Incorporation of Acyl Groups into the Anticodon of *Escherichia coli* Glutamic Acid Transfer Ribonucleic Acid†

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ABSTRACT: In contrast to other tRNAs, *Escherichia coli* $tRNA^{Glu}$ is very reactive with aromatic anhydrides and acylimidazoles. The reaction velocity, as measured by acid-precipitable radioactivity incorporated after reaction with labeled reagents, is dependent on and increases with the pH. The pancreatic ribonuclease digest of labeled acyl-$tRNA^{Glu}$ indicated that there is one main site of acylation, since most radioactivity is found in one peak. When the [^{14}C]benzoyl-$tRNA^{Glu}$ is subjected to complete hydrolysis and the resulting nucleosides chromatographed on thin-layer chromatographic plates, one radioactive spot was found by autoradiography. The product of benzoylation of 2-thio-5-(methylaminomethyl)-uridine upon chromatography in the solvent has the identical R_F as the aforementioned nucleoside derivative. Enzymatic hydrolysis data permit the deduction that this product is 2-thio-5-(methylbenzamidomethyl)uridine. The $tRNA^{Glu}$ can, thus, be selectively acylated in high yields in the wobble position of its anticodon.

Aqueous solutions of acetic anhydride react with tRNAs giving 2′-*O*-acyl derivatives (Knorre *et al.*, 1965), whereas organic solvents favor N-acetylation of cytidine residues in tRNA (Keith and Ebel, 1968). These modifications derived from either N- or O-acylations have been of some use in studies of the physical properties of tRNAs (Knorre *et al.*, 1969; Knorre and Shamovsky, 1967). A recent publication has reported the effect of phenoxyacetylation on the displacement of specific tRNA activities in BD-cellulose[1] chromatography (Friedman, 1972). In addition, an unidentified compound was isolated which may be the product of the acylating reagent with a particularly reactive nucleotide.

Our interest in nucleotide and tRNA acylation was kindled by the hope of using acylating reagents as chemical probes of tRNA structure (Cedergren *et al.*, 1971; Toupin, 1972). These hopes were largely unfulfilled due to the low reactivity of tRNAs as compared with mononucleotides under the conditions of acylation. This report deals with an exception; *Escherichia coli* $tRNA^{Glu}$ (Ohashi *et al.*, 1972) is easily acylated by a series of acid anhydrides.

Experimental Section

Materials. The [7-^{14}C]benzoic acid (14.3 Ci/mol) and the [7-^{14}C]phthalic anhydride (10.5 Ci/mol) were purchased from New England Nuclear Corp. *N*-Benzoyl- and *N*-acetylimidazoles were obtained from Pierce Chemicals. Pancreatic ribonuclease A (EC 2.7.7.16) was purchased from Sigma Chemicals and T_1 (EC 2.7.7.26) and T_2 ribonucleases came from Calbiochem. Snake venom phosphodiesterase (EC 3.1.4.1) and bacterial alkaline phosphatase (EC 3.1.3.1) were from Worthington Biochemicals. The purified $tRNA^{Glu}$ was a generous gift of Mr. A. D. Kelmers of the Oak Ridge National Laboratory, and had a stated acceptance activity of 1210 pmol of glutamic acid/A_{260}. Using the conditions stated in this report the identical aminoacyl acceptance was obtained. Authentic s^2U*, 2-thio-5-(*N*-methylaminomethyl)-uridine was a gift from Dr. John Carbon.

Methods. All reactions were performed using a Metrohm pH-Stat loaded with 0.1 N NaOH in a 1-ml microcell thermostated at 25°. Chromatography was performed on DEAE-cellulose, and the effluents were passed through a uv monitor operating at 254 or 265 nm and/or anthracene flow cell in a Nuclear-Chicago radioactivity counter.

Preparation of [7-^{14}C]Benzoic Anhydride. To 4.3 mg of [7-^{14}C]benzoic acid was added 20 mg of benzoic anhydride in 5 ml of anhydrous hexane. The exchange was allowed to proceed for 2 weeks at 25°. The resulting solution was used to acylate guanosine 5′-phosphate as previously reported (Cedergren *et al.*, 1971). The specific activity of the benzoic anhydride was thereby estimated from the specific activity calculation of the *O*-benzoylguanosine 5′-phosphate, the product of the reaction.

