Derivatives of *N*-[*N*-(9-β-D-Ribofuranosylpurin-6-yl)carbamoyl]threonine in Phosphodiesterase Hydrolysates of Wheat Embryo Transfer Ribonucleic Acid†

R. S. Cunningham‡ and M. W. Gray*

ABSTRACT: One major and one minor purinylcarbamoyl amino acid nucleoside have been isolated from wheat embryo transfer RNA. The major nucleoside has been characterized as *N*-[*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (t^6A), while the minor nucleoside has been identified as a methylated derivative of t^6A, *N*-[*N*-methyl-*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (mt^6A). Both t^6A and mt^6A have been isolated as the 5′-mononucleotides, pt^6A and pmt^6A, from snake venom phosphodiesterase hydrolysates of wheat embryo transfer RNA. Because of the additional negative charge contributed by the carboxyl function, these nucleotides elute from DEAE-cellulose after the bulk of the mononucleotides at pH 7.8 and so are readily concentrated. Under the conditions of hydrolysis used in this study, both t^6A and mt^6A were only partially released as the expected 5′-mononucleotides. These two nucleosides were also present in the form of dinucleotides having the structures $pUpt^6A$ and $pUpmt^6A$. These dinucleotides persist in phosphodiesterase hydrolysates of wheat embryo transfer RNA because the phosphodiester bond linking the 5′-hydroxyl of the purinylcarbamoyl amino acid nucleoside residue to the 3′-hydroxyl of the adjacent nucleoside residue is exceptionally resistant to hydrolysis, due to the presence of the bulky side chain in t^6A and mt^6A. Since in *Escherichia coli*, t^6A has been shown to be present in those transfer RNA species which recognize codons beginning with adenosine, and to be specifically located next to the 3′-nucleotide residue of the anticodon sequence in such tRNA species (Nishimura, S. (1972), *Progr. Nucl. Acid Res. Mol. Biol. 12*, 49), it would be expected that a sequence 5′···$pUpt^6A$···3′ should be found in all tRNAs containing t^6A. The isolation of the dinucleotides $pUpt^6A$ and $pUpmt^6A$ from phosphodiesterase hydrolysates of unfractionated wheat embryo transfer RNA is consistent with this expectation and provides circumstantial evidence that in wheat embryo, also, t^6A and mt^6A occur next to the 3′-terminal residue (uridine) of the anticodon sequence in tRNAs recognizing codons beginning with adenosine. The presence of t^6A as the major purinylcarbamoyl amino acid nucleoside in the transfer RNA of a higher plant as well as in bacterial, fungal, and animal tRNAs suggests an important function for this nucleoside in protein biosynthesis in both prokaryotic and eukaryotic organisms.

The modified base *N*-[*N*-(purin-6-yl)carbamoyl]threonine has been isolated from acid hydrolysates of the transfer RNA of calf liver, rat liver, yeast, and *E. coli* (Chheda *et al.*, 1969; Schweizer *et al.*, 1969). The corresponding ribonucleoside, *N*-[*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (t^6A),[1] has been found in enzymic hydrolysates of baker's yeast tRNA (Schweizer *et al.*, 1969). The presence of t^6A in the tRNA of a higher plant (tobacco) has been inferred by the use of a radioactive labeling technique (Dyson *et al.*, 1970), and the occurrence of t^6A in human urine (Chheda, 1969) suggests that this nucleoside is present in human tRNA, as well. Microanalysis of samples of tRNA from a variety of organisms has indicated the consistent presence of t^6A, the only known exception to date being the tRNA of *Mycoplasma* (Miller and Schweizer, 1972).

Takemura *et al.* (1969) first showed that t^6A is located next to the 3′-nucleotide residue of the anticodon sequence in *Torulopsis utilis* $tRNA^{Ile}$, and sequence studies of additional tRNAs have confirmed this specific localization of t^6A (Nishimura, 1972). Since t^6A has been shown to be present in *Escherichia coli* tRNA species which recognize codons beginning with adenosine (Ishikura *et al.*, 1969; Kimura-Harada *et al.*, 1972a; Powers and Peterkofsky, 1972), it is possible that this amino acid–adenosine derivative plays a role in the recognition of such codons (Nishimura, 1972). Certain ureidopurine derivatives related to t^6A have been shown to have cytokinin activity (Dyson *et al.*, 1970), but the significance of this observation with regard to the biological function of tRNA is not clear at the present time.

Although the major purinylcarbamoyl amino acid (PurCbm-AA) nucleoside present in transfer RNA appears to be t^6A, there is evidence for the occurrence of derivatives of t^6A in minor amounts in tRNA. The isolation and characterization of the glycine analog of t^6A from yeast tRNA has been described (Schweizer *et al.*, 1970), and it is possible that other analogs of t^6A exist in which threonine is replaced by other amino acids. A methylated derivative of t^6A, *N*-[*N*-methyl-*N*-

† From the Department of Biochemistry, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada. *Received September 19, 1973.* Supported by a grant (MA-4124) from the Medical Research Council of Canada.

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[1] Abbreviations used are: t^6A, *N*-[*N*-(9-β-D-ribofuranosylpurin-6-yl)-carbamoyl]threonine; pt^6A, the 5′-phosphoric ester of t^6A; mt^6A, *N*-[*N*-methyl-*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine; pmt^6A, the 5′-phosphoric ester of mt^6A; PurCbm-AA, purinylcarbamoyl amino acid; ms^2i^6A, 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine; A_{260} unit, that amount of material having an absorbance of 1.0 at 260 nm when dissolved in 1 ml of water and measured in a 1-cm light path. As simpler and more descriptive alternatives to the above systematic names, Dr. W. E. Cohn of the Office of Biochemical Nomenclature has suggested the following: t^6A, *N*-[(N^6-adenosyl)carbonyl]threonine or N^6-(*N*-threonylcarbonyl)adenosine; mt^6A, *N*-[(N^6-methyl-N^6-adenosyl)-carbonyl]threonine or N^6-methyl-N^6-(*N*-threonylcarbonyl)adenosine.

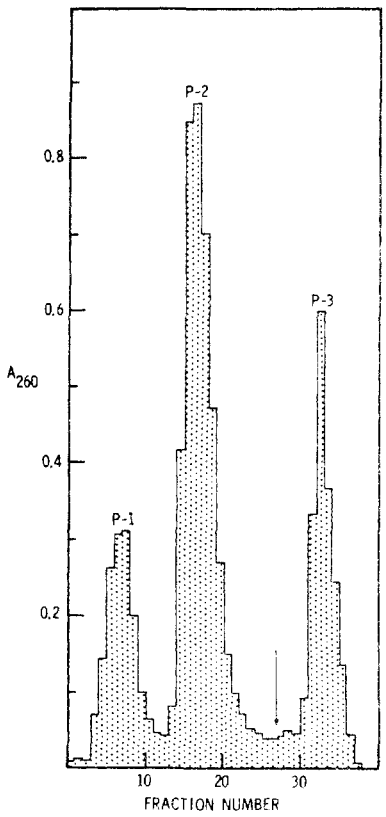

FIGURE 1: Fractionation of the post-mononucleotide material of a phosphodiesterase hydrolysate of wheat embryo transfer RNA. A neutralized hydrolystate containing 49,100 A_{260} units was diluted to a formate ion concentration of about 0.085 M and applied to a column (4.5 cm × 30 cm) of DEAE-cellulose equilibrated with 0.025 M Tris formate (pH 7.8). Nucleoside 5′-monophosphates were eluted with 0.085 M Tris–formate (pH 7.8) in 7.3 M urea. Subsequent elution with 0.17 M Tris–formate (pH 7.8) in 6.6 M urea brought off fractions P-1 and P-2, while application of 0.25 M Tris–formate (pH 7.8) in 6.0 M urea (arrow) resulted in elution of fraction P-3.

(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (mt^6A), has recently been shown to be present in *E. coli* $tRNA^{Thr}$ (Kimura-Harada *et al.*, 1972b), and several other unidentified nucleosides which appear to have structures related to that of t^6A have been reported (Nishimura, 1972).

