Reaction mechanism analysis of chemical ligation for long-chain elongation of L-*a*TNA

(*Graduate School of Engineering, Nagoya University*) ○Hikari Okita, Keiji Murayama, Hiroyuki Asanuma

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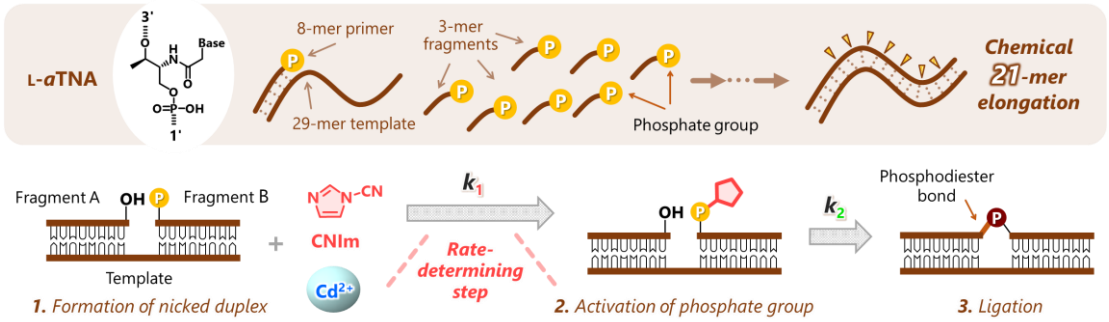
DNA functions as a versatile biological tool due to its sequence specificity, but it is vulnerable to enzymatic degradations. Many artificial nucleic acids have been developed by chemical modification of DNA scaffold to provide enzyme resistivity. We have designed *acyclic* L-threoninol nucleic acids (L-*a*TNA) by changing D-ribose scaffold to acyclic scaffold. L-*a*TNA can form highly stable homo-duplex compared with DNA and it can hybridize with complementary strands of DNA and RNA.[1] However, since natural enzyme does not recognize L-*a*TNA, it cannot be a substrate of useful enzymes such as polymerase and ligase.

We have recently developed nonenzymatic pseudo-primer extension reactions by using *N*-cyanoimidazole (CNIm) and Mn^{2+} instead of enzymes, which enabled template-directed elongation of 9-mer L-*a*TNA from random trimer fragments.[2] If we achieve much efficient template-directed synthesis, design of L-*a*TNA aptamer, creation of artificial life, and nanotechnology based on L-*a*TNA will be possible. For this purpose, we focused on CNIm/M^{2+} system and ligation mechanisms were analyzed in detail to improve the efficiency of L-*a*TNA replication. It was revealed that Cd^{2+}, Ni^{2+}, and Co^{2+} dramatically increased the ligation rate more than Mn^{2+} for not only L-*a*TNA but also DNA ligations.[3] Furthermore, we performed kinetic analysis of chemical ligation of L-*a*TNA. The ligation proceeds mainly via three steps: (i) duplex formation between fragments and a template, (ii) activation by CNIm binding to phosphate group, and (iii) ligation of two fragments by generating a phosphodiester bond. We found that the activation was rate-determining step and stabilization of 3'-phopahte group of L-*a*TNA at nick site accelerated the ligation rate. Based on these results, we finally achieved elongation of 21-mer L-*a*TNA with Cd^{2+} and random trimers by reversing the elongation direction suitable for stabilizing phosphate group.[3]



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