Aminoacylation of tRNA by antisense molecule

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ABSTRACT
Aminoacylation of tRNA was attempted through formation of tRNA/DNA/aa-PNA (N-aminoacylated peptide nucleic acid) ternary hybrid. A 23-mer DNA, that is complementary to a 3'-terminal of tRNA and to a 9-mer PNA carrying an amino acid unit, was designed to achieve close proximity between the amino acid and the 3'-OH group of tRNA. The aminoacylation was carried out in a buffer solution containing imidazole. The aminoacylation was detected by nuclease S1 treatment followed by HPLC and MALDI-TOF MS. This novel methodology will open a way for easy and versatile aminoacylation of nonnatural amino acids onto specific tRNAs.

INTRODUCTION
Aminoacylation of a tRNA with a nonnatural amino acid is a key step for nonnatural mutagenesis. To achieve the nonnatural aminoacylation onto a specific tRNA, a molecular system that recognizes the tRNA sequence and transfers an amino acid to its 3'-end of tRNA has to be designed. In this study, N-aminoacyl PNA was employed as an aminoacyl donor, and a 23-mer DNA was used as a template for the tRNA and the PNA (Fig. 1). This ternary system is expected to aminoacylate the specific amino acid to a specific tRNA. 3-(2-Naphthyl)-L-alanine (napAla) was chosen as the nonnatural amino acid for its easy detection by fluorescence.

Fig.1. Aminoacylation of tRNA by aa-PNA and DNA.
RESULTS AND DISCUSSION
A napAla-PNA (napAla-thioester-TTTTGATT-LysLysNH₂, Fig. 2) carrying an amino acid activated ester at the \( N \)-terminal of a PNA was synthesized by the solid phase method. The product was purified by HPLC and was characterized by MALDI-TOF MS. A 23-mer DNA, that is complementary to a 3'-terminal of tRNA and to a 9-mer PNA carrying an amino acid unit, was designed to achieve close proximity between the amino acid and the 3'-OH group of tRNA. Hybridization of the ternary system (tRNA/DNA/aa-PNA) was evidenced by a gel mobility shift assay. The aminoacylation reaction mixture contained 50 mM imidazole-AcOH (pH 6.5), 200 \( \mu \)M tRNA, 200 \( \mu \)M DNA, and 200 \( \mu \)M napAla-PNA. After incubation at \( 0°C \) for 2h, 1.5 M NaOAc solution (pH 4.5) was added to stop the reaction. The reaction product was purified by ethanol precipitation, and digested by nuclease S1 for 10 min at 37°C. The digested product was analyzed by HPLC and MALDI-TOF MS. NapAla-AMP was detected only in the presence of tRNA, DNA, and napAla-PNA (Fig. 3). These results suggest that aminoacylation of adenosine at the position of CCA terminal of tRNA. The yield of the aminoacylation was evaluated from the peak area of napAla-AMP to be approximately 2%. This novel methodology will open a way for easy and versatile aminoacylation of nonnatural amino acids onto specific tRNAs.

![Fig. 2. Structure of napAla-PNA.](image)

![Fig. 3. HPLC analysis of aminoacylation product after nuclease S1 digestion.](image)