Nucleic Acids Research

The identification of the tRNA substrates for the supK tRNA methylase

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ABSTRACT

Purified preparations of the tRNA methylase deficient in supK strains of Salmonella typhimurium transfer methyl groups from S-adenosylmethionine (SAM) to at least two tRNA species, an alanine tRNA and a serine tRNA. The identity of the tRNA substrates for this enzyme was determined by a change in the elution position of the methyl-labeled tRNA from BND-cellulose columns before and after aminoacylation with a specific amino acid followed by derivatization of the free primary amino group with phenoxy- or naphthoxyacetate. The radioactive methyl group enzymatically added to these tRNAs is both acid and base labile and can be hydrolyzed to a volatile product at pHs above 7.5 and also at pH 1. The methylated 3'-nucleotide isolated from digested tRNA is a pyrimidine derivative and chromatographs like a modified uridylic acid. Its identity has not been established, but it is likely that it corresponds to the methyl ester of V, uridin-5-oxyacetic acid.

INTRODUCTION

The supK strains of Salmonella typhimurium were first isolated by their suppressor activity for the nonsense codon, UGA.[3] Later they were reported to be deficient in a single tRNA methylase.[4] This deficiency results in the under-methylation of transfer RNA, and it has been suggested that the UGA suppressor activity observed in these strains is due to one or more undermethylated tRNAs.[3,4] Frameshift suppression has also been observed in supK strains, and similar explanations have been presented to account for this type of suppression.[5]

The identification of the tRNA species and the nucleoside within these tRNAs that act as substrates for the supK methylase is critical for understanding the mechanism of translational suppression. We have isolated and purified the supK

methylase and are able to specifically methylate tRNA in vitro with this enzyme.[6,7]

We report here the use of the procedure of Gillam et al.[8] to identify two tRNAs that accept methyl groups using the purified methylase. One is an alanine tRNA, the other a serine tRNA. The methylated nucleotide within these tRNAs has the interesting property of being both acid and alkali labile, and even at pH 7.5 the methyl group is slowly hydrolyzed from the tRNA. Recently Lesiewicz and Duddock[9] have reported a similar methylated product in Escherichia coli alanine tRNA which they tentatively identified as the methyl ester of V, uridin-5-oxyacetic acid.[10] In this report we describe some of the properties of this unusual methylated nucleotide.

MATERIALS AND METHODS

The methods for the preparation of the purified tRNA methylase and unfractionated tRNA from S. typhimurium LT2 (wild type) as well as the assay for the supK methylase were described by Pope and Reeves.[7] These methods are modifications of the earlier procedures of Reeves and Roth.[4]

Batch Methylation of tRNA. Approximately 100 A_{260} units (5 mg) of unfractionated tRNA were methylated with ^{3}H-methyl-S-adenosylmethionine (Amersham/Searle) using 2 μg of purified supK tRNA methylase. The tRNA was previously incubated at pH 9.0 for 2 hr at 37°C to deacylate the tRNA and to remove methyl groups added in vivo by the supK tRNA methylase. The methylation reaction was carried out at 37°C in a total volume of 2.5 ml containing: 40 mM KH_2PO_4, pH 7.5, 4 mM $MgCl_2$, 2 mM L-methionine and 20 μM ^{3}H-methyl-S-adenosylmethionine (250 Ci/mole). The extent of methylation was followed by measuring the trichloroacetic acid (TCA) precipitable radioactivity as described by Reeves and Roth.[4] After 60 min the pH was lowered to 5.0 and the tRNA was recovered by DEAE-cellulose chromatography.[11]

Aminoacylation and derivatization of the methyl-labeled tRNA. The aminoacylation of the tRNA was carried out as described by Reeves and Roth,[4] except that as many as 6 amino acids were used in one reaction mixture. The final concentration of

each amino acid was 40 μM. One ^{14}C-labeled amino acid (50 Ci/mole, Schwartz/Mann) was added to each group, and the reaction was carried out for 30 minutes. Using this procedure the tRNA is saturated with all amino acids tested except glycine which is esterified more slowly. The methyl-labeled, aminoacylated tRNA was recovered from the reaction as previously described.[11]

The aminoacylated-tRNA was derivatized with either napth-oxyacetyl or phenoxyacetyl groups by the procedure of Gillam et al.[8] as modified by Roy et al.[12] After derivatization the tRNA was precipitated with 2 volumes of ethanol, washed with ethanol and dried in a gentle air stream. The tRNA was dis-solved in 100 mM sodium acetate, pH 4.5, 10 mM $MgCl_2$. Chro-matography was carried out within 24 hours.

BND-cellulose column chromatography. BND-cellulose (benzo-ylated, naphthoylated DEAE-cellulose, Boehringer Mannheim Corp.) of mesh size > 200 was packed in a 0.9 x 15 cm high-pressure column (Glenco).[13] Resin was added and packed under pressure until a bed height of 8 cm was maintained. This produced a flow rate of 37 ml/hour at approximately 200 PSI. The column was equilibrated with 0.4 M NaCl, 10 mM sodium acetate, pH 4.5, 10 mM $MgCl_2$. Transfer RNA was added in a volume of from 1 to 3 ml of the equilibrating buffer. The column was then washed with approximately 25 ml of the equili-brating buffer, followed by approximately 25 ml of 0.8 M NaCl, 10 mM sodium acetate, pH 4.5, 10 mM $MgCl_2$ (high salt or HS buffer) and 20 ml of 1.0 M NaCl, 10 mM sodium acetate, pH 4.5, 10 mM $MgCl_2$, 20% V/V EtOH (high salt-ethanol or HSE buffer). The column effluent was monitored at 260 nm. Elution with the ethanol containing buffer was not initiated until the A_{260} dropped to 0.050. Fractions of 1.1 ml were collected directly into scintillation vials. To each of the vials 10 ml of Aquasol (New England Nuclear) was added followed by vigorous shaking. The vials were then counted in a liquid scintilla-tion counter. Following re-equilibration the column was used for subsequent runs.

Reversed-phase Column Chromatography. Reversed-phase column chromatography (RPC-5) was performed according to

Kelmers and Heatherly.[14] RPC-5 resin (a gift from J. Katze)
was packed in a 0.6 x 30 cm high-pressure column. The column
was equilibrated with 10 mM sodium acetate, pH 4.5, 10 mM
$MgCl_2$, containing 0.45 M NaCl. Elution was carried out at
room temperature with a linear gradient (100 ml) of NaCl
(usually 0.45 to 0.8 M) in the same acetate buffer. Fractions
of 1.1 ml were collected in scintillation vials every two
minutes which required a pressure of approximately 300 PSI.
To each vial was added 10 ml of Aquasol followed by vigorous
shaking. Radio-activity in each vial was counted in a liquid
scintillation counter. Following re-equilibration the column
was used for subsequent runs.

Hydrolysis of the methylated tRNA. The rate of hydrolysis of
the methylated nucleotide in tRNA was performed with in vitro
methyl-labeled tRNA from S. typhimurium LT2. Each reaction
mixture contained approximately 2 A_{260} units of ^{14}C-methyl
labeled tRNA (approximately 4×10^4 CPM) in 0.4 ml of either 50
mM Tris-acetate, pH 5.2 to pH 11.6, or 0.1 M HCl, pH 1.0.
Each reaction mixture was incubated at $37^\circ C$ in a sealed tube
for up to 72 hr. Aliquots (50 μl) were withdrawn at appro-
priate times and applied to 2.2 cm Whatman 3MM filter discs
which were washed in cold trichloroacetic acid (TCA).[4]
Radioactivity was determined by scintillation counting in a
Packard scintillation spectrometer. This method detects TCA
insoluble radioactive methyl groups, those still bound to
tRNA. A second method was also used to determine radio-
activity, which measures remaining, non-volatile methyl
groups. After each aliquot was removed, it was brought to a
pH between 4.5 and 5.0 by the addition of 50 μl of 0.5 M
soldium acetate, pH 4.5. This mixture was dried overnight at
$80^\circ C$. The residue was dissolved in 1.0 ml H_2O and 10 ml of
Aquasol (New England Nuclear) was added. Radioactivity was
determined by liquid scintillation counting.

Enzymatic digestion of tRNA. Methyl-labeled tRNA was digested
with either pancreatic ribonuclease (RNAse A), ribonuclease
T_1, T_2 or with spleen phosphodiesterase. The digestion condi-
tions were: 1. Ribonuclease A (Type IIA, Sigma Chem. Corp.,
0.2 mg per mg tRNA) was incubated with tRNA in 10 mM sodium

acetate, pH 6.0, for 4.5 hr at 37°C. The volume was usually 50 µl per mg tRNA. 2. Ribonuclease T_1 (Sigma, 2000 units per mg tRNA) was incubated with tRNA in 10 mM sodium acetate, pH 6.0, for 4.5 hr at 37°C. 3. Ribonuclease T_2 (Sigma, 20 units per mg tRNA) was incubated with tRNA in 50 mM potassium acetate, pH 4.7, for 4 hr at 37°C. 4. Spleen phosphodiesterase (Sigma, 0.03 mg per mg tRNA) was incubated with tRNA in 10 mM ammonium acetate, pH 5.5, 2 mM sodium EDTA for 20 hr at 37°C.

The nucleoside was obtained from the free nucleotide produced by procedures 1, 2 or 4 by further incubation with acid phosphatase from wheat germ (Worthington Biochemicals, 0.2 mg per mg tRNA). Incubation was for 24 hr in 50 mM sodium acetate, pH 4.5, at 37°C. All digestions were stored frozen until used for chromatography or electrophoresis.

Chromatography of the methylated nucleotide. Thin layer chromatography was performed on cellulose TLC sheets (Eastman Chemicals) in the solvent systems described in Table I. The

Table 1. R_f values of the unknown (N) and major nucleosides and 3'-nucleotides.

Nucleosides and Nucleotides	R_f Values in Solvent Systems					
	A	B	C	D	E	F
Adenosine	0.84	0.14	0.61	0.65	----	0.64
Guanosine	0.67	----	0.44	0.59	----	0.56
Uridine	0.69	0.68	0.62	0.74	----	0.70
Cytidine	0.77	0.51	0.56	0.71	----	0.68
N	0.71	0.59	0.53	0.77	----	0.76
A_p	0.74	0.20	0.26	0.22	----	0.14
G_p	0.48	0.45	0.16	0.18	0.54	0.09
U_p	0.51	0.74	----	0.33	0.92	0.21
C_p	0.67	0.77	----	0.24	0.67	0.17
N_p	0.61	0.61	0.30	0.35	0.59	0.21

R_f values were determined by ascending chromatography on cellulose thin layer sheets (Eastman No. 13255).

The solvents are: (A) Isobutyic acid: 0.5 N NH_4OH, 5:3 (v/v), (B) 0.1 M NaH_2PO_4, pH 6.8, 4.5 M $(NH_4)_2SO_4$: isopropanol 100:2 (v/v), (C) Isopropanol:H_2O:NH_4OH, 7:3:1 (v/v/v), (D) ethanol: 1 M ammonium acetate, pH 5.0, 7:3 (v/v), (E) Isopropanol: HCl: H_2O, 680:175:144 (v/v/v), and (F) ethanol:1 M ammonium acetate, pH 7.5, 7:3 (v/v).

position of the radioactive products was determined by scanning
the dried chromatograms with a Varian Aerograph Model 6000
Radioscanner.

RESULTS

The specificity of the procedure used to identify the
methyl accepting tRNAs is based on two reactions: first, the
enzymatic aminoacylation of tRNA and second, the increased
retention of phenoxyacetylated aminoacyl-tRNA on BND-cellulose
columns.[8] Only tRNA species which are aminoacylated (those
for which the amino acid was present in the aminoacylation re-
action) are derivatized in the second step and thus show
increased retention on BND-cellulose chromatography. A typical
BND-cellulose column elution profile for both derivatized and
nonderivatized aminoacyl-tRNA is shown in Figure 1. In this
case ^{14}C-seryl-tRNA elutes predominantly (81%) with the high
salt (HS) elution (Fig. 1A) while the phenoxyacetylated seryl-
tRNA elutes predominantly (90%) with the high salt-ethanol
(HSE) elution (Figure 1B). Therefore the specificity of the
enzymatic aminoacylation of tRNA allows the determination of a
labeled but unknown tRNA species by its elution position from
BND-cellulose after phenoxyacetylation.

This procedure was used to determine which amino acid
accepting tRNA species are substrates for the supK tRNA
methylase. Unfractionated tRNA was first exhaustively methy-
lated in vitro with ^{3}H-methyl-SAM and the purified supK tRNA
methylase. This methyl-labeled tRNA was aminoacylated in
three different batches each containing six different amino
acids. All amino acids except for proline and glycine were
included in these groups. Under our aminoacylation conditions
glycine reacts very slowly, and prolyl-tRNA is difficult to
derivatize.[8] A control was made in which no amino acids were
added to the otherwise complete reaction mixture. In all
cases except for the control a ^{14}C-labeled amino acid was
included in each batch in order to ascertain if the acylation
and derivatization reactions were going to completion. The
elution pattern of ·the methyl-labeled tRNA which had been
through the sham aminoacylation and derivatization is almost
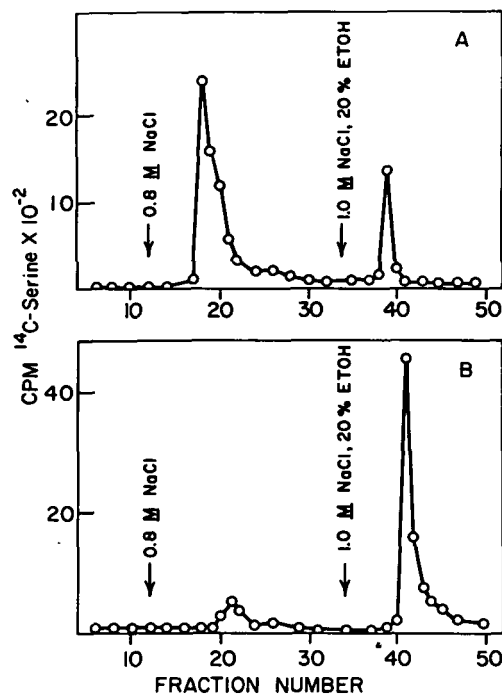identical to the pattern for underivatized seryl-tRNA shown

