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2'-O-METHYL-1-METHYL ADENOSINE: A NEW MODIFIED NUCLEOSIDE IN RAGI (Eleusine coracana) tRNA

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SUMMARY: A new modified nucleoside 2'-O-methyl-1-methyl adenosine has been found to be present in the tRNA of Eleusine coracana (ragi) seedlings. The sequence of the dinucleotide of which this modified nucleoside is a part suggests its presence in phenylalanine-tRNA. The structural implications of the presence of this new modification are discussed.

INTRODUCTION: It is well established that some of the modified nucleosides in tRNA are specific to certain organisms and occur at specific positions. Methylation is one of the most frequent modifications found in tRNA, and numerous examples of base methylation and 2'-ribose methylation are known. However, methylation both in the base and the ribose of a nucleoside is rare. Only two such nucleosides have been reported earlier : 2'-O-methyl ribothymidine in rabbit liver tRNA (1) and 2'-O-methyl-N^4-methyl cytidine in E. coli 16S rRNA (2). 1-Methyl adenosine (m^1A) usually occurs in eukaryotic tRNA at the 58th position, and the residue at the 58th position has been implicated in more than one kind of tertiary interaction in the three-dimensional structure of tRNA (3). In this paper, we report the presence of 2'-O-methyl-1-methyl adenosine (m^1Am) in ragi tRNA. The presence of this nucleoside in tRNA may have important structural and functional implications.

MATERIALS AND METHODS: Ribonuclease T2, bovine spleen phosphodiesterase, and nuclease P1 were from Sigma Chemical Co., USA. Cellulose acetate membrane strips were from Schleicher and

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Schuell Inc. Keene, New Hampshire, USA. Carrier-free 32p-labelled orthophosphoric acid was from Bhabha Atomic Research Center, Bombay, India.

Ragi seeds (1g) were germinated at 30°C in the dark for 24 h distilled water. The germinated seeds were grown in the dark for a further 72 h period in the presence of neutralized carrier-free 32P-orthophosphoric acid (5 mCi). 50 µg/ml chloramphenicol was added during culture to maintain asceptic conditions. ^{32}P-labelled total tRNA was extracted from the whole seedlings by the SDS-phenol method and DEAE-cellulose chromatography. Contaminating polysaccharides were removed by extraction of tRNA into 2-methoxyethanol and precipitation by cetyltrimethylammoniumbromide (4), followed by electroelution of tRNA from a 8% polyacrylamide gel.

Ribonuclease T2, spleen phosphodiesterase and nuclease P1 digestions were carried out at 37°C, in 20mM ammonium acetate, pH 4.5, pH 6.0 and pH 5.3 respectively. m^1Amp was converted to m^6Amp by treatment with 0.1 N NaOH for 16 h at 37°C (5). High voltage electrophoresis on Whatman 3 MM paper and on cellulose acetate was run at pH 3.5 (pyridine-acetate) at about 75V/cm.

RESULTS: ^{32}P-labelled tRNA from ragi seelings was digested with RNase T2 and the products were separated by high voltage electrophoresis on Whatman 3 MM paper. RNase T2 produces, in addition to the 3'-mononucleotides, dinucleotides from sites at which the 2'-oxygen of a nucleotide is methylated. The autoradiogram showed several minor spots numbered 1 through 9, in addition to the spots due to the four major mononucleotides (Fig.1). Spots 1 and 9 were further analysed and shown to be 5-methyl cytidine-3'-phosphate (m^5Cp) and 3', 5'-guanosine diphosphate (pGp) respectively (data not shown). In order to determine the identities of spots 2 to 8, each of the spots was eluted from the paper and the eluate was digested with spleen phosphodiesterase and the products were analysed by reelectrophoresis on Whatman 3 MM paper. The products of each of the spots are shown in Table 1. Spot No.4 produced in equimolar amounts, Up and a second nucleotide migrating just below the origin, as phosphodiesterase products (Fig. 2b). It was inferred that this slow moving species was either m^1Ap or m^7Gp

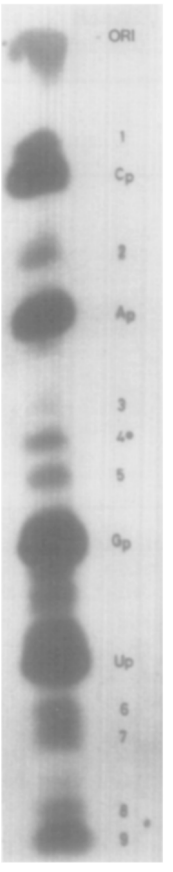

Fig. 1 Autoradiogram showing the high voltage electrophoretic separation of RNase T2 digestion products of ^{32}P-labelled tRNA.

which frequently occur in eukaryotic tRNA and because of an additional positive charge on the base, are expected to have low electrophoretic mobilities at pH 3.5. The identity of the slow moving spot was established by its change in electrophoretic mobility upon treatment with alkali. Alkali treatment is known to convert m^7Gp to 4-amino-5 (N-methyl) formamido isocytosine ribotide which migrates just ahead of Gp on electro phoresis at pH 3.5, and m^1Ap to m^6Ap which comigrates with Ap (6). This slow moving spot (Fig. 2b) upon alkali treatment moved with the mobility of Ap (Fig. 2d). In addition, the slower moving phosphodiesterase product (Fig. 2b) migrated towards the cathode during electrophoresis on cellu-

TABLE 1
Nucleotide compositions of dinucleotides from spots 2 to 8

Spot No	Nucleotide composition	Identity
2	Cp	CmpCp
3	Ap	AmpAp
4	m^1Ap, Up	m^1AmpUp
5	Cp, Gp	CmpGp/GmpCp
6 and 7	Gp, Up	GmpUp/UmpGp
8	Up	UmpUp

The radioactive material in each spot was eluted, digested using spleen phosphodiesterase and the products were identified by electrophoresis on Whatman 3MM paper.

