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Recognition of Individual *Escherichia coli* Transfer Ribonucleic Acids by 1-Adenine-Specific Methyltransferase from Rat Liver

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Purified 1-adenine-specific tRNA methyltransferase from rat liver preferentially methylated *Escherichia coli* tRNA species containing the target adenylate residue in a G-T-Ψ-C-G-A-A-U-C sequence. The results of methylation of various tRNA species are discussed.

1-Methyladenosine (m¹A) is not a natural component of *Escherichia coli* tRNA (Dunn, 1961); however, its content in yeast and rat liver tRNA is rather high, 0.9 and 1.3 mol% respectively (Baguley & Staehelin, 1969). It occurs in pancreatic-ribonuclease digests of yeast and rat liver tRNA in R-m¹A-A-Yp and in R-m¹A-Yp sequences. Methylation, to an extent of 1 mol of 1-methyladenosine formed per mol of bulk *E. coli* tRNA with purified enzyme from either rat liver or leukaemic-rat spleen, yielded predominantly the sequence G-m¹A-A-Up with some A-m¹A-A-Up and G-m¹A-Up (Baguley & Staehelin, 1969). The 1-adenine methylation site was located 19 nucleotides from the amino acceptor end in yeast tRNA^{Ser}, next to the common G-T-Ψ-C-R sequence (Baguley *et al.*, 1970). In all sequenced tRNA species an adenosine or 1-methyladenosine residue occurs in this position, the adjacent position is occupied by a purine or a pyrimidine, and in positions 17 and 16 there are always pyrimidine residues. Two exceptions to this general pattern were reported in two mammalian tRNA species. Firstly, tRNA^{Phe} from various mammalian tissues (Keith *et al.*, 1973, 1974; Roe *et al.*, 1975; Ortwirth *et al.*, 1975) contains two 1-methyladenosine residues per chain, one being in the T-Ψ-C loop and the second one in the dihydrouridine loop. Secondly, the m¹A residue in tRNA^{Met} from several mammalian sources (Simsek *et al.*, 1974; Piper, 1974) is contained in a G-m¹A-A-A-C sequence. An attempt was made in the present study to find a correlation between the extent of methylation (by using a purified 1-adenine methyltransferase) and nucleotide sequences neighbouring the target adenylate residue in a number of homogeneous tRNA species from *E. coli*.

Experimental

1-Adenine methyltransferase (EC 2.1.1.36) was partially purified, approx. 40-fold, from rat liver. The

purified enzyme had a specific activity of 0.7 nmol of methyl group/h per mg of protein, and its methylating activity, as shown by nucleoside analysis of the methylated bulk *E. coli* tRNA, was 96% specific for 1-adenine (Kraus & Staehelin, 1974a). All other materials, techniques, sources of tRNA species and the numbering system of the isoaccepting tRNA species were previously described (Kraus & Staehelin, 1974a).

Results and Discussion

The site of 1-adenine methylation was located in *E. coli* tRNA^{Leu}₃ 19 nucleotides from the 3'-end in a sequence A-m¹A-G-Up (Kraus & Staehelin, 1974b). The same adenylate residue has been shown by others (Baguley *et al.*, 1970; Kuchino & Nishimura, 1974; Pegg, 1974) to be methylated in yeast tRNA^{Ser}, and in *E. coli* tRNA^{Ser}₃, tRNA^{His}₁, tRNA^{Glu}₂, tRNA^{Met} and tRNA^{Val}₁. Several individual tRNA species were tested for their ability to serve as methyl acceptors for the 1-adenine methyltransferase from rat liver. As shown in Table 1, the extents of methylation varied greatly. The best methyl acceptor was tRNA^{Glu}₂. There was also extensive methylation of tRNA^{Ser}₁, tRNA^{Ser}₃, tRNA^{Tyr} and tRNA^{Arg}. All these tRNA molecules contain an identical sequence of nucleotides in the T-Ψ-C loop, namely G-T-Ψ-C-A-A-U-C. Other tRNA molecules having an adenosine in position 20 or a base different from adenosine in position 18 were methylated to significantly lower extents. These findings are in good agreement with the previous report that 80% of 1-methyladenosine formed *in vitro* in bulk *E. coli* tRNA was found in the G-m¹A-A-Up sequence (Baguley & Staehelin, 1969). The presence of this sequence at the methylation site was clearly not the sole factor determining the extent of methylation, since this varied significantly within the group of tRNA species containing this sequence. The methylation of tRNA^{Glu}₂, tRNA^{Tyr} and tRNA^{Phe} was examined more closely as a function of time; tRNA^{Glu}₂ was chosen as the best

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Table 1. Methylation of individual *Escherichia coli* tRNA species with rat liver 1-adenine methyltransferase
The numbering system of the isoaccepting tRNA species and the conditions of the enzyme assay were defined previously (Kraus & Staehelin, 1974a)

tRNA		Extent of methylation (nmol of methyl group/3 h per mg of tRNA)	Sequence at 1-adenine-methylation site Position from 3'-end			
			20	19	18	17
<i>E. coli</i>	Bulk	3.8				
	Ser 1	9.6				
	Ser 3	10.3				
	Glu 2	26	G	A	A	U
	Tyr	7.5				
	Arg	9.8				
	Phe	3.9	G	A	U	U
	Val	4.1	G	A	U	C
	Leu 1	3				
	Leu 2	5.5				
	Leu 3	4.5	A	A	G	U
	fMet	5	A	A	A	U
Yeast	Bulk	1.2				
	Asp	0.6	A	A	U	U
Rat liver	Bulk	0.1

Fig. 1. Time course of 1-adenine methylation of individual *E. coli* tRNA species

Assay tubes contained 0.48 mg of enzyme protein and 2.5 μ g of tRNA^{Glu} (○), 2.5 μ g of tRNA^{Tyr} (●) or 4 μ g of tRNA^{Phe} (△).

acceptor of methyl groups, tRNA^{Tyr} as a medium one and tRNA^{Phe} as a relatively poor one.

Fig. 1 demonstrates that the extent of methylation of all three tRNA species increased with time even after a long exposure to the enzyme, tRNA^{Glu} was methylated to an extent of 29.3 nmol of methyl group/mg of tRNA, which corresponds to about

0.9 mol of methyl group incorporated/mol of tRNA. tRNA^{Tyr} and tRNA^{Phe} were methylated to much lower extents, namely 0.3 and 0.17 mol of methyl group/mol of tRNA respectively. These methylation extents represented 1-adenine methylation exclusively; the trace amounts of *N*²-guanine methyltransferase I present in the preparation did not contribute to the total methylation, since all tRNA species used in the present study, except tRNA^{Val}, tRNA^{Phe} and tRNA^{Arg}, were previously shown (Kraus & Staehelin, 1974a) not to act as substrates for purified *N*²-guanine methyltransferase I. The threefold difference in the extent of methylation between tRNA^{Glu} and tRNA^{Tyr} is surprisingly high in view of the fact that the entire T- Ψ -C loop and two adjacent G-C pairs of the arm are identical. The most striking difference between those two tRNA molecules is the presence of two 4-thiouridine residues between the amino acid arm and the dihydrouridine arm in tRNA^{Tyr}, whereas in tRNA^{Glu} there is an unmodified U-C sequence there. It is noteworthy that the 4-thiouridine modification is also absent from mammalian tRNA. Further, tRNA^{Glu} has a short extra arm and tRNA^{Tyr} contains a long extra arm.

As the results in Table 1 show, the nucleotides directly surrounding the target adenylate residue are an important factor influencing the extent of methylation. It appears, however, that there must be another factor involved in the recognition process *in vivo* in addition to the sequence surrounding the adenine methylation site. tRNA^{Trp} from brewer's yeast (Keith *et al.*, 1971), for instance, contains 1-methyladenosine in a sequence G-T- Ψ -C-A-m¹A-U-U-C, whereas the identical sequence in tRNA^{Asp} from the same yeast is not methylated (Gangloff *et al.*, 1972). It has been suggested that 1-methyladenosine is formed primarily during the early and intermediate stages of maturation of a precursor tRNA (Munns & Sims, 1975). It is therefore possible that the enzyme recognizes the target sequence at the level of the larger precursor molecule. McClain & Seidman (1975) have shown that point mutation in a tRNA-precursor sequence was sufficient to inhibit modification of proximal and distal nucleotides in the secondary structure, thereby demonstrating the involvement of the tertiary structure in the recognition process.

The combinations of the nucleotides neighbouring the m¹A residue in yeast tRNA species result in eight slightly different sequences, and so there may be more than one 1-adenine methyltransferase, with different recognition specificities, needed in eukaryotic cells. Agris *et al.* (1974), indeed, separated the activity for the formation of 1-methyladenine from HeLa cells into more peaks of activity. However, the separated activities showed no difference in recognition specificity when tested with *E. coli* tRNA^{fMet}.

The results show, in agreement with those of Kuchino & Nishimura (1974), preferential methylation of *E. coli* tRNA containing the target adenylate residue in a G-T-Ψ-C-G-A-A-U-C sequence with rat liver enzyme. On the other hand, only approx. 30% of 1-methyladenosine formed *in vivo* in rat liver tRNA is found (Baguley & Staehelin, 1969) in a corresponding sequence Y-G-m¹A-A-U. The significantly lower extents of methylation found in tRNA molecules containing other sequences may be the result of conditions *in vitro*, or may be due to the use of a heterologous substrate or other factors. It became clear that the problem of 1-adenine methyltransferase specificity cannot be solved entirely, even when pure tRNA substrates and purified enzymes are used. These questions can perhaps be answered by using tRNA species from mutants lacking the methylating activity, precursor tRNA molecules or tRNA synthesized *in vitro*.

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