【Grant-in-Aid for Scientific Research (S)】

Broad Section E

Title of Project : Creation of artificial genetic system with acyclic artificial nucleic acids and application to evolutionary molecular engineering

ASANUMA Hiroyuki

(Nagoya University, Innovative Research Center for Preventive Medical Engineering, Professor)

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Keyword : Pre-RNA world hypothesis, L-*a*TNA, Chemical ligation, Origin of life

【Purpose and Background of the Research】

RNA world hypothesis, a widely accepted origin of life, prompted prebiotic chemists to synthesize ribonucleotide and self-replication of RNA oligomers under plausible prebiotic conditions. However, since non-enzymatic ribonucleotide synthesis and self-replication of RNA was very difficult to realize under abiotic conditions, alternative hypothesis called "pre-RNA" world was introduced. This hypothesis postulates existence of primitive genetic material, i.e., "pre-RNA" (artificial nucleic acid), which should have much more simple structure than ribonucleotide, and is then evolved into RNA. But none of

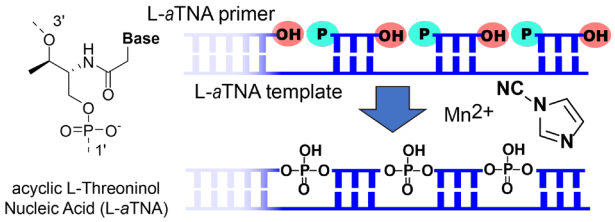

Fig.1. Chemical ligation of L-*a*TNA.

artificial nucleic acids satisfied the requisites of pre-RNA so far, there has been no progress on this hypothesis.

Our group has originally developed several acyclic artificial nucleic acids, and found that L-*a*TNA (Fig.1) could recognized both DNA and RNA. Recently, we also found that L-*a*TNA could be efficiently ligated in the presence of metal ion and *N*-cyanoimidazole, and achieved its pseudo-primer extension reaction non-enzymatically (Fig.1). In this project, we will create a new artificial genetic system with L-*a*TNA (Fig.2); self-replication (amplification), transcription, and reverse-transcription of L-*a*TNA. Here, we regard L-*a*TNA as "genome", and 1) template-directed synthesis of L-*a*TNA (self-replication, amplification), 2) "transcription" of L-*a*TNA to DNA(RNA), and 3) "reverse-transcription" of DNA(RNA) to L-*a*TNA will be realized non-enzymatically. This artificial genetic system enables sequencing of L-*a*TNA strand via transcribed DNA. PCR amplification of transcribed DNA and subsequent reverse-transcription also enables evolutionary molecular engineering as DNA-protein world. We will also create L-*a*TNA aptamer by making full use of the L-*a*TNA-based evolutionary molecular engineering, as an application of artificial genetic system.

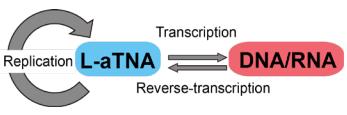

Fig.2. Artificial genetic system

【Research Methods】

Template-directed self-replication of L-*a*TNA is realized by using a pool of random L-*a*TNA trimers as substrates. Next, PCR-like self-amplification is investigated.

We next investigate transcription of L-*a*TNA to DNA(RNA) to prepare for the L-*a*TNA aptamer. For this purpose, metal ions and metal-complexes that activate nucleophilicity of -OH are designed. If we transcribe L-*a*TNA to DNA, L-*a*TNA sequence can be decoded via DNA sequencing.

We next achieve reverse-transcription of DNA(RNA) to L-*a*TNA by using a pool of random L-*a*TNA trimers as self-replication of L-*a*TNA.

After realizing the artificial genetic system, evolutionary molecular engineering is applied to L-*a*TNA as DNA engineering. First, L-*a*TNA oligomer that can bind to specific target is fished from a random pool of L-*a*TNA oligomers. Then the caught L-*a*TNA oligomer is transcribed into DNA(RNA), which is amplified by PCR, followed by reverse-transcription to L-*a*TNA. By repeating this evolution cycle, L-*a*TNA will be obtained by decoding the transcribed DNA.

【Expected Research Achievements and Scientific Significance】

If we realize the artificial genetic system in Fig.2, primitive nucleic acid that satisfies the requisites for pre-RNA is for the first time evidenced with L-*a*TNA, which should contribute to the study on the origin of life. Furthermore, obtained L-*a*TNA aptamer should be available as a new nucleic acid medicine.

【Publications Relevant to the Project】

- K. Murayama, H. Kashida, H. Asanuma, Acyclic L-Threoninol Nucleic Acid (L-*a*TNA) with Suitable Structural Rigidity Cross-pairs with DNA and RNA., *Chem. Commun*., **51**, 6500-6503(2015).
- K. Murayama, H. Okita, T. Kuriki, H. Asanuma, Nonenzymatic polymerase-like template-directed synthesis of acyclic L-threoninol nucleic acid, *Nat. Commun.*, **12**, 804(2021).

【Homepage Address and Other Contact Information】

http://www.chembio.nagoya-u.ac.jp/labhp/bioanal3/index-e.html