The selective reaction of methoxyamine with cytidine residues in mammalian initiator transfer ribonucleic acid

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ABSTRACT

Methoxyamine reacts selectively with tRNA molecules at certain exposed cytosine residues usually located in non base-paired regions of the two dimensional clover leaf structure. Here methoxyamine is used for the first time in a study of a mammalian tRNA structure. One of the sequence abnormalities of myeloma initiator tRNA is a cytosine instead of the usual uracil immediately preceding the anticodon. A study of the reaction of the cytosine residues with methoxyamine indicates that the accessibility of bases to chemical reagents in the anticodon loop of this mammalian initiator tRNA is very similar to that observed for the bacterial initiator tRNA.

INTRODUCTION

At the initiation stage of protein synthesis in mammalian cells a special initiator tRNA (tRNA_f^{Met}) donates the methionine residue which is to become the N-terminus of the newly synthesised protein¹. The role of methionyl-tRNA_f^{Met} is limited to the initiation step and it does not function apparently in polypeptide chain elongation. We have recently determined the complete nucleotide sequence of the tRNA_f^{Met} species present in the cytoplasm of mouse myeloma cells². Two unusual features of the primary structure of this molecule are:-

(1) The presence of a cytosine as the base immediately preceding the anticodon in a position which has hitherto always been found to be occupied by a uracil in tRNA sequences.

(2) The abnormal sequence in loop IV of the tRNA clover leaf (-A-U-C-G-m¹A-A-A-). This sequence has also been demonstrated in loop IV of tRNA_f^{Met} isolated from other mammalian sources as well as in yeast tRNA_f^{Met} by Simsek, Petrisant and RajBhandary³.

Neither of these two sequence abnormalities occur in *E. coli* tRNA_f^{Met} despite the otherwise very considerable homology between the primary structures of the initiator tRNAs of *E. coli*⁴ and mouse myeloma cells². These two molecules are evidently closely similar in structure since both are efficient substrates for *E. coli*

methionyl-tRNA synthetase and *E. coli* methionyl-tRNA transformylase.

The selective reactivity of certain regions of tRNA molecules with chemical reagents has been used to yield information concerning the three dimensional structure of transfer RNA. One such reagent, methoxyamine, reacts with cytosine bases which are sterically exposed within the unpaired regions of the tRNA clover leaf⁵. The reaction of methoxyamine with *E. coli* tRNA_f^{Met} has already been investigated in some detail⁶, and we report here the results of an investigation to establish whether the modification of mammalian tRNA_f^{Met} by the same reagent might reflect special features of mammalian initiator tRNA structure.

METHODS

Purified ³²P-labelled mouse P3 myeloma cell tRNA_f^{Met} was prepared from cells cultured with ³²P-phosphate². Treatment with methoxyamine was for 20 hr at 37°C in the presence of 3 M methoxyamine (pH 5.5) containing 10 mM MgCl₂, as detailed by Chang⁶. After ethanol precipitation to remove the reagent, the RNA was digested with T₁ RNase and the products of RNase digestion were separated using two-dimensional electrophoresis⁷. Individual oligonucleotides were analysed by pancreatic RNase digestion and the digestion products separated by electrophoresis at pH 3.5 on Whatman DE 81 paper. Pancreatic RNase digestion products were subsequently analysed by T₂ RNase digestion^{5, 6}.

RESULTS

Fig. 1 shows a radioautograph of the fingerprint of T₁ RNase digested myeloma tRNA_f^{Met} after methoxyamine treatment. A fingerprint of a T₁ RNase digest of the same molecule as it appears before chemical modification is published elsewhere². Table 1 summarises the structures of the oligonucleotides present in Fig. 1 together with the relative yields in which they were found.

Methoxyamine treatment of mouse myeloma tRNA_f^{Met} causes two oligonucleotide spots (corresponding to C-U-A-C-C-A_{OH} and C-C-C-A-U-t₆A-A-C-C-C-A-Gp) to completely disappear from the T₁ RNase fingerprint and creates six new spots. However, since methoxyamine reacts with cytosine bases to give approximately equal amounts of two derivatives, thereby causing certain modified oligonucleotides to migrate as two spots in the second dimension of the fingerprinting procedure⁵, 6a and 6b (Fig. 1) are in reality both C^{*}-Gp⁺ and 12a and 12b are different forms of C-U-A-C^{*}-C^{*}-A_{OH}. For each case of methoxyamine modified oligonucleotide in Fig. 1 it was possible to determine which cytidine residue had reacted on the basis of

Figure 1

Radioautograph of the fingerprint of a T_1 RNase digest of methoxyamine-modified mouse myeloma $tRNA_{Met}$. The numbers refer to the digestion products listed in Table 1. Oligonucleotides were separated⁷ in the first dimension (1) by electrophoresis on cellulose acetate in 5% acetic acid, 7 M urea 0.002 M EDTA, pH 3.5 and in the second dimension (2) by electrophoresis on Whatman DE 81 paper in 7% formic acid, 0.002 M EDTA.

† ABBREVIATION

* C is used to denote methoxyamine modified cytidine residues, the type of modification being unspecified (methoxyamine reaction can yield either N-4-methoxycytidine or 6-methoxyamine-5, 6-dihydro-N-4-methoxycytidine⁵).

TABLE 1

Oligonucleotide No. ^a	Sequence	Molar yield	
		Control	Methoxyamine modified
1	Gp	3.9	3.8
2	C-Gp	3.1	2.2
6a + 6b	C [*] -Gp	-	0.7
3	A-Gp	2.1	2.2
4	C-A-Gp	2.0	2.0
5	A-A-Gp	1.0	0.9
7a + 7b	U-m ² Gp and U-m ² G p	0.8	0.6
8	m ⁷ G-D-m ⁵ C-Gp	0.9	1.0
9	C-U-Gp	1.1	1.1
10	pA-Gp	1.0	0.6
11	A-U-Gp	1.0	1.0
-	C-U-A-C-C-A ^{OH}	1.0	0
12a + 12b	C-U-A-C-C-A ^{OH}	-	0.9
13	A-U-C-Gp	1.0	1.0
14a + 14b	U-m ¹ Gp and U-m ¹ G-m ² Gp	0.9	0.7
-	C-C-C-A-U-t ⁶ A-A-C-C-C-A-Gp	0.9	0
15a	C-C-C [*] -A-U-t ⁶ A-A-C-C-C-A-Gp	-	0.5
15b	C-C-C [*] -A-U-t ⁶ A-A-C-C-C-A-Gp	-	0.4
16	m ¹ A-A-A-C-C-A-U-C-C-U-C-U-Gp	0.9	0.8

^aThe numbers refer to the oligonucleotides in Fig. 1

Quantitations of the oligonucleotides present in a T₁ RNase digest of methoxyamine modified mouse myeloma tRNA_f^{Met}

the pancreatic RNase degradation products and the known sequence of the oligonucleotide from which it had been derived. The phosphodiester linkage adjacent to a cytidine residue is rendered considerably less susceptible to pancreatic RNase hydrolysis after the cytidine has been modified by methoxyamine.⁵ Thus 15a and 15b could be characterised by the presence of CAU and CCAU respectively amongst their pancreatic RNase degradation products.

Mammalian tRNA_f^{Met} contains eight cytosines which are not involved in hydrogen bonding when the molecule is folded as a clover leaf (Fig. 2). Of the modified T₁ RNase oligonucleotides, 15a and 15b are derived from the anticodon region and 12a and 12b originate from the 3' terminus of the initiator tRNA. Since methoxyamine apparently only modifies cytosine bases which are within the non base-paired regions of the clover leaf model, 6a and 6b (C-Gp) are evidently derived from reaction at residue 16 of loop 1. Residue 16 has invariably been found to be partially or wholly susceptible to methoxyamine modification in different tRNA sequences^{5,6}, thus rendering it rather improbable that C-Gp could have originated from a base paired region of the structure as it is represented in Fig. 2. In conclusion, it appears that of the eight unpaired cytosine bases in Fig. 2, three are reactive towards methoxyamine (C-33, C-73 and C-74), two exhibit partial reactivity (C-16 and C-32) and three are unreactive (C-31, C-47 and C-55).

DISCUSSION

There is striking similarity between the methoxyamine reactive exposed cytosine positions of *E. coli* tRNA_f^{Met 6} and mammalian tRNA_f^{Met}. Despite the differences in primary structure in the loop IV regions of these molecules², C-47 is unreactive in mouse myeloma initiator tRNA, as in those tRNA molecules which have the more usual -G-T-Ψ-C- loop IV sequence⁸.

The chemical reactivity of the anticodon loop (loop II) is of considerable interest since the conformation of an anticodon loop may be primarily determined by its nucleotide sequence. In both *E. coli* and mammalian tRNA_f^{Met} the first cytidine residue at the 5' side of the anticodon loop (C-31 in Fig. 2) exhibits no susceptibility to methoxyamine modification. In contrast in su_{III}⁺ tyrosine tRNA this residue is partially reactive⁵. In both tRNA_f^{Met} species as well as in su_{III}⁺ tyrosine tRNA the cytidine at the 5' end of the anticodon itself (C-33 in Fig. 2) is very reactive towards methoxyamine. Conformational differences between anticodon loop structures may be responsible for the variations which have been observed in the reac-

Figure 2

The nucleotide sequence² of mouse myeloma cell tRNA^{Met} drawn as a clover leaf. * denotes cytidine residues which are partially susceptible to reaction with methoxyamine and C indicates residues which are very extensively modified. The extent of modification under the reaction conditions used is shown. The figures in brackets, taken from Chang⁶, show the degree to which cytidine residues which occupy the corresponding locations in the clover leaf of *E. coli* tRNA^{Met} react under the same conditions.

tivities of bases which occupy the same positions within the anticodon loops of different tRNA species⁸. Mouse myeloma tRNA_f^{Met} is the first tRNA which has been found with a cytidine and not a uridine preceding the anticodon². This cytidine (C-32) is partially modified by methoxyamine (Table 1), although it is quite possible that this reaction occurs only in response to a structural change elicited by the modification of C-33 with the reagent and that C-32 is not available for reaction in the normal anticodon structure of mammalian tRNA_f^{Met}. In the anticodon loop of *E. coli* tRNA_f^{Met} this residue is a uridine and adenosine replaces the t⁶A at position 36 of the mammalian initiator tRNA structure. The uridine preceding the anticodon of *E. coli* tRNA_f^{Met} is only partially reactive towards a substituted carbodiimide reagent⁶. These variations in anticodon loop primary structure do not create anticodon loop conformations which are sufficiently different to be characterised by substantial differences in the abilities of individual anticodon loop bases to react with methoxyamine.

Both the 3' terminal cytidine residues (C-73 and C-74) of myeloma tRNA_f^{Met} are modified by the reagent, as in other tRNAs for which the pattern of methoxyamine modification has been studied⁸. Similarly, cytidines within loop 1 are generally found to be reactive.

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