Figure 1. BND-cellulose chromatography of seryl-tRNA and phenoxyacetylseryl-tRNA. Unfractionated tRNA (10 A_{260} units) containing either ^{14}C-seryl-tRNA or phenoxyacetyl-^{14}C-seryl-tRNA was applied to a 0.9x10 cm BND-cellulose column and eluted as described in Materials and Methods. The high salt-ethanol (HSE) elution was started at Fraction 33. Fractions contained 1.1 ml. A. ^{14}C-seryl-tRNA; B. phenoxyacetyl-^{14}C-seryl-tRNA.

in Fig. 1A. The high salt (HS) region contains 81% of the total ^{3}H-methyl label which indicates the technique should work for determining the methyl containing tRNAs which elute in this region.

Only two amino acids were found which gave a shift of greater than 7% of the total methyl label. Values of less than 7% were considered of questionable validity due to the inherent variance in the column elution procedure. The two amino acids resulting in significant shifts are alanine and serine. The elution profiles for aminoacylation and derivatization with alanine and serine are shown in Figure 2. With alanine (Figure 2A) 53% of the methyl-labeled tRNA is shifted
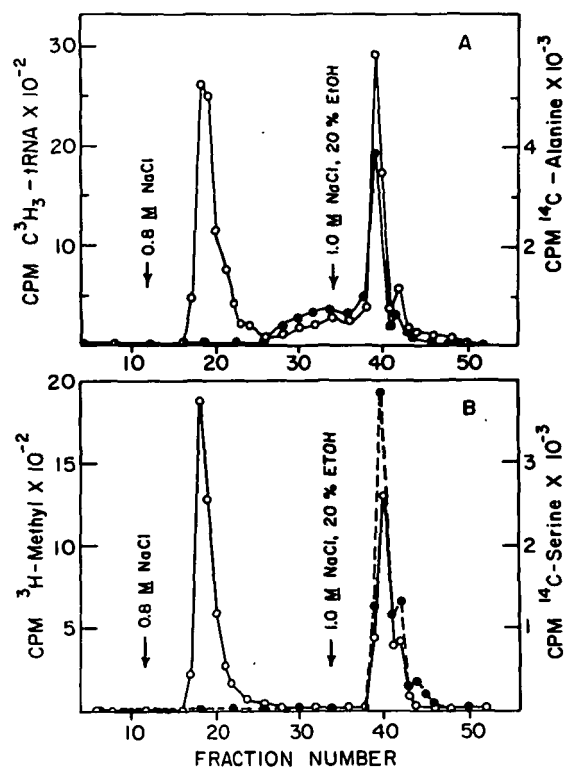
Figure 2. BND-cellulose chromatography of methyl-labeled, unfractionated tRNA containing either phenoxyacetyl-^{14}C-alanyl-tRNA or phenoxyacetyl-^{14}C-seryl-tRNA. Approximately 10 A_{260} units of unfractionated tRNA was methylated in vitro with the purified supK tRNA methylase and ^{3}H-methyl-SAM. The methyl-labeled tRNA was then aminoacylated with either ^{14}C-alanine or ^{14}C-serine as described in Materials and Methods. After derivatization with phenoxyacetyl groups, the tRNA was chromatographed on BND-cellulose as described (Fig. 1 and Materials and Methods). A. methyl-labeled, phenoxyacetyla-lanyl-tRNA; o—o , ^{3}H-methyl; ●—● , ^{14}C-alanine   B. methyl-labeled, phenoxyacetylseryl-tRNA; o—o , ^{3}H-methyl; ●—● , ^{14}C-serine.

away from the HS peak, and with serine (Figure 2B) 38% is shifted. After correcting for methyl-labeled tRNA that elutes in the HSE region prior to aminoacylation (19%) we find that 34% of the methylated tRNA is alanine tRNA and 19% is serine tRNA. We have estimated that per A_{260} unit of crude tRNA there are 170 pmoles of methyl accepting sites (see below). Thus in 1 A_{260} unit of crude tRNA (about 1750 pmoles tRNA)[15]

we estimate that 58 pmoles of alanine tRNA and 32 pmoles of
serine tRNA are substrates for the supK methylase. We have
accounted for only 53% of the methyl-labeled tRNA. However,
the above values are probably minimal values since incomplete
aminoacylation, subsequent hydrolysis of the aminoacyl bond or
incomplete derivitization would all result in lower amounts of
methyl-labeled tRNA shifted from the HS region.