The result of this exchange was a hexane stock solution containing [7-^{14}C]benzoic anhydride with a specific activity of 1.83 Ci/mol. In an analogous procedure, [^{14}C]acetylimidazole or [^{14}C]benzoylimidazole was prepared respectively from [^{14}C]acetic anhydride or [^{14}C]benzoic acid exchange with the corresponding *N*-acylimidazoles. Prior to use on tRNAs, the appropriate quantity of the hexane solution was evaporated, and the residue redissolved in 100 μl of anhydrous dimethylformamide.

Acylation of $tRNA^{Glu}$. Approximately 1 mg of $tRNA^{Glu}$ was dissolved in 1 ml of either a 0.03 M Tris-HCl buffer (pH 7.5) containing 0.01 M $MgCl_2$ or 0.02 M NaH_2PO_4 (pH 8.0) containing 0.01 M $MgCl_2$. To the microcell of a pH-Stat set to the desired pH was added 1.0–1.5 mg of solid phthalic anhydride. The reaction started immediately upon the addition of tRNA solution. In the case of *N*-acetylimidazole, *N*-benzoylimidazole, and benzoic anhydride, 1.0–1.5 mg of either was dissolved in 100 μl of dimethylformamide which was then added to the microcell containing the tRNA solution. The pH-Stat was started and the reaction was judged complete when the addition of 0.1 N NaOH had ceased. The time of reaction was from 2 to 3 hr. The reaction mixture was applied to a Sephadex G-25 (coarse) column (1.2 × 67 cm) which was eluted with a 0.02 M Tris-HCl (pH 7.0) buffer. The fractions eluting in the void volume containing the tRNA were

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[1] Abbreviations used are: BD-cellulose, benzoylated diethylaminoethylcellulose; s^2U*, 2-thio-5-(*N*-methylaminomethyl)uridine; $tRNA^{Glu}$, glutamic acid tRNA.

concentrated to 1 ml in a Diaflo cell equipped with a UM-10 membrane. This solution was dialyzed against 0.02 M Tris-HCl (pH 7.0) for 2 hr. Three volumes of ethanol were added to the solution and the resulting precipitate was filtered and dried.

*Benzoylation of s^2U**. To a 60-μg sample of s^2U* solubilized in 1 ml of 0.03 M Tris-HCl (pH 8.0) containing 0.01 M $MgCl_2$ in a pH-Stat was added 250 μg of [^{14}C]benzoic anhydride dissolved in 100 μl of dimethylformamide. After 2 hr at 25° no further NaOH was added and the reaction was terminated. The resulting solution was acidified to pH 3.2 with 0.5 N formic acid and extracted three times with four volumes of benzene. The aqueous layer was adjusted to pH 8.0 with 1 N NaOH, and the solution concentrated to 40 μl by a stream of nitrogen.

Determination of Kinetics of Acylation. For some purposes it was necessary to follow the incorporation of radioactive label into the tRNA^{Glu}. Using identical reaction conditions as those described above, aliquots of 100 μl of the reaction solution were taken at appropriate times and deposited on a glass fiber filter disk. These disks were plunged into 10% cold trichloroacetic acid and washed with 5% trichloroacetic acid, three times with Hokin's reagent (1.6 ml of 10 N NaOH and 125 ml of glacial acetic acid made to 2 l. with 95% ethanol) and finally with ether. These disks were then dried and transferred to scintillation vials containing a toluene-based scintillation cocktail. The radioactivity was determined in a Packard Tri-Carb counter with an efficiency of 50%.

Aminoacylation of tRNA^{Glu}. The aminoacylation of tRNA^{Glu} was performed as previously described using the aminoacyl synthetases prepared from *E. coli* strain B (Beauchemin *et al.*, 1973). The incubation mixture contained in 250 μl: 0.1 A_{260} of tRNA, 0.125 mg of the aminoacyl-tRNA synthetase preparation, 25 μmol of Tris-HCl at pH 7.5, 30 μmol of $MgCl_2$, 2.5 μmol of ATP and CTP, 0.5 μmol of EDTA, and 0.125 μCi of L-[^{14}C]glutamic acid (50 Ci/mol). Incubations were carried out for 10 min at 37°. Samples of 100 μl were pipetted on filter disks which were washed as described in the above section.

Aminoacyl Acceptance of Benzoylated tRNA^{Glu}. A sample of tRNA^{Glu} was reacted with benzoic anhydride for 2 hr at pH 8.0 as described above. The reaction mixture was dialyzