Modification of the amino acid acceptor stem of *E. coli* $tRNA_f^{Met}$ by ligation of chemically synthesized ribooligonucleotides

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The single-stranged region of the amino acid acceptor stem corresponding to the 3'-end of *E. coli* $tRNA_f^{Met}$ was replaced by ligation of chemically synthesized ribooligonucleotides, in order to change the length of the single-stranded CCA terminus. The chemically synthesized ribooligomers, CCA, ACCA, AACCA and CAACCA, were ligated to nuclease-treated *E. coli* $tRNA_f^{Met}$, which lacked the ACCA sequence at the 3'-end. The methionine acceptor activities of these modified tRNAs were examined using *E. coli* methionyl-tRNA synthetase. Ligation of the chemically synthesized pentamer (AACCA) to the acceptor terminus restored the methionine acceptor activity, whereas ligation of the hexamer (CAACCA) or trimer (CCA) to the acceptor terminus did not Modification of the acceptor terminus had no effect on the formylation of accepted methionine.

Aminoacylation *Formylation* *Elongated aminoacylation end* *Truncated aminoacylation end ligation* *Synthetic oligonucleotide*

1. INTRODUCTION

Transfer RNA is one of the smallest nucleic acids that has a biological function in living cells. In many laboratories structure requirements for the recognition of tRNAs by aminoacyl-tRNA synthetases have been concerned with the interaction between nucleic acids and proteins [1–14].

We have synthesized various analogues of *E. coli* $tRNA_f^{Met}$ by ligating chemically synthesized ribooligomers to natural $tRNA_f^{Met}$ fragments using T4 RNA ligase [12–14].

Replacement of the anticodon trimers in *E. coli* $tRNA_f^{Met}$ caused a large decrease in the amino acid acceptor activity with *E. coli* methionyl-tRNA synthetase [14]. On the other hand, changes in the discriminator position of the amino acid acceptor stem had little influence on the affinity for the synthetase [13].

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E. coli $tRNA_f^{Met}$ has a unique structure at the anticodon loop and the amino acid acceptor stem [15,16] where the last 5 bases from the 3'-terminus curl back in contrast to other tRNAs [16]. We assumed that the acceptor end directly binds methionine, and therefore investigated the correlation between the length of the protruding 3'-terminus and the methionine acceptor activities of analogs having a different protruding end. Here, we describe the syntheses of several modified *E. coli* $tRNA_f^{Met}$ formed by ligating CCA, ACCA, AACCA or CAACCA to a $tRNA_f^{Met}$ lacking the 3'-ACCA. The methionine acceptor activities of these analogues were examined using the *E. coli* methionyl-tRNA synthetase.

2. MATERIALS AND METHODS

Ribooligonucleotides were synthesized by the triester method [17]. [γ-^{32}P]ATP and L-[U-^{14}C]-methionine were obtained from NEN and Amer-

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sham, respectively. *E. coli* $tRNA_f^{Met}$ was a gift from Dr D. Söll. T4 RNA ligase was purified as described [18]. Polynucleotide kinase and *E. coli* alkaline phosphatase were obtained from Takara Shuzo and nuclease S1 from Sankyo. Other enzymes used for the analyses of products were obtained as in [14,19,20]. Crude *E. coli* aminoacyl-tRNA synthetase was purified as described [21]. Folinic acid-SF as a formyl donor was obtained from Lederle.

Partial digestion of *E. coli* $tRNA_f^{Met}$ with nuclease S1 and aminoacylation reaction were performed as in [13]. Phosphorylation with polynucleotide kinase, dephosphorylation with alkaline phosphatase and ligation with T4 RNA ligase were performed according to [12,14]. Formylation was performed under the conditions for aminoacylation containing 0.7 mM folinic acid-SF at 37°C for 30 min in 30 μl formylation reaction mixture. Half of the mixture was treated with the same volume of 50 mM $CuSo_4$, 0.3 M Tris-HCl (pH 7.5) at 37°C for 10 min.

3. RESULTS AND DISCUSSION

A scheme for the synthesis of modified *E. coli* $tRNA_f^{Met}$ is shown in fig.1. *E. coli* $tRNA_f^{Met}$ was treated with a limited amount of nuclease S1 to remove the 3'-terminal ACCA. The remaining fragment with 73 nucleotides [tRNA(73)] was isolated on 10% polyacrylamide gel electrophoresis (PAGE). Four ribooligonucleotides, CCA, ACCA, AACCA, CAACCA, were synthesized by the triester method as in [17]. These chemically synthesized ribooligonucleotides were phosphorylated with [γ-^{32}P]ATP and polynucleotide kinase and then joined to the tRNA(73) with T4 RNA ligase. The ligated products were isolated on 10% PAGE and subjected to a nearest neighbor analysis to confirm the junction point. The yields of these ligase reactions were below 10%.

The ligated molecules, tRNA(76), tRNA(77), tRNA(78) and tRNA(79), were dephosphorylated with alkaline phosphatase and tested for their methionine acceptor activities (fig.2). The tRNA(77)

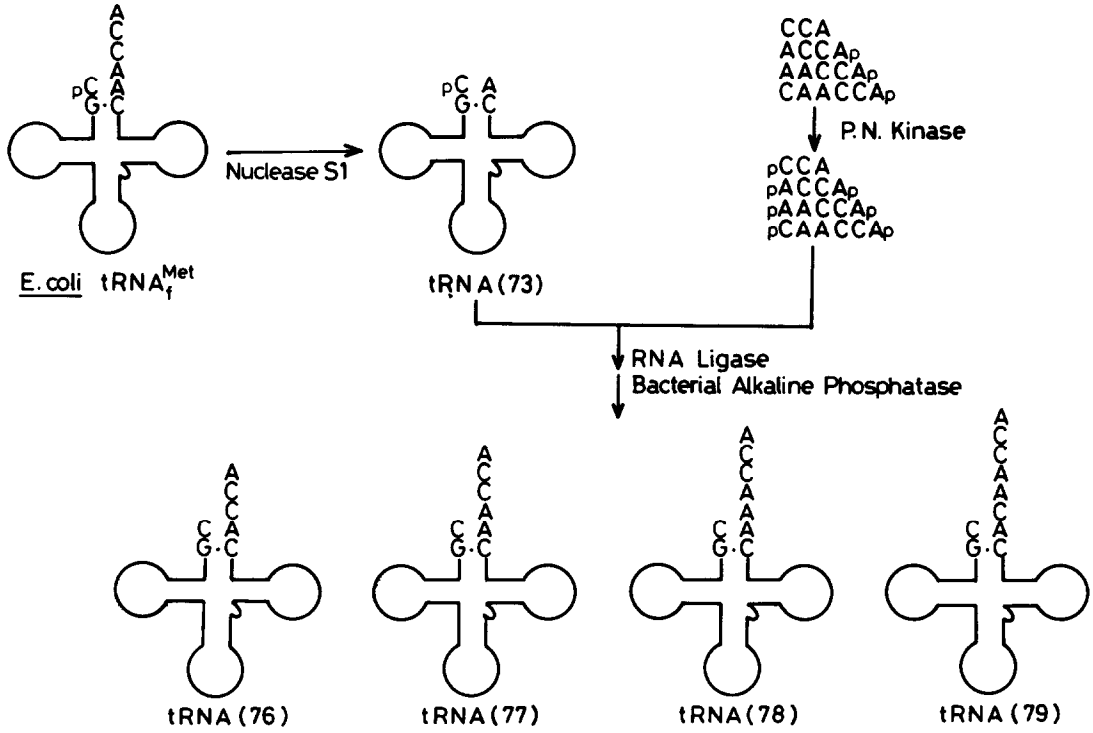

Fig.1. Scheme for the modification of the amino acid acceptor stem of *E. coli* $tRNA_f^{Met}$.

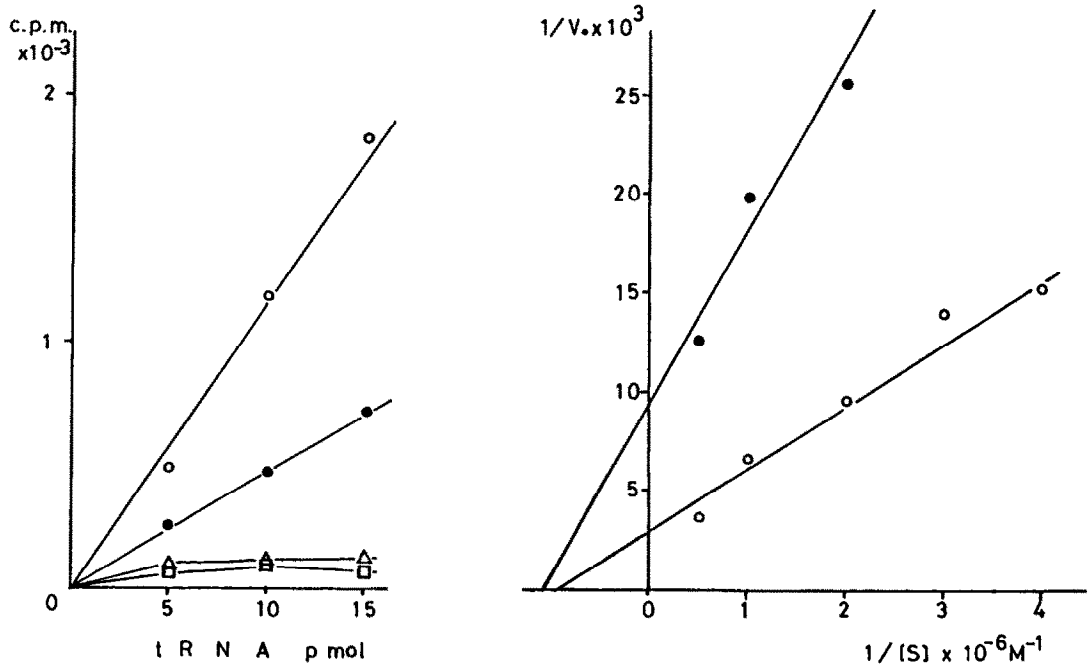

Fig.2.Aminoacylation of modified tRNAs. Methionine acceptor activities of tRNAs (left) and kinetic studies for aminoacylation (right). (○—○) Control tRNA(77), (△—△) tRNA(76), (●—●) tRNA(78), (□—□) tRNA(79).

molecule was synthesized as a control since it had the same sequence as the natural $tRNA_f^{Met}$. Fig.2 shows that tRNA(76), 1 base shorter, and tRNA(79), 2 bases longer, had no methionine acceptor activity, but tRNA(78), 1 base longer, could accept methionine. The kinetic studies on aminoacylation showed that the differences in aminoacylation activity were reflected only in the V_{max} values since the K_m values of these tRNAs were almost the same. Therefore, both tRNA(77) and tRNA(78) had similar affinities for *E. coli* methionyl-tRNA synthetase. It appears that the 3′-terminus of $tRNA_f^{Met}$ does not influence the affinity of methionyl-tRNA synthetase. Consequently we can assume that both tRNA(76) and tRNA(79) presumably form a complex with the synthetase, but are unable to position the 3′-end adenosine moiety at the catalytic site of the synthetase. One extra base in the 3′-terminus region may not prevent the required contact of the 3′-end adenosine with the catalytic site.

The recognition site of methionyl-$tRNA_f^{Met}$ by the transformylase in prokaryotes is still unclear. We have investigated the formylation of the tRNA(78) under the same assay system conditions as for the aminoacylation reaction except for the presence of formyl donor. The formylation ratio for tRNA(78) and tRNA(77) was 0.87 and 0.74, respectively. This may indicate that the addition of 1 extra base in the 3′-terminus region of $tRNA_f^{Met}$ does not affect the shape of the molecule or that the transformylase may recognize not only the 3′-terminal region but also another area as can be seen by comparison of the sequence of $tRNA_f^{Met}$ [22] and $tRNA_m^{Met}$ [23].

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