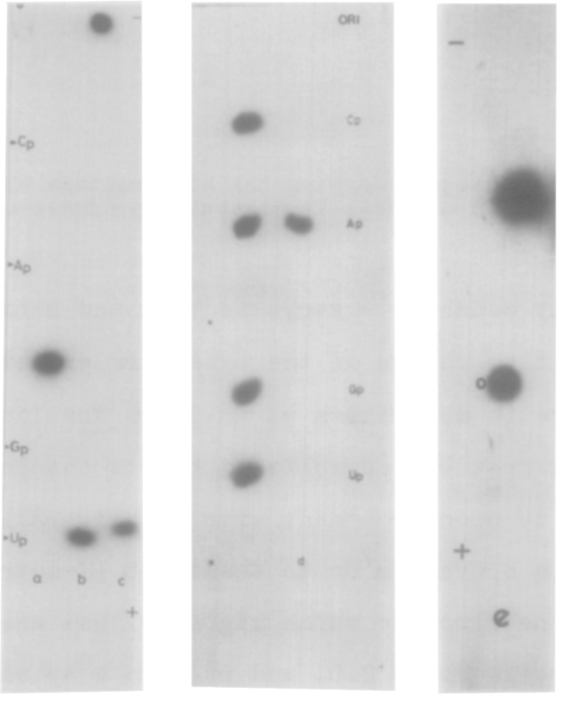

Fig. 2 Analysis of the dinucleotide from spot No.4. The dinucleotide was eluted and, (a) reincubated with RNase T2, (b) digested with spleen phosphodiesterase (c) digested with nuclease P1, and in each case the products were analyzed by Electrophoresis on Whatman 3MM paper. The slow moving spot from (b) was eluted and (d) treated with 0.1N NaOH at 37°C for 16h and electrophoresed on Whatman 3MM paper, (e) reelectrophoresed on cellulose acetate at pH 3.5.

lose acetate at pH 3.5 (Fig. 2e). This is characteristic of m^1Ap (7). These established the composition of the dinucleotide spot No.4 (Fig. 1) as m^1Ap + Up. This meant that either Up or m^1Ap has a 2'-O-methylated ribose moiety. The resistance of spot No.4 (Fig. 1) to RNase T2 was confirmed by reincubation of the eluted dinucleotide with the enzyme, followed by electrophoresis (Fig. 2a).

Determination of the 3'-end nucleotide of the dinucleotide spot No.4 (Fig.1) would indicate which of the two nucleotides (m^1Ap or Up) is 2'-O-methylated. The dinucleotide spot No.4 was eluted and digested with nuclease P1. Nuclease P1 has a 3',5'-phosphodiesterase activity as well as 3' phosphomonoesterase activity (8). Therefore, a structure XmpYp, upon hydrolysis by nuclease P1 would yield the nucleotide at the 3' end as its 5' phosphate (pY). The dinucleotide spot No.4, gave pU as the product of nuclease P1 hydrolysis (Fig. 2c), showing that the 2'-O-methylation is on m^1Ap which is at the 5' end of the dinucleotide. This established the presence of a new modified nucleotide 2'-O-methyl-1-methyl adenylic acid in ragi tRNA, and the structure of the dinucleotide spot No.4 as m^1AmpUp.

DISCUSSION: The ribose 2'-hydroxyls play a crucial role in stabilizing the structure of tRNA (3). Oxygen at 2'-position of ribose at locations 8, 21 and 58 of the tRNA structure is known to form tertiary hydrogen bonds (3, 9). The 2'-oxygen in ribose 58 forms a hydrogen bond with the phosphate at position 60, and this tertiary backbone-backbone interaction stabilizes the stacking of bases in the TψC loop (3). Nearly 60% of eukaryotic tRNAs whose sequences are known contain m^1A at the 58th position (10). Here, we have shown the 2'-ribose methylation of 1-methyl-adenosine in ragi tRNA. We have also

shown that the RNase T2 resistant dinucleotide containing m^1Amp has the sequence m^1AmpUp. Although m^1A is known to occur at positions other than 58 in a few cases, the dinucleotide sequence m^1A-U is found only at position 58-59 (10). tRNAs for as many as 13 amino acids have this sequence at position 58-59, but only in the case of phenyl alanine-tRNA all the known eukaryotic sequences have m^1A-U at position 58-59. We therefore speculate that in ragi tRNA, the new modification (m^1Am) is present at the 58th position and in phenylalanine tRNA. The presence of this modification abolishes the tertiary interaction between the ribose 58 and the phosphate 60. Loss of this teriary interaction may destabilize the TΨC loop somewhat and whether such structural variations impart such subtlety on the molecule as necessary for a particular function is at the present, a matter of conjecture.

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