows low sequence identity (<30%) to the members of the type-A family of DNA polymerases, except for two yet uncharacterized DNA polymerases of *T. thermophilus* phages: φYS40 (91,3%) and φTMA (90,6%). The Tt72 *polA* gene does not complement the *Escherichia coli polA*$^{-}$ mutant in replicating *polA*-dependent plasmid replicons. It encodes a 703-aa protein with a predicted molecular weight of 80 490 and an isoelectric point of 5,49. The enzyme contains a nucleotidyltransferase domain and a 3′→5′ exonuclease domain that is engaged in proofreading. Recombinant enzyme with His-tag at the *N*-terminus was overproduced in *E. coli*, subsequently purified by immobilized metal affinity chromatography, and biochemically characterized. The enzyme exists in solution in monomeric form and shows optimum activity at pH 8,5, 25 mM KCl, and 0,5 mM Mg^{2+}. Site-directed analysis proved that highly-conserved residues D^{15}, E^{17}, D^{78}, D^{180}, and D^{184} in 3′→5′ exonuclease and D^{384}
and D^{615} in the nucleotidyltransferase domain are critical for the enzyme’s activity. Despite the source of origin, the Tt72 DNA polymerase has not proven to be highly thermoresistant, with a temperature optimum at 55°C. Above 60°C, the rapid loss of function follows with no activity >75°C. However, during heat treatment (10 min. at 75°C), trehalose, trimethylamine *N*-oxide, and betaine protected the enzyme against thermal inactivation. A midpoint of thermal denaturation at T_m=74,6°C and circular dichroism spectra >60°C indicate the enzyme’s moderate thermal stability.