Because t^6A may have a specific role in the functioning of those tRNA species containing it, and since this highly modified nucleoside seems to be present in both prokaryotic and eukaryotic transfer RNA, it is of interest to examine its distribution and localization in the transfer RNA of a range of organisms. In this report, we describe the isolation and characterization of PurCbm-AA compounds from the transfer RNA of a higher plant tissue, wheat embryo. It had previously been observed (Gray, 1968) that when phosphodiesterase hydrolysates of wheat embryo tRNA were fractionated on DEAE-cellulose at pH 7.8, the post-mononucleotide fraction contained compounds having spectral properties suggestive of PurCbm-AA nucleoside derivatives. Two major and one minor derivative could be separated by two-dimensional paper chromatography, and it was considered possible that this separation might be based on the presence of a different amino acid in the three compounds. The study reported here was undertaken to explore this possibility further.

Materials and Methods

Isolation and Purification of Wheat Embryo Transfer RNA. A method for the purification of transfer RNA from commercially milled wheat embryo has been described in detail (Gray, 1968). Briefly, total RNA is first isolated by phenol extraction of the fresh (nondefatted) embryo, essentially as described by Glitz and Dekker (1963). The combined aqueous phases are made 3 M in NaCl to selectively precipitate the high molecular weight (26 S + 18 S) rRNA components (Crestfield *et al.*, 1955), and the RNA remaining soluble in 3 M NaCl (largely tRNA) is recovered by ethanol precipitation and further purified to remove polysaccharides by treatment with 2-methoxyethanol (Kirby, 1956; Glitz and Dekker, 1963). The transfer RNA is then recovered as the cetyltrimethylammonium salt (Ralph and Bellamy, 1964), and, after conversion to the sodium salt, the purified RNA is washed and stored at −20° as an anhydrous, salt-free powder (Gray and Lane, 1967). Wheat embryo transfer RNA prepared in this manner contains, in addition to tRNA, about 10% 5S rRNA, as judged by polyacrylamide gel electrophoresis (unpublished observations).

Purification of Phosphodiesterase from Snake Venom. Phosphodiesterase was purified from *Vipera russelli* venom (Ross Allen Reptile Institute, Silver Springs, Fla.) by a combination of acetone fractionation (Koerner and Sinsheimer, 1957) and chromatography on cellulose (Hurst and Butler, 1951), as described by Lane *et al.* (1963). Aliquots of the enzyme preparation were passed through 1-g cellulose columns as often as required to reduce contamination by the venom ribonuclease (McLennan and Lane, 1968) to a low level. Preparations of snake venom phosphodiesterase used in this study contained no detectable phosphomonoesterase activity and yielded predominantly 5′-mononucleotides (>95% of the hydrolysis products) from wheat embryo transfer RNA.

Chromatographic, Electrophoretic, and Desalting Techniques. Preparative and analytical paper chromatography and paper electrophoresis were carried out according to techniques previously used in the characterization of other minor components of tRNA (Gray and Lane, 1967, 1968). The following chromatographic systems were used: (A) 95% ethanol–water (4:1, v/v), in conjunction with ammonium sulfate impregnated Whatman No. 1 chromatography paper (Lane, 1963); (B) saturated ammonium sulfate–2-propanol (40:1, v/v), also used with ammonium sulfate treated paper; (C) 1-butanol–water–95% ethanol (50:35:17, v/v/v). Buffers used for electrophoresis were: (D) 1 M formic acid (pH 1.8); (E) 0.025 M ammonium acetate (pH 3.5); (F) 0.025 M ammonium acetate (pH 5.0); (G) 0.025 M ammonium formate (pH 9.2); (H) 0.025 M triethylammonium acetate (pH 4.7).

When chromatographic systems A and B were employed for the preparative isolation of nucleotide derivatives, the compounds were desalted on DEAE-cellulose (carbonate form) as described by Rushizky and Sober (1962), except that the desalted compounds were recovered in 1 M pyridine formate (pH 4.5). Charcoal desalting was carried out as described previously (Gray and Lane, 1967), except that the compounds were eluted from charcoal with 95% ethanol–water–concentrated ammonium hydroxide (25:25:1, v/v/v). Desalting on DEAE-cellulose (formate form) was done according to the method of Lane (1965).

Hydrolysis of RNA and Fractionation of the Products. Wheat embryo transfer RNA was incubated with purified phosphodiesterase under conditions previously found to give apparently complete hydrolysis (Hudson *et al.*, 1965). Hydrolysates consisted of: 200 ml of a 1% aqueous solution of transfer RNA (approximately 40,000 A_{260} units), 100 ml of 0.5 M ammonium formate (pH 9.2), and 100 ml of purified

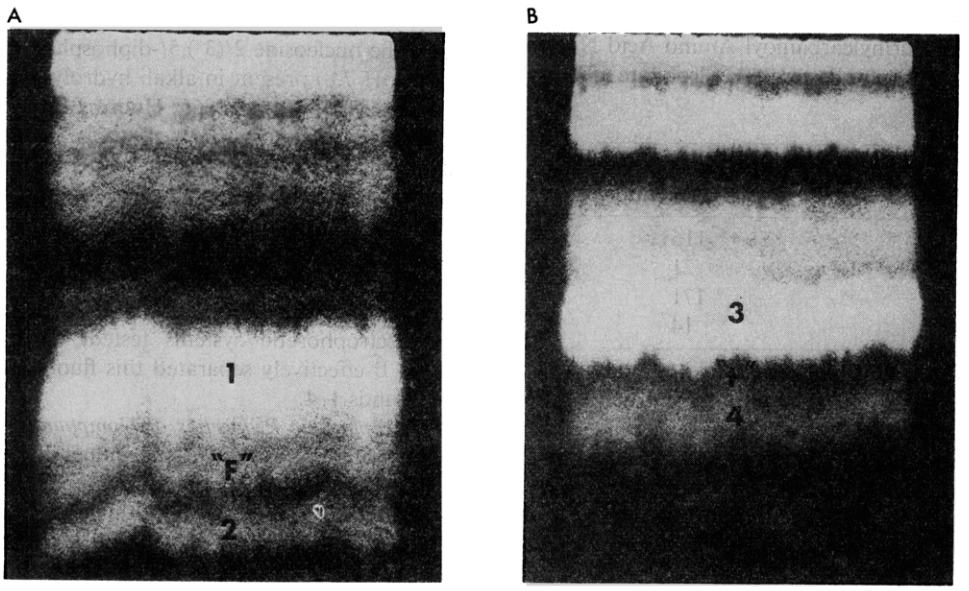

FIGURE 2: Representative ultraviolet contact photographs illustrating the resolution of the components of fractions P-1 (A) and P-3 (B) by paper chromatography in system A. Approximately 100 A_{260} units of each fraction were applied in a 7-in. strip on each chromatogram. Development time was 18 hr in (A), 22 hr in (B); increased resolution could be obtained by developing for a longer time or by a second development in the same solvent. The bands marked "F" displayed an intense whitish fluorescence under uv light.

phosphodiesterase (10,000 units[2]). The mixture was incubated at 37° for 21 hr and then adjusted to pH 7.8 with formic acid and diluted to 1200 ml to reduce the formate ion concentration to about 0.085 M. Fractionation was carried out on DEAE-cellulose according to the stepwise elution protocol which has been applied to alkali hydrolysates of wheat embryo 18S + 26S rRNA (Lane, 1965) and wheat embryo transfer RNA (Hudson *et al.*, 1965). This procedure resolved the post-mononucleotide material into three fractions (P-1, P-2, and P-3), as shown in Figure 1.

Paper chromatography in system A indicated that P-1 contained one major (**1**) and one minor (**2**) ultraviolet (uv) absorbing compound having spectra suggestive of PurCbm-AA derivatives, while a second major compound (**3**) with similar spectra was present in P-3. Compounds **1**, **2**, and **3** correspond to the compounds designated Y_2, Y_3, and Y_1, respectively, previously noted in the post-mononucleotide fraction of phosphodiesterase hydrolysates of wheat embryo transfer RNA (Gray, 1968). Fraction P-2 consisted almost entirely of nucleoside 2′(3′)-cyclic 5′-diphosphates (pN>p, with pU>p the major component), resulting from traces of venom RNase contaminating the purified phosphodiesterase preparations (McLennan and Lane, 1968). Less than 0.5% of the A_{260} units originally in the hydrolysate remained on the column after the elution of P-3, and further treatment of this material with a mixture of phosphodiesterase and *E. coli* alkaline phosphatase did not yield any uv-absorbing product having the spectral properties of a PurCbm-AA nucleoside.

Isolation and Purification of Purinylcarbamoyl Amino Acid Derivatives. Paper chromatography of P-1 and P-3 in system A was used to prepare **1**, **2**, and **3** in quantities sufficient for structural analysis (Figure 2). This procedure revealed an additional minor PurCbm-AA derivative (**4**), which migrated as a faint uv-absorbing band ahead of **3** during chromatography of P-3 in system A (Figure 2B). The four derivatives were recovered by desalting on DEAE-cellulose (carbonate).

At this stage, each of the compounds was still contaminated with material which on chromatograms fluoresced intensely under uv light but which absorbed uv light weakly (see Figure 2). This material overlapped each of the compounds in most chromatographic and electrophoretic systems tested, with the exception of chromatographic system B. In addition, **3** was contaminated with pUp, derived from pU>p in the course of hydrolysis. The four compounds could be freed of these contaminants by paper chromatography in system B, in which the fluorescent material migrated well behind the various purinylcarbamoyl amino acid derivatives, while pUp migrated well ahead of **3**. After desalting on DEAE-cellulose (carbonate), the compounds were further purified by electrophoresis (buffer D). The final product in each case proved to be homogeneous in all chromatographic and electrophoretic systems tested.

Table I indicates the yield of each derivative from a representative hydrolysate of wheat embryo transfer RNA.

Treatment of Purinylcarbamoyl Amino Acid Derivatives with Alkaline Phosphatase. Incubation mixtures consisted of approximately 30 A_{260} units of compound in 300 μl of water, 150 μl of 1 M ammonium formate (pH 9.2), and 15 μl of *E. coli* alkaline phosphatase (Worthington BAP-C; 10 mg/ml; 31 units/mg). Hydrolysates were proportionately reduced for treatment of smaller amounts of compound. After 2 hr at 37°, each incubation mixture was taken to dryness and the residue redissolved in water and desalted by electrophoresis in buffer D. Further purification of the uv-absorbing product was sometimes carried out by a second electrophoresis in buffer H.

Results

Separation of the Purinylcarbamoyl Amino Acid Nucleoside Derivatives in Snake Venom Phosphodiesterase Hydrolysates of Wheat Embryo Transfer RNA. It was expected that PurCbm-

[2] One unit of phosphodiesterase activity is defined as that amount which releases 1 A_{260} unit of acid-soluble nucleotide from wheat embryo high molecular weight (18 S + 26 S) rRNA per hr in the standard assay (1 ml of 1% RNA, 0.5 ml of 0.5 M ammonium formate (pH 9.2), and 0.5 ml of enzyme solution incubated at 37° for 1 hr; the reaction was stopped by the addition of 4 ml of cold 5% trichloroacetic acid).

TABLE 1: Amounts of Purinylcarbamoyl Amino Acid Nucleoside Derivatives in a Phosphodiesterase Hydrolysate of Wheat Embryo Transfer RNA.

Compd	A_{260} Units/100,000 A_{260} Units of Hydrolysate[a]
1	116
2	21
3	171
4	14

[a] The number of A_{260} units of compounds **1**, **2**, and **4** was estimated after the initial chromatography of fractions P-1 and P-2 in system A (Figure 2). In the case of compound **3**, the amount was estimated after a second chromatography in system B, and the latter value has been corrected for 90% recovery of uv-absorbing material during desalting of **3** on DEAE-cellulose (carbonate) after the initial chromatography in system A.

AA nucleosides such as t^6A would be released as nucleoside 5′-monophosphates during phosphodiesterase hydrolysis of tRNA, and that, because of the free carboxyl function, such nucleotides would elute from DEAE-cellulose later than the bulk of the 5′-nucleotides during fractionation of the hydrolysis products at pH 7.8. In fact, it was found that the PurCbm-AA nucleoside derivatives present in the post-mononucleotide fraction of phosphodiesterase hydrolysates of wheat embryo transfer RNA could be resolved into two groups (eluting in fractions P-1 and P-3, respectively) by the column fractionation technique described. The elution pattern of the post-mononucleotide material (Figure 1) suggested that P-1 and P-3 had net charges of −3 and −4, respectively, at pH 7.8, since P-1 was eluted from DEAE-cellulose with a buffer which brings off alkali-stable dinucleotides (NmpNp, net charge −3 at pH 7.8) during similar fractionations of alkali hydrolysates of RNA, while P-3 came off the column in a buffer which elutes the nucleoside 2′(3′),5′-diphosphates (pNp, net charge −4 at pH 7.8) present in alkali hydrolysates of RNA (Singh and Lane, 1964; Lane, 1965; Hudson *et al.*, 1965).

Fractions P-1 and P-3 were each found to contain one major and one minor PurCmb-AA derivative, which could be resolved from one another by chromatography in system A (Figure 2). An intensely uv-fluorescent band of unknown origin was consistently positioned between compounds **1** and **2** and between compounds **3** and **4** after chromatography of fractions P-1 and P-3 in system A. Of the chromatographic and electrophoretic systems tested, only chromatographic system B effectively separated this fluorescent material from compounds **1**–**4**.

Electrophoretic Properties of Compounds **1**–**4**. Compounds **1**–**4** migrated as anions at pH values between 1.8 and 9.2 (Figure 3A). All were more negatively charged than adenosine 5′-monophosphate (pA) within this pH range.

Treatment of each of the compounds with *E. coli* alkaline phosphatase resulted in a substantial decrease in net negative charge (Figure 3B), consistent with the removal of a monoester phosphate group in each case. The products of phosphatase treatment showed no increase in electrophoretic mobility between pH 5 and 9 (in contrast to pA), confirming the absence of a secondary phosphoryl dissociation. The dephosphorylation products fell into two groups, **3′** and **4′** having similar electrophoretic mobilities and being more negatively charged than **1′** and **2′** at pH values between 1.8 and 9.2. At pH 9.2, compounds **1′**–**4′** had apparent net charges of −1.12, −1.04, −1.66, and −1.66, respectively, relative to an assigned net charge of −2 for pA at this pH. These results suggested that **1′** and **2′** contained a single acidic group, whereas **3′** and **4′** contained two such groups.

The properties deduced for compounds **1** and **2** from this preliminary characterization were consistent with their being the (5′)-phosphoric esters of monoacidic PurCbm-AA nucleosides. To account for the additional anionic dissociation in **3** and **4**, three possibilities were considered: (1) compounds **3**

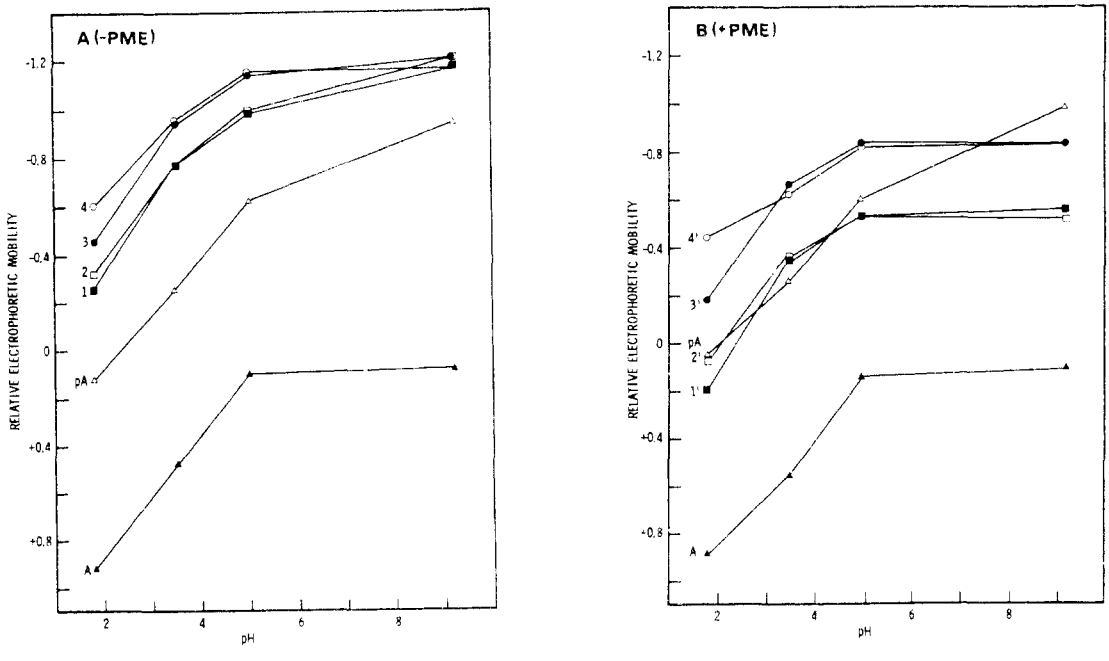

FIGURE 3: Relative electrophoretic mobilities of PurCbm-AA nucleoside derivatives isolated from a phosphodiesterase hydrolysate of wheat embryo transfer RNA. Mobilities are relative to that of the picrate marker, and the sign indicates migration as an anion (−) or cation (+). Relative mobilities are shown before (A) and after (B) treatment of each of the derivatives with *E. coli* alkaline phosphatase (PME).

and **4** contained an acidic amino acid residue such as aspartate or glutamate instead of a neutral amino acid such as threonine; (2) these two compounds contained a neutral amino acid but had an additional acidic group elsewhere in the molecule; (3) compounds **3** and **4** were *dinucleotides* of the form pNpA* or pA*pN, where A* is a monoacidic PurCbm-AA nucleoside and N is another nucleoside. Further characterization showed the third alternative to be correct.

