SYNTHESIS OF FMET-TRNA FROM E. COLI

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In order to elucidate the structure-function relationship of fMet-tRNA from Escherichia coli, fragments of this tRNA were synthesized chemically and joined together to form the tRNA molecule.

1) Fragments synthesized by phosphodiester methods.

Fragments 1a, 1b, 8,9 and 10 were synthesized either by stepwise condensation or by block condensation method using DCC or TPS. Yields were rather moderate but this method would be suitable for obtaining limited amounts of pure oligonucleotides with chain length up to octanucleotide.

2) Fragments synthesized by a mixed di- and triester method.

Fragments 9, 10 and 11a-d were synthesized by this method. Protecting groups used for phosphates were cyanoethyl and acyl or o-nitrobenzyl were used for 2'-OH. A separation of the intermediates was effected by variously modified methods which enabled rapid separation of the final products.

3) Fragments synthesized by triester method.

Fragments 2, 3, 4, 5 and 6 were synthesized by a triester method using p-chlorophenyl for internal phosphate, o-nitrobenzyl for 2'-OH, p-chlorophenyl and anilidate for terminal phosphates. These protections afford a general procedure for synthesizing fully protected oligonucleotide blocks suitable for elongation either to the 3'- or 5'-direction.

4) Joining of synthetic fragments with RNA ligase.

RNA ligase seems to be suitable for joining these chemically synthesized fragments to afford longer olionucleotides. 5'-End eicosanucleotide GGUCCGACCGAGGUGGGGCGC and 3'-end heptadecanucleotide UCCGGCCCCCGCAACCA were synthesized by using this enzyme. The structure of the former oligonucleotide was confirmed by reconstituting it with the 3/4 molecule obtained from fMet-tRNA. The joining of the rest of the molecule is now in progress.