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The role of active-site amino acids in template-independent DNA synthesis by human terminal deoxynucleotidyl transferase

Senchurova S.I.[1]*, Kuznetsova A.A.[1], Laprina D.S.[1], Fedorova O.S.[1], Kuznetsov N.A.[1, 2]

[1] *Institute of Chemical Biology and Fundamental Medicine, SB RAS, Novosibirsk, Russia*

[2] *Department of Natural Sciences, Novosibirsk State University, Novosibirsk, Russia*

* *svetasenchurova@gmail.com*

Terminal deoxynucleotidyl transferase (Tdt) is a template-independent DNA polymerase belonging to the DNA polymerase X family. Tdt is only present in vertebrate cells and responsible for adding nucleotides to the 3'-end of single strand DNA at V-D and J-D junction of immunoglobulins and TCR receptor genes during V(D)J recombination. Being isolated and described in 1960 it remains one of the least studied enzyme catalyzing DNA synthesis. Although kinetic mechanisms have been identified for many template-dependent polymerases, it is clear that the absence of a template chain should have a significant impact on the kinetic parameters of DNA and nucleotide binding, catalysis and product dissociation. Herein, we aimed to gain a better understanding of the role of active-site amino acids R336, H342, D345 and L397. According to X-ray structural data, H342 and L397 form contacts with primer, R336 and H342 form contacts with deoxynucleoside triphosphates and D345 forms contacts with catalytic divalent metal ion. The studies were carried out under single-turnover kinetic conditions. The kinetic and thermodynamic parameters of the untemplated DNA synthesis by R336Q, H342A, D345E, L397A mutant forms were obtained. It was shown that substitution of these residues lead to decrease in Tdt activity. Highest efficiency of nucleotide incorporation was observed in the presence of Mn^{2+} compared to Co^{2+} and Mg^{2+} (Mn^{2+} > Co^{2+} >> Mg^{2+}). In the case of mutant D345E, a significant decrease in the binding constant with nucleoside triphosphate compared to WT Tdt was shown.

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