Further Characterization of Purinylcarbamoyl Amino Acid Nucleoside Derivatives from Wheat Embryo Transfer RNA. COMPOUND **1** FROM FRACTION P-1. The ultraviolet absorption spectra of **1** (Figure 4A) were identical with those of *N*-[*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (Hall, 1971) and *N*-[*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]-glycine (Schweizer *et al.*, 1970). As shown in Figure 3, treatment of **1** with phosphatase yielded a product (**1'**) displaying a decreased electrophoretic mobility relative to the parent compound at all pH values, consistent with the removal of a

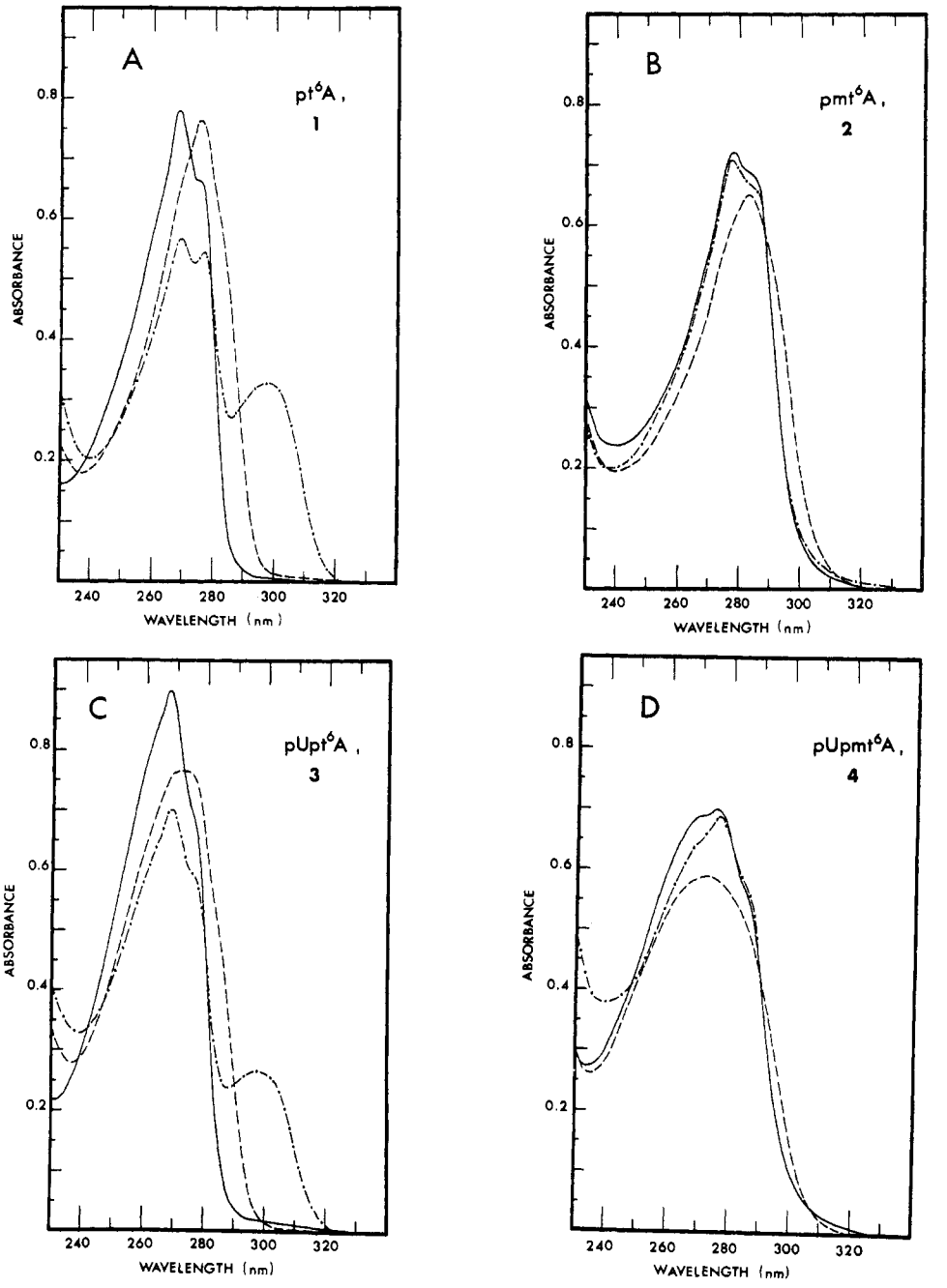

FIGURE 4: Ultraviolet absorption spectra of wheat embryo tRNA PurCbm-AA nucleoside derivatives. Spectra were recorded on a Bausch and Lomb Spectronic 505 spectrophotometer: (– –) 0.1 N HCl (A and C) or 1 N HCl (B and D); (——) 0.025 M potassium phosphate buffer (pH 6.8); (–·–) 0.1 N NaOH.

Compd	λ_{max} Acidic	Neutral	Alkaline
1	276	269, 276 (s)	269, 277, 297
2	283	278, 285 (s)	278, 285 (s)
3	270–274	268	268, 276 (s), 297
4	272	268 (s), 275	268 (s), 276

TABLE II: Chromatographic and Electrophoretic Mobilities of PurCbm-AA Nucleoside Derivatives from Wheat Embryo Transfer RNA.

Compd	Rel Chromat Mobil (R_{Ado})[a] for System A	Rel Chromat Mobil (R_{Ado})[a] for System B	Rel Electro Mobil (R_{pic})[b] for Buffer D	Rel Electro Mobil (R_{pic})[b] for Buffer E	Rel Electro Mobil (R_{pic})[b] for Buffer F	Rel Electro Mobil (R_{pic})[b] for Buffer G
1	1.12	1.03	−0.26	−0.77	−0.99	−1.17
HO^--treated **1**	0.62	1.71	+0.08	−0.27	−0.60	−0.92
pA	0.63	1.75	+0.12	−0.25	−0.62	−0.94
2	1.28	0.93	−0.32	−0.77	−1.00	−1.20
HO^--treated **2**	0.99	1.43	+0.09	+0.27	−0.60	−0.99
pm^6A	1.00	1.42	+0.12	−0.25	−0.62	−0.94
3	0.76	0.92				
4	0.96	0.90				

[a] The relative chromatographic mobilities of Ado, pA, and Ap in system A, calculated from the data of Lane (1965), are 1.00, 0.62, and 0.78, respectively; subscript Ado, adenosine. [b] The sign indicates migration as an anion (−), or cation (+); pic, picrate.

monoester phosphate group. The continuing presence in **1′** of an acidic group was shown by the fact that it migrated as an anion at pH 9.2, whereas A is uncharged at this pH and remains at the origin. Acid hydrolysis of **1′** (2 A_{260} units in 0.2 ml of 0.5 M HCl heated for 10 min at 100° in a sealed tube) yielded a single compound having uv absorption spectra identical with those of *N*-[*N*-(purin-6-yl)carbamoyl]threonine (Chheda *et al.*, 1969).

TABLE III: Analysis of Alkaline Hydrolysates of **1′**.