Serine, alanine and valine tRNAs from E. coli contain V
(uridin-5-oxyacetic acid) or a V-like base in the wobble posi-
tion of their anticodons.[10,16,17] Below we show that the
methylated nucleotide is probably a methyl ester of a uridine
derivative, very likely the methyl ester of V. Since both
alanine and serine tRNAs accept the methyl group, we further
analyzed valine tRNA to see if it was also a substrate for the
supK methylase. Previous tests indicated that valyl-tRNA was
not a substrate for methylation, but the tests may have failed
due to poor aminoacylation or derivatization. We applied the
same procedure again, aminoacylation and derivatization, but
this time used RPC-5 column chromatography instead of BND-
cellulose to monitor the derivatized tRNAs. Figure 3 shows
the elution pattern of the nonacylated, methyl-labeled tRNA
on RPC-5. One major peak of methylated tRNA appears at frac-
tion 40, and a minor peak appears at fraction 68. Approxi-
mately 20 A_{260} units of unfractionated tRNA containing 150,000
CPM of ^{3}H-methyl groups were aminoacylated to saturation with
^{14}C-valine. The tRNA was recovered from the reaction and
half was chromatographed on RPC-5 using a linear salt gradient
(Figure 4A). The valine and methyl radioactive profiles are
not superimposable, but they do overlap to some extent. The
other half of the valyl-tRNA was derivatized with naphthoxy-
acetyl groups and also chromatographed on RPC-5. From the
results in Figure 4B it is clear that valyl-tRNA is not
methylated by the supK methylase. The derivatized valyl-tRNA
elutes much later in two peaks at Fractions 68 and 76 while
the majority of the methyl label remains unmoved at about
Fraction 35. These results rule out valyl-tRNA as a sub-
strate for the supK methylase.
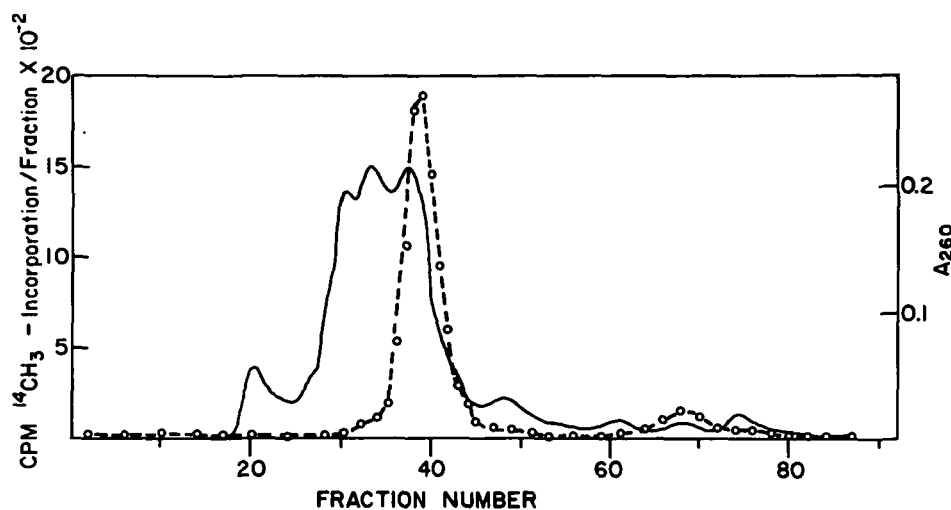The lability of the methylated tRNA. The specific methyl

Figure 3. RPC-5 column chromatography of unfractionated, methylated tRNA. Approximately 5 A_{260} units of tRNA was exhaustively methylated with ^{14}C-methyl-SAM and the purified supK tRNA methylase. The tRNA was applied to a 0.6x30 cm RPC-5 column and eluted with a 0.45 to 0.80 M linear NaCl gradient (see Materials and Methods for details). —— , A_{260}; o---o , ^{14}C-methyl.

group added by the supK methylase is easily hydrolyzed from tRNA and much of it can be lost during standard tRNA extraction procedures. When we isolate crude tRNA from wild type S. typhimurium by phenol extraction and DEAE-cellulose chromatography at pH 7.5 we find that between 50 and 100 pmoles of methyl group can be transferred to 1 A_{260} unit of tRNA in vitro using the purified methylase. However, if the pH during the extraction is kept between 4.5 and 6 and the temperature below 4°C, the methyl acceptance of tRNA from wild type cells is only about 8.0 pmoles per A_{260} unit tRNA using the purified supK tRNA methylase, indicating that the methyl group added in vivo has not been lost during extraction. Transfer RNA extracted from supK strains by the same low pH procedure gives a methyl acceptance value of about 30-40 pmoles per A_{260} unit indicating that these strains are somewhat deficient in the specific methyl group added by the supK tRNA methylase. If any of these tRNAs are pre-incubated for 2 hours at pH 9.5, the methyl accepting activity of the tRNA
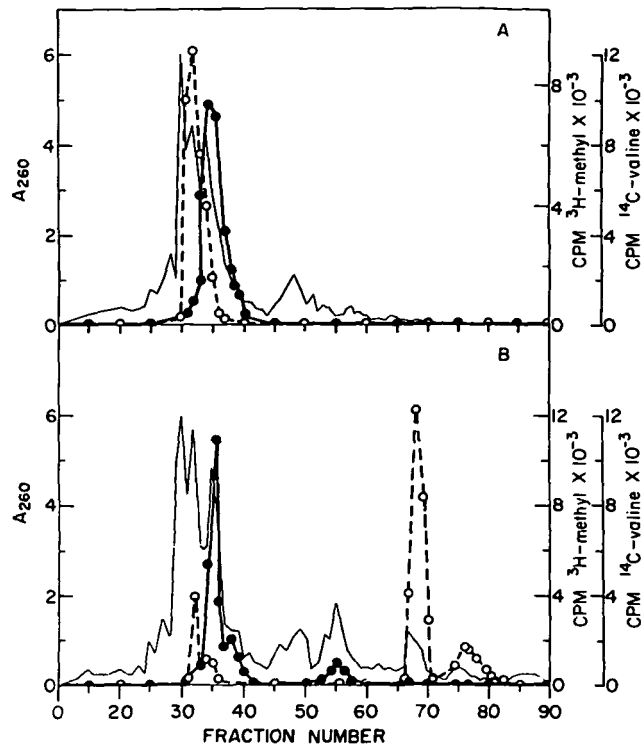
Figure 4. RPC-5 column chromatography of methyl labeled, valyl-tRNA before and after derivatization with naphthoxyacetyl groups. Unfractionated tRNA (100 A_{260} units) containing approximately 10^5 CPM ^{3}H-methyl groups was aminoacylated with ^{14}C-valine (50 Ci/mole, Schwarz/Mann). The recovered tRNA sample was divided and half was derivatized with naphthoxyacetyl groups. Each sample was applied to a 0.6x30 cm RPC-5 column and eluted with a 0.50 to 1.2 M linear NaCl gradient (see Materials and Methods for details). ——— , A_{260}; ●———● , ^{3}H-methyl; ○— — —○ , ^{14}C-valine. A. Methyl-labeled, valyl-tRNA. B. Methyl-labeled, naphthoxyacetylvalyl-tRNA.

rises to 150 pmoles per A_{260} unit (Figure 5A). Thus, the same procedure used to strip tRNA of amino acids can be used to remove this specific methyl group. We have found that storing tRNA at pH 7.5 at -20°C does not prevent loss of the methyl group. In vitro methyl-labeled tRNA slowly loses radioactivity upon storage, and the methyl acceptance of stored tRNA slowly increases (Figure 5B).
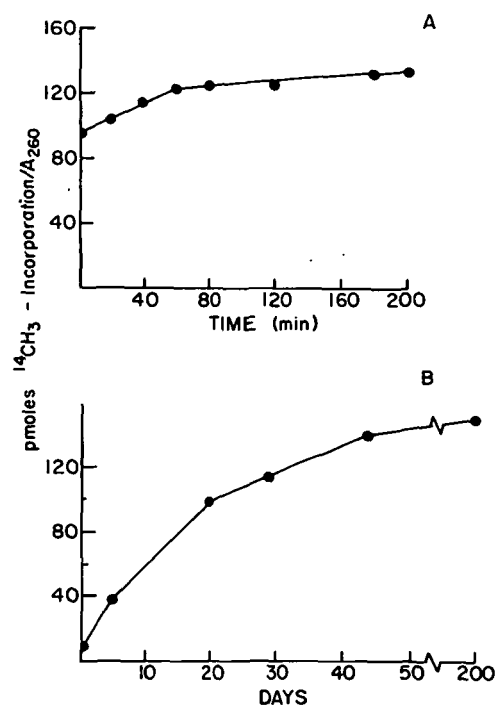
Figure 5. The increase of methyl acceptance of tRNA stored at pH 7.5. A. A sample of tRNA prepared and stored at pH 7.5 was incubated at 37°C in 10 mM MgCl$_2$, 10 mM Tris-HCl, pH 7.5. Samples were removed at the times indicated and assayed for methyl acceptance. B. A sample of tRNA prepared at low pH to preserve in vivo added methyl groups was stored frozen in 10 mM MgCl$_2$, 10 mM Tris-HCl pH 7.5. At various times samples were thawed and assayed for methyl acceptance with the puri- fied supK methylase. Note that in both A and B, the pmoles of methyl group incorporation per A$_{260}$ unit of tRNA approach the same level.

Rate of Hydrolysis of the methylated tRNA. The rate of hydro- lysis of the in vitro ^{14}C-methyl-labeled tRNA was determined at pHs between 1 and 11.7 (Figure 6). At pH 7.5, 37°C, the half time of hydrolysis is about 20 hours. The radioactive methyl group is hydrolyzed to a volatile product. The rate of hydrolysis at each pH value is the same whether TCA precipi- table radioactivity or remaining non-volatile radioactivity is measured. All rates show first order kinetics since the logarithm of remaining radioactivity plotted against time is linear. This would be expected for the hydrolysis of a methyl
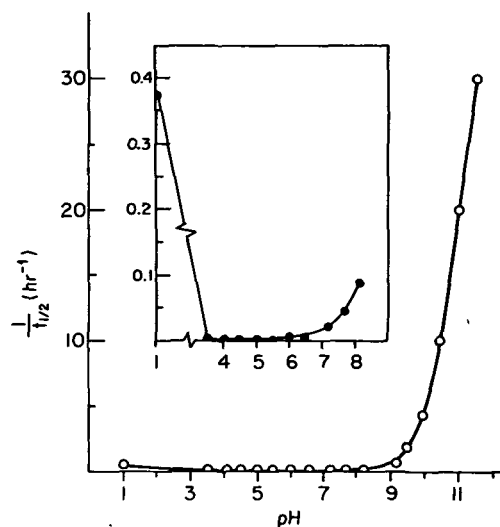
Figure 6. The hydrolysis rate of methyl-tRNA vs. pH. The reciprocal of the half times of hydrolysis is plotted vs. pH (see Materials and Methods).

ester in aqueous solution. It is also apparent from the data in Figure 6 that the rate is dependent on hydroxide ion concentration at high pH. Data not shown also indicates that general base catalyzed hydrolysis occurs in Tris buffers. The rate of hydrolysis at pH 7.5 is directly proportional to the Tris concentration.

There are 150 pmoles of methyl accepting sites in 1.0 A_{260} unit of tRNA determined by our standard methylation assay procedure using the purified supK tRNA methylase.[7] We have found, however, that even at pH 7.5, the pH of the assay, there is a slow but detectable rate of chemical hydrolysis of the methylated tRNA (Figure 6). We have estimated that the actual number of methyl accepting sites in tRNA is about 10% higher than the values we determined in the assay. Therefore, there are about 170 pmoles of methyl accepting sites in 1.0 A_{260} unit (1750 pmoles) of unfractionated tRNA.

Enzymatic hydrolysis of methylated tRNA. A methylated-3'-mononucleotide product results from either ribonuclease T_2 or pancreatic ribonuclease (RNAse A) digestion of the methyl-labeled tRNA. Figure 7 shows the results of chromatography
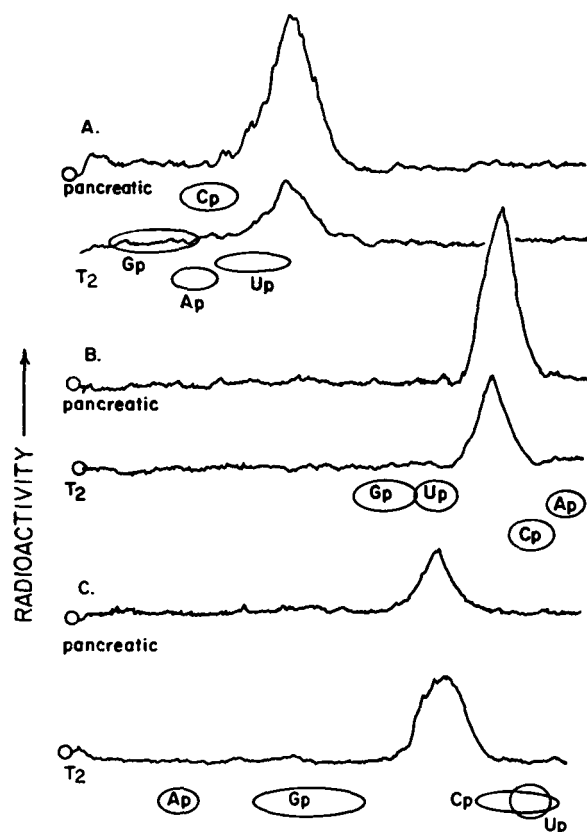
Figure 7. Cellulose thin layer chromatography of the methyl-labeled product after RNAse A or RNAse T_2 digestion. Unfrac-tionated ^{14}C-methyl label tRNA was digested as described in Materials and Methods with either pancreatic RNAse or RNAse T_2. Aliquots of the digestion mixtures were applied to cellulose thin layer sheets along with the four major 3'-nucleotides. A. developed in solvent D; B. solvent A; C. solvent B.

of the digested methyl-labeled tRNA in three solvent systems. The radioactive nucleotide has the same mobility whether RNAse T_2, RNAse A or spleen phosphodiesterase is used to digest the tRNA. With RNAse A all the radioactivity in the tRNA sample is recovered in this mononucleotide. This result indicates that the methylated nucleotide is a pyrimidine derivative and always occurs in tRNA on the 3'-side of another pyrimidine. This 3'-methylated nucleotide coelutes

with 3'-UMP when chromatographed on DEAE-cellulose at pH
3.5 and is well separated from the earlier eluting 3'-CMP peak.

If the 3'-nucleotide is further hydrolyzed with acid
phosphatase a labeled nucleoside results. The nucleoside has
no electrophoretic mobility at pH 3.5 and 7.0. At pH 9.0
only very limited electrophoresis can be carried out due to
the instability of the labeled methyl group. The radioactive
methyl group in the nucleoside is hydrolyzed at all pHs with
about the same rates observed with the intact methyl-labeled
tRNA. With limited electrophoresis at pH 9.0 the nucleoside
moves toward the anode indicating a net negative charge for
the nucleoside at high pH values. These mobilities are
consistent with our tentative identification of the nucleo-
side as the methyl ester of V.

Digestion of methyl-labeled tRNA with RNAse T_1 results in
a labeled RNA fragment which appears to be a tetra- or penta-
nucleotide. This fragment elutes just behind the TψCG frag-
ment on a DEAE-cellulose column using the procedure of
Tomlinson and Tener.[18] In tRNA$_{ala\ 1}$ from $\underline{E.\ coli}$ the RNAse
T_1 fragment containing V is a pentanucleotide.[16,17] This is
also true for the V-containing RNAse T_1 fragment from
tRNA$_{ser\ 1}$.[19]

Table I summarizes the chromatographic mobilities of the
nucleotide and the nucleoside in various solvent systems.
Two-dimensional thin layer chromatography of the 3'-nucleotide
was also performed in the system described by Nishimura.[10]
Using this system only a short run in solvent II can be per-
formed due to its low pH. A single radioactive spot appears
on the chromatogram just overlapping Cp in the same relative
position as 5-methylaminomethyl-2-thiouridylic acid, s^2U^*p
(Figure 1, ref. 10).

DISCUSSION

We have identified two tRNA species, an alanine tRNA and
a serine tRNA, that act as substrates for the supK tRNA
methylase. The methylated nucleoside in these tRNAs appears
to be a methyl ester of a uridine derivative. In both tRNAs
this is probably V or the V-like nucleoside reported to be in
the wobble position of their anticodons.[10,16,17,19] Since the

supK mutants of S. typhimurium are deficient in this methy-
lated nucleoside, and such mutants are slow growing and sup-
press the UGA triplet, this methyl group appears to be
critical for proper codon-anticodon recognition. Of the two
tRNA species that have been identified, seryl-tRNA would be
the likely choice for the tRNA species that incorrectly pairs
with the UGA nonsense triplet. One codon for serine, UCA, is
related to UGA by one base change.

There is probably one other tRNA species that is an
acceptor of methyl groups. On BND-cellulose chromatography
there is a methylated tRNA that elutes in the ethanol con-
taining buffers.[4] A second, small peak of methylated tRNA
is also apparent on RPC-5 column chromatography (Figure 3)
which elutes much later than the main peak. In E. coli there
also appears to be at least three tRNA species which accept
an alkali-labile methyl group (B. Dudock, personal communica-
tion) and one of these has been identified as alanine tRNA.[9]

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