DOI 10.18699/SBB-2022-591

The pre-steady state analysis of human terminal deoxynucleotidyltransferase conformational dynamics under DNA synthesis

Kuznetsova A.A.*, Fedorova O.S., Kuznetsov N.A.
Institute of Chemical Biology and Fundamental Medicine, SB RAS, Novosibirsk, Russia
* *sandra-k@niboch.nsc.ru*

Terminal deoxynucleotidyltransferase (TdT) is a member of the DNA polymerase X family that is responsible for random addition of nucleotides to single-stranded DNA at V-D and D-J junctions in genes of heavy chains of immunoglobulins and T-cell receptors. We examined pre-steady-state state kinetics and MD simulations of nucleotide incorporation by TdT. It was demonstrated that the incorporation efficiency decreases in the order dGTP > dTTP ≈ dATP > dCTP in the presence of Mg^{2+}. It was shown that the elongation mode, namely the ratio of translocation and dissociation rate constants, as well as the catalytic rate constant are dependent on the nature of the nucleobase. The MD data indicate that during the recognition of various nucleotides, TdT comes into specific contacts with the nucleotide being incorporated. It turned out that guanine forms a broad network of contacts with amino acid residues in the active-site pocket, which allow dGTP to get efficiently accommodated at the correct position and facilitate the catalytic reaction. According to the MD simulations, it is possible that interactions with Asp395, Val394 and Arg453 are responsible for efficient processive dGTP incorporation by TdT. We have applied stopped-flow kinetics using intrinsic tryptophan fluorescence of the enzyme to analyze the conformational dynamics of TdT during processing of DNA synthesis. Similar to other DNA polymerases, TdT seems to undergo a multistep conformational transition during substrate binding and further conformational changes during catalysis. Such behavior illustrates the concept of the dynamic model of enzyme catalysis, in which conformational changes in the enzyme molecule drive the substrate-to-product conversion.

Acknowledgements: This work was supported by Russian Science Foundation grant No. 21-64-00017.