Anal.	Condns of Hydrolysis	μmol of Ado	μmol of Thr	μmol of Gly	Molar Ratio Thr:Ado	Molar Ratio Gly:Ado
1	100° oven, 180 min	0.357	0.236	0.008	0.66	0.022
2	100° oven, 40 min	0.362	0.080	n.d.[a]	0.22	
3	boiling water bath, 180 min	0.290	0.254	0.026	0.88	0.090

[a] Not detectable. Replicate samples of compound **1′** (3 A_{260} units in 0.2 ml of 0.1 M NaOH) were heated in stoppered tubes under the conditions indicated. In analysis 3, evaporation from the sample occurred during hydrolysis, effectively raising the NaOH concentration of the hydrolysis mixture. No evaporation of the sample occurred in the case of analyses 1 and 2. Upon completion of heating, the samples were taken to dryness and redissolved in 1 ml of 0.2 N sodium citrate (pH 2.2). An aliquot (0.5 ml) of the hydrolysate was taken for amino acid analysis (Beckman amino acid analyzer). Another portion of the hydrolysate was used for spectrophotometric quantitation of adenosine, which was then isolated by charcoal desalting and characterized by chromatography.

TABLE IV: Chromatographic and Electrophoretic Mobilities of Phosphatase-Treated PurCbm-AA Nucleoside Derivatives from Wheat Embryo Transfer RNA.

Compd	Rel Chromat Mobil (R_A) for System A	Rel Chromat Mobil (R_A) for System B	Rel Chromat Mobil (R_A) for System C	Rel Electro Mobil R_{pic} for Buffer D	Rel Electro Mobil R_{pic} for Buffer E	Rel Electro Mobil R_{pic} for Buffer F	Rel Electro Mobil R_{pic} for Buffer G
1′	1.47	0.68	0.48	+0.19	−0.35	−0.55	−0.58
t^6A	1.47	0.68	0.48	+0.19	−0.36	−0.57	−0.57
2′	1.61	0.62	0.64	+0.07	−0.31	−0.53	−0.56
mt^6A	1.62	0.64	0.61	+0.05	−0.33	−0.59	−0.59
3′	1.30	0.53	0.26				
4′	1.45	0.48	0.35				
m^6A	1.44	0.81	1.36				

Alkaline hydrolysis of *N*-[*N*-(purin-6-yl)carbamoyl]threonine has been shown to liberate adenine and threonine (Chheda *et al.*, 1969). When a sample of **1** was treated with alkali (2 A_{260} units in 0.5 ml of 1 M NaOH, room temperature, 90 hr), the uv-absorbing product was found to be retained on DEAE-cellulose at pH 7.8 at low salt concentration (0.025 M Tris–formate). The product was identified as adenosine 5′-monophosphate (pA) on the basis of its spectral, chromatographic, and electrophoretic properties (Table II). Notably, the product was readily distinguishable from adenosine 2′(3′)-monophosphate (Ap) by chromatography in system A. Alkaline hydrolysis of **1′** gave a uv-absorbing product which was not retained on DEAE-cellulose at low salt concentration and neutral pH and which was identified as adenosine.

Alkaline hydrolysis of **1′** according to the conditions of Chheda *et al.* (1969) followed by amino acid analysis indicated that threonine was the major amino acid liberated by this treatment (Table III). The molar ratio of threonine to adenosine in such hydrolysates was found to vary widely (from 0.22 to 0.88 in the analyses presented), depending upon the heating conditions employed. Small amounts of glycine were also detected, but it is not known whether this was in fact derived from a glycine-containing PurCmb-AA derivative present in small amounts in **1′**. More vigorous hydrolysis conditions (contrast analyses 1 and 3, Table III) resulted in only a slight (8%) increase in the amount of threonine liberated but a substantial (threefold) increase in the amount of glycine, while a significant decrease (about 20%) in the amount of adenosine formed was noted. On the other hand, alkaline hydrolysis of either pure threonine or adenosine under the conditions used in analysis 1 (Table III) did not yield detectable amounts of glycine or any other amino acid, and quantitative recovery of threonine was noted.

Finally, comparison of the electrophoretic and chromatographic properties of **1′** with those of synthetic t^6A (Chheda, 1969) showed the two compounds to be identical (Table IV).

These data establish the structure of **1** as *N*-[*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine 5′-phosphate (pt^6A), *i.e.*, the 5′-phosphoric ester of t^6A (Figure 5).

COMPOUND **2** FROM FRACTION P-1. The ultraviolet absorption spectra of **2** (Figure 4B) were essentially identical with those of the minor nucleoside component recently isolated from *E. coli* $tRNA^{Thr}$ and identified as *N*-[*N*-methyl-*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (Kimura-Har-

ada *et al.*, 1972b). Except at pH 1.8, the electrophoretic mobility of **2** was similar to that of **1** (Figure 3A). As in the case of **1**, phosphatase treatment of **2** gave a product (**2'**) having a decreased electrophoretic mobility at all pH values, suggesting the removal of a monoester phosphate group. The electrophoretic mobility of **2'** was similar to that of **1'** except at pH 1.8 (Figure 3B).

Alkaline hydrolysis of **2** yielded a uv-absorbing product characterized as N^6-methyladenosine 5'-phosphate (pm^6A) from its spectral, chromatographic, and electrophoretic properties (Table II), while alkaline hydrolysis of **2'** yielded a derivative having the properties of N^6-methyladenosine (m^6A). Quantitative amino acid analysis of an alkaline hydrolysate of **2'** gave yields of 0.56 mol of Thr and 0.13 mol of Gly/mol of m^6A. The molar ratio of threonine to N^6-methyladenosine was similar to the ratio of threonine to adenosine observed in the case of **1'** treated with alkali under identical conditions (hydrolysis 1, Table III), while the yield of glycine was about sixfold higher from **2'** than from **1'** under the same conditions. In addition, a small amount of serine (0.05 mol/mol of m^6A) was detected in the alkaline hydrolysate of **2'**. As in the case of **1'**, it is not known at present whether the detection of amino acids other than threonine implies the presence in **2'** of small amounts of PurCbm-AA nucleosides containing amino acids other than threonine.

The chromatographic properties of **2** and **2'** (Tables II and IV) suggested that they were more nonpolar than **1** and **1'**, respectively, consistent with the additional presence of a methyl group in the former two compounds. Thus, compounds **2** and **2'** had greater chromatographic mobilities than **1** and **1'**, respectively, in systems A and C (nonpolar solvents), but migrated more slowly in system B (a polar solvent). Similar relative chromatographic mobilities in these systems are displayed by pA and A compared to their N-methylated derivatives, pm^6A and m^6A.

On the basis of these data, compound **2** has been assigned the structure *N*-[*N*-methyl-*N*-(9-β-D-ribofuranosylpurin-6-yl)-carbamoyl]threonine 5'-phosphate (pmt^6A), *i.e.*, the 5'-phosphoric ester of mt^6A (Figure 5).

It is noteworthy that although **1'** and **2'** migrate as cations at pH 1.8 (Figure 3B), their considerably lower mobility relative to A at this pH suggests the presence of a basic group with a lower pK_a than that in A. The basic dissociation in **1'** and **2'** is undoubtedly the same as that noted in *N*-[*N*-(purin-6-yl)carbamoyl]threonine by Chheda *et al.* (1969), which these authors attributed to ring protonation. The consistently lower net positive charge of **2'** relative to **1'** at pH 1.8 suggests that the basic dissociation in mt^6A has a significantly lower pK_a than that in t^6A. Thus, electrophoresis in 1 M HCOOH (pH 1.8) can readily distinguish between t^6A and mt^6A (Figure 3B) and between the 5'-phosphoric esters of these compounds (Figure 3A).

COMPOUND **3** FROM FRACTION P-3. The ultraviolet absorption spectra of **3** (Figure 4C) were similar in certain respects to those of **1** (*e.g.*, the peak in the alkaline spectrum at 297 nm) but showed distinct differences. In particular, **3** displayed a significantly higher A_{260}/A_{280} ratio than **1** at all pH values. These differences suggested the possible presence of an additional chromophore in **3**.

Electrophoresis indicated that **3** was more negatively charged than **1** at all pH values (Figure 3A). Treatment of **3** with phosphatase yielded a product (**3'**) having a reduced net negative charge at all pH values (Figure 3B), consistent with the removal of a monoester phosphate group. The fact that **3'** had a greater electrophoretic mobility than **1'** at all pH values and migrated as an anion even at pH 1.8 suggested the presence of a strongly acidic group in **3'**.

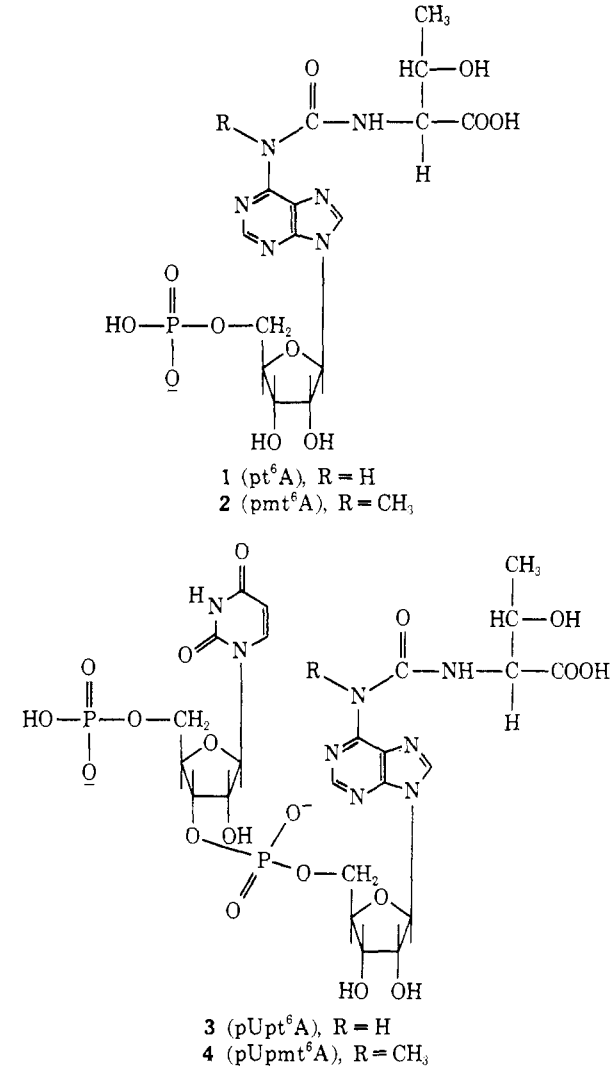

FIGURE 5: Structures of the purinylcarbamoyl amino acid nucleoside derivatives isolated from phosphodiesterase hydrolysates of wheat embryo transfer RNA.

When a sample of **3** was treated with alkali and the resulting hydrolysate fractionated on DEAE-cellulose at pH 7.8, part of the uv-absorbing material was eluted from the column while part remained on the column. From its uv absorption spectra and chromatographic mobility, the nonretained material was identified as adenosine, while the retained material proved to be uridine 2'(3'),5'-diphosphate (pUp) (Table V). Hydrolysis of **3'** and fractionation under the same conditions gave the same results, except that the uv-absorbing material retained on DEAE-cellulose proved to be uridine 2'(3')-monophosphate (Up) rather than pUp (Table V). Direct analysis of alkali hydrolysates of **3** and **3'** by paper chromatography indicated that A and pUp were produced in approximately equimolar amounts from **3**, while **3'** yielded equimolar amounts of A and Up on alkaline hydrolysis (Table VI). Since the uv absorption spectra of **3** were distinctly different from those of a dinucleotide containing adenosine and uridine, and since t^6A is converted to A under these conditions of alkaline treatment, the above results suggested that **3** was a dinucleotide, $pUpt^6A$ (Figure 5), and that **3'** was the corresponding dinucleoside phosphate, Upt^6A.

TABLE V: Chromatographic Mobilities (System A) of the Products of Alkaline Hydrolysis of **3** and **3′**.[a]

Compound	R_F				
	Unretained Material (Adenosine Spectrum)	Retained Material (Uridine Spectrum)	A	pUp	Up
HO^--treated **3**	0.42	0.24			
			0.42	0.25	0.46
HO^--treated **3′**	0.43	0.47			

[a] Alkaline hydrolysates were prepared by incubating approximately 4 A_{260} units of **3** and **3′** in 1 ml of 1 M NaOH at room temperature for 90 hr. After adjusting the pH to 7.8 with formic acid, each hydrolysate was diluted to 40 ml and passed through a 2.5 cm × 10 cm column of DEAE-cellulose (formate) equilibrated with 0.025 M Tris–formate (pH 7.8). The column was washed with the same buffer until no further uv-absorbing material eluted, and the uv-absorbing material appearing in the effluent during charging of the sample and the subsequent wash ("unretained material") was desalted on charcoal. Ultraviolet-absorbing material remaining on the DEAE-cellulose column during elution with 0.025 M Tris–formate (pH 7.8) ("retained material") was recovered in 1 M pyridine formate (pH 4.5), after first washing the column to remove salt.

These structural assignments were confirmed by treating **3** and **3′** with pancreatic RNase and separating the products by electrophoresis (Figure 6A). Equimolar amounts of pUp and t^6A were obtained from **3**, while **3′** gave equimolar amounts of Up and t^6A (Table IV). The t^6A isolated from RNase hydrolysates of **3** was spectrally, chromatographically, and electrophoretically identical with **1′** and with synthetic t^6A, and yielded the same amino acids as **1′** on amino acid analysis (*i.e.*, mainly Thr with a trace of Gly). The pUp isolated from RNase hydrolysates of **3** showed as a single band during electrophoresis at pH 1.8 or chromatography in system B, both of which resolve the **2′** and **3′** isomers of pUp. Only the 3′ isomer of pUp would be expected to be produced by RNase hydrolysis of pUpt^{6}A. No evidence was obtained for the presence of nucleosides other than U and t^6A in significant amounts in **3**.

COMPOUND **4** FROM FRACTION P-3. The characterization of compound **3** as a dinucleotide having the structure pUpt^{6}A prompted us to search for an analogous dinucleotide containing mt^6A instead of t^6A. Such a dinucleotide would be expected to migrate ahead of pUpt^{6}A during chromatography in system A, and careful examination of chromatograms of fraction P-3 developed in this solvent did reveal a faint uv-absorbing band (designated **4**) migrating ahead of **3** in this system (Figure 2B).

The ultraviolet absorption spectra of compound **4** are shown in Figure 4D. At each of the three pH values, the spectra were those expected to be generated by a dinucleotide containing U and mt^6A. Relative to the spectra of **2** (pmt^{6}A), for example, the spectra of **4** displayed absorbance maxima shifted to lower wavelengths, broadening of the absorption curves, and a substantial increase in A_{260}/A_{280} ratios.

The electrophoretic properties of **4** and **4′** were closely similar to those of **3** and **3′**, respectively, at all pH values except 1.8 (Figures 3A and 3B). At this low pH, **4** and **4′** had distinctly greater net negative charges than **3** and **3′**. These electrophoretic properties were consistent with the presence of mt^6A rather than t^6A in **4** and **4′**, since mt^6A carries significantly less positive charge than t^6A at pH 1.8 (Table IV).

Treatment of **4** with pancreatic RNase (Figure 6B) yielded two products, identified as mt^6A and pUp, in approximately equimolar amounts (Table VI). The limited amount of material available precluded a quantitative amino acid analysis of the mt^6A derived from **4**, but on the basis of the result obtained with compound **3**, it would seem unlikely that the major amino acid constituent would be other than threonine. There

TABLE VI: Quantitative Analysis of Alkaline and Ribonuclease Hydrolysates of Purinylcarbamoyl Amino Acid Derivatives.

Compd	Hydrolysis by	Products Separated by	Found (nmol)		Molar Ratio
3	Alkali[a]	Chromatography in system B	pUp	A	pUp:A
			67.5	58.3	1.16
3′	Alkali	Chromatography in system B	Up	A	Up:A
			75.4	64.5	1.17
3	RNase A[b] (3 anal.)	Electrophoresis in buffer D	pUp	t^6A	pUp:t^6A
			37.8	41.4	0.91
			41.0	47.7	0.86
			47.4	51.0	0.93
3′	RNase A	Electrophoresis in buffer D	Up	t^6A	Up:t^6A
			43.6	50.8	0.86
4	RNase A	Electrophoresis in buffer D	pUp	mt^6A	pUp:mt^6A
			73.3	61.3	1.20

[a] Approximately 4 A_{260} units of **3** or **3′** was dissolved in 50 μl of 1 M NaOH and allowed to stand at room temperature. After 90 hr, 5 μl of glacial acetic acid was added to each hydrolysate, which was then directly spotted on ammonium sulfate impregnated Whatman No. 1 paper and developed in system B. Uv-absorbing areas plus appropriate blanks were eluted in 0.1 M HCl and the amount of each compound was measured spectrophotometrically. The following molar extinction coefficients (260 nm) were used: pUp and Up, 9950; A, 14,300. [b] Carried out as described in the legend to Figure 6. After elution in water, the amount of each compound (corrected for an appropriate blank) was estimated spectrophotometrically. The following molar extinction coefficients were used: pUp and Up, 10,000 at 262 nm; t^6A, 25,000 at 268 nm; mt^6A, 25,000 at 278 nm.

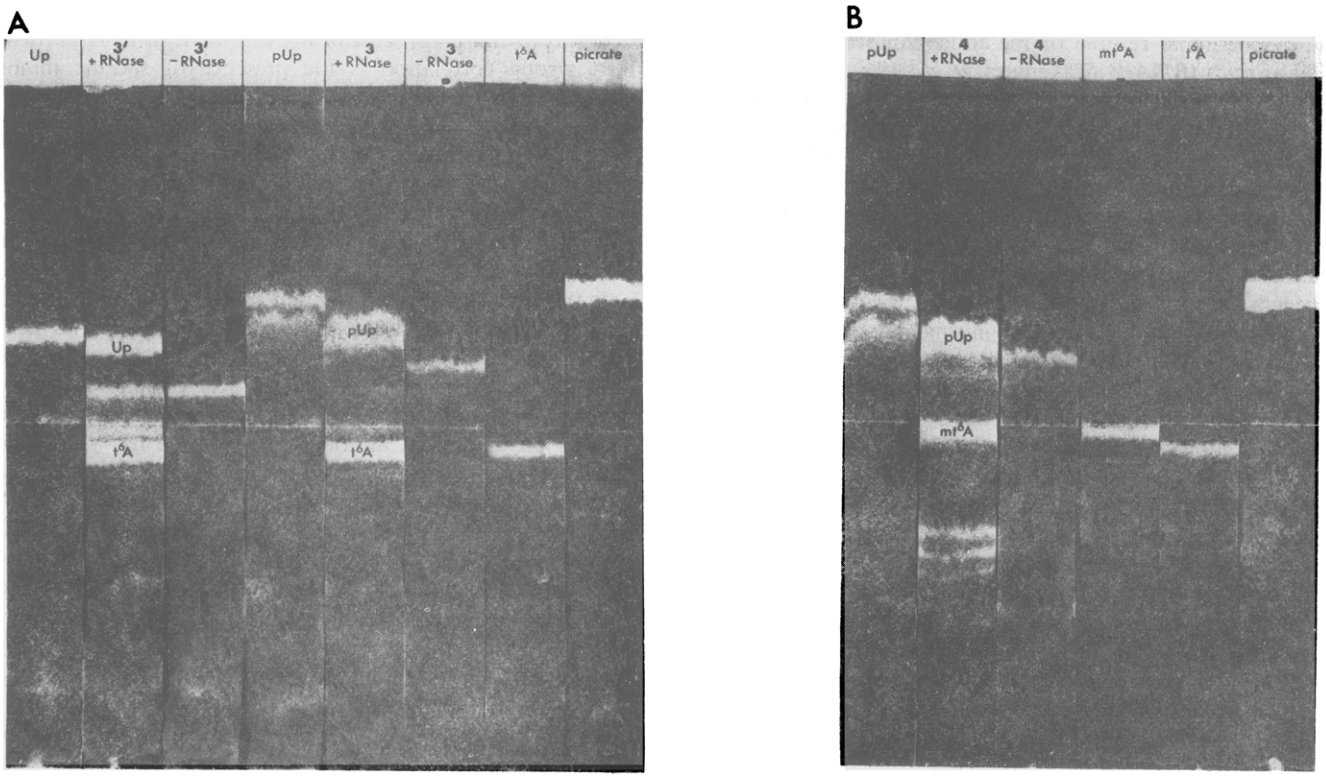

FIGURE 6: Electrophoretic separation of the products of ribonuclease hydrolysis of wheat embryo transfer RNA PurCbm-AA nucleoside derivatives. Approximately 2 A_{260} units of compound was dissolved in 0.025 M Tris–formate (pH 7.5), 10 μl of RNase A (Worthington; 0.5 mg/ml) was added, and the mixture was incubated at 37° for 22 hr. The hydrolysates were taken to dryness, redissolved in 50 μl of water, and spotted on strips of Whatman No. 1 paper moistened with buffer D, along with appropriate markers. Electrophoresis was for 1 hr at 500 V: (A) untreated and RNase-treated **3** and **3′** (a small amount of **3′**, about 5% on a molar basis, remained unhydrolyzed under the conditions used); (B) untreated and RNase-treated **4**. Spreading of the pUp band and lack of correspondence with marker pUp probably reflect the presence of salt in the applied sample, since re-electrophoresis of the pUp band in buffer D gave a single narrow band corresponding in mobility to that of the slower moving (presumably 3′) isomer of the pUp marker (which had been derived from an alkaline hydrolysate of RNA).

was no evidence for the presence of nucleosides other than U and mt^6A in **4**.

Compounds **4** and **4′** had the expected increased mobility relative to **3** and **3′**, respectively, during paper chromatography in nonpolar systems (A and C), while showing a slightly decreased mobility in a nonpolar system (B) (Tables II and IV). On the basis of the above results, compound **4** was assigned the structure pUmt^6A (Figure 5).

Discussion

The major purinylcarbamoyl amino acid nucleoside in wheat embryo tRNA, as in the tRNA of other organisms examined to date, is *N*-[*N*-(9-β-D-ribofuranosylpurin-6-yl)-carbamoyl]threonine (t^6A). The present study has also identified a methylated derivative of t^6A, *N*-[*N*-methyl-*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (mt^6A), as a minor constituent of wheat embryo tRNA. This latter nucleoside has previously been isolated from *E. coli* $tRNA^{Thr}$ (Kimura-Harada *et al.*, 1972b), but this is the first report of the occurrence of mt^6A in eukaryotic transfer RNA.

The PurCbm-AA nucleosides of wheat embryo tRNA are only partially released as the expected 5′-nucleotides, pt^6A and pmt^6A, during phosphodiesterase hydrolysis of wheat embryo tRNA under the conditions described in this report. They are also present in the form of dinucleotides having the structures pUpt^6A and pUpmt^6A. These dinucleotides apparently persist in phosphodiesterase hydrolysates of wheat embryo tRNA because the phosphodiester bond linking the 5′-hydroxyl of the PurCbm-AA nucleoside residue to the 3′-hydroxyl of the adjacent nucleoside residue in tRNA is exceptionally resistant to hydrolysis by purified phosphodiesterase. An analogous situation has been noted by Cory *et al.* (1969), who found that the phosphodiester linkage between uridine and t^6A in a purified *E. coli* $tRNA^{Met}$ was split only slowly by pancreatic RNase, indicating some kind of steric hindrance. Inhibition of phosphodiesterase hydrolysis of internucleoside phosphodiester bonds by base-methylated (Baev *et al.*, 1963) and sugar-methylated (Gray and Lane, 1967) nucleosides has been noted previously. In addition, product inhibition has been shown to be a factor affecting the rate of phosphodiesterase hydrolysis of phosphodiester bonds in RNA (Singer and Fraenkel-Conrat, 1963) and low molecular weight substrates (Gray and Lane, 1967). Since product inhibition would be most pronounced as hydrolysis approaches completion, phosphodiester bonds which are intrinsically resistant to phosphodiesterase (due to the presence of modified nucleoside components) would be the ones most affected by the additional inhibitory effect of 5′-nucleotides generated in the digest. On the basis of these results, it would be anticipated that the dinucleotides isolated in the present study should be reduced in quantity or entirely absent in hydrolysates conducted at higher enzyme:tRNA ratios, and this has been verified.

Since t^6A has been postulated to play a role in the recognition of codons starting with adenosine (Ishikura *et al.*, 1969;

Nishimura, 1972), and has been shown to be located next to the 3′-terminal residue of the anticodon sequence in *Torulopsis utilis* $tRNA^{Ile}$ (Takemura *et al.*, 1969) and in several *E. coli* tRNAs (Nishimura, 1972), it would be expected that a sequence 5′···pUpt^6A···3′ (or a similar one containing a derivative of uridine) should be found in all tRNAs containing t^6A. The isolation of the dinucleotide pUpt^6A from phosphodiesterase hydrolysates of unfractionated wheat embryo tRNA is consistent with this expectation, and provides circumstantial evidence that in wheat embryo, also, t^6A occurs next to the 3′-terminal residue (uridine) of the anticodon sequence in tRNAs recognizing codons beginning with adenosine. The isolation of pUpmt^6A during the present study suggests a similar localization of mt^6A in wheat embryo tRNA, and, in fact, the sequence 5′···pUpmt^6A···3′ has been demonstrated in *E. coli* $tRNA^{Thr}$ (Kimura-Harada *et al.*, 1972b). It is significant that no dinucleotides containing t^6A or mt^6A and a nucleoside other than uridine were encountered in the present study.

Although threonine was the major amino acid detected in alkali hydrolysates of t^6A and mt^6A isolated in this study, small amounts of glycine were also derived from t^6A, while hydrolysates of mt^6A contained small amounts of both glycine and serine in addition to threonine. These results raise the possibility that analogs of t^6A and mt^6A containing amino acids other than threonine may be present as rare minor components of wheat embryo tRNA, particularly since these additional amino acids were not observed in alkali hydrolysates of adenosine or threonine. The glycine analog of t^6A, *N*-[*N*-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]glycine, has in fact been isolated from yeast tRNA (Schweizer *et al.*, 1970). Other workers have noted the presence of small amounts of amino acids (usually glycine and serine), in addition to threonine, in alkali hydrolysates of t^6A and mt^6A isolated either from unfractionated tRNA (Chheda *et al.*, 1969) or from individual purified acceptors (Kimura-Harada *et al.*, 1972a,b). Further studies will be required to establish definitively the origin of the additional amino acids detected during the present study in alkali hydrolysates of t^6A and mt^6A from wheat embryo tRNA. The observation that quantitative conversion of t^6A and mt^6A to A and m^6A, respectively, occurs under alkaline conditions accounts for the fact that no PurCbm-AA nucleotides were found in the dinucleotide fraction of alkali hydrolysates of wheat embryo tRNA in a previous study (Hudson *et al.*, 1965).

Providing conditions are chosen which ensure the complete release of PurCbm-AA nucleosides as the 5′-nucleotides during phosphodiesterase hydrolysis of transfer RNA, the method described in this report appears to be suitable for the isolation and quantitative analysis of the PurCbm-AA nucleoside constituents of RNA, since pt^6A and pmt^6A are easily separated from the bulk of the 5′-nucleotides by a preliminary fractionation on DEAE-cellulose, and subsequently from each other by two-dimensional paper chromatography. We are currently refining such a quantitative analytical technique and applying it to the bulk transfer RNA of other organisms. On the basis of preliminary results, it appears that pt^6A and pmt^6A are both present in phosphodiesterase hydrolysates of *E. coli* tRNA, as expected, but that phosphodiesterase hydrolysates of yeast tRNA contain only pt^6A. It should be emphasized, however, that our isolation procedure would not detect PurCbm-AA nucleoside derivatives in which the carboxyl group is blocked.

As discussed by Nishimura (1972), it is possible to correlate the specific locationing of certain modified components of tRNA molecules with the properties of those tRNAs in codon recognition, at least in the case of *E. coli*. A particularly strong correlation is the presence of specific minor nucleosides adjacent to the 3′ residue of the anticodon sequence. Thus *E. coli* tRNAs that recognize codons starting with uridine always contain 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine (ms^2i^6A) while tRNAs that recognize codons starting with adenosine contain t^6A or a derivative of t^6A. It is likely that such regularities extend to the tRNA of other organisms.

In the case of ms^2i^6A, Gefter and Russell (1969) have shown that this minor nucleoside is essential for the amino acid transfer function of tRNAs containing it. Likewise, the specific localization of t^6A in transfer RNA implies a specific role for this modified nucleoside in the functioning of those tRNAs containing it, although such a role has yet to be directly demonstrated. Moreover, the presence of t^6A as the major purinylcarbamoyl amino acid nucleoside in the tRNA of a higher plant as well as in bacterial, fungal, and animal tRNA suggests that, whatever the role of t^6A in protein biosynthesis, it has a similar function in both prokaryotic and eukaryotic organisms. In light of the almost universal occurrence of t^6A in transfer RNA, it is particularly noteworthy that the tRNA of *Mycoplasma* apparently lacks this modified nucleoside (Miller and Schweizer, 1972).

Finally, in view of the apparently rigid functional constraints which have conserved t^6A throughout the evolutionary history of transfer RNA molecules, it is most interesting that analogs and derivatives of t^6A should occur in very minor amounts in tRNA. It remains to be determined how the function of such derivatives differs at the molecular level from that of t^6A, and what mechanism restricts additional modification (such as methylation in the case of mt^6A) to a limited number of the total t^6A residues in a tRNA population.

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3′-*O*-Aminoacyl-2′-deoxyadenosines and 2′-*O*-Aminoacyl-3′-deoxyadenosines Related to Charged Transfer Ribonucleic Acid Termini†

Morris J. Robins,* Roger A. Jones, and Malcolm MacCoss‡

ABSTRACT: Aminoacyl nucleosides derived from 2′-deoxyadenosine and 3′-deoxyadenosine have been isolated as pure solids and completely characterized for the first time. Reaction of 5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosine (**1**) with *N*-trityl blocked amino acid anhydrides (**2a–c**) (generated *in situ* from the corresponding *N*-tritylamino acid and dicyclohexylcarbodiimide) in the presence of 4-*N*,*N*-dimethylaminopyridine gave the 3′-*O*-(*N*-tritylaminoacyl)-5′-*O*-(di-*p*-methoxytrityl)-2′-deoxyadenosines (**3a–c**) in high yields. This coupling reaction was unsuccessful using pyridine. Analogous treatment of 5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosine (**6**) gave the corresponding 2′-*O*-(*N*-tritylaminoacyl)-5′-*O*-(mono-*p*-methoxytrityl)-3′-deoxyadenosines (**7a–c**). The L-leucine (**a**), L-phenylalanine (**b**), and L-methionine (**c**) compounds were prepared in each series. Complete deblocking was effected using formic acid–1-butanol–toluene (1:1:1) at room temperature. Under these conditions the 3′-*O*-(L-aminoacyl)-2′-deoxyadenosines (**4a–c**) and 2′-*O*-(L-aminoacyl)-3′-deoxyadenosines (**8a–c**) were obtained in high yield with no detectable hydrolysis of either the aminoacyl ester or glycosidic bonds.

N-Formylmethionyl and *N*-acetylphenylalanyl derivatives were prepared in each series by subsequent acylation of the free aminoacyl compounds with acetic formic anhydride and *p*-nitrophenyl acetate, respectively. Biochemical rationale for the use of these compounds in the study of protein biosynthesis and initiation processes and preliminary biochemical data are discussed.

It is well established that the aminoacyl nucleoside antibiotic puromycin inhibits protein biosynthesis by simulating a charged terminus of tRNA. Acting in this role it accepts the growing peptide chain from peptidyl-tRNA and the covalently linked peptidylpuromycin dissociates from the ribosomal complex (for example, see Nathans (1964), Traut and Monro (1964), and Coutsogeorgopoulos (1967); for a recent concise review, see Suhadolnik (1970)). Various 3′(2′)-*O*-aminoacylnucleosides and nucleotide derivatives have been synthesized and evaluated as analogous peptide receptors in protein biosynthetic systems (see, for example, Rammler and Khorana, 1963; Waller *et al.*, 1966; Žemlička *et al.*, 1969; Rychlik *et al.*, 1969; Chládek *et al.*, 1970; Černá *et al.*, 1970a,b; Chládek, 1972, and other papers of the Czech group; Gottikh *et al.*, 1970; Tarusova *et al.*, 1971; Pozdnyakov *et al.* (1972), and other papers of the Russian group), and base as well as amino acid variation has been explored especially by the Czech group.

An example of such protein biosynthesis blockage by 2′,3′-bis(*O*-aminoacyl)adenosines (Černá *et al.*, 1970a) added

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‡ University of Alberta Postdoctoral Fellow, 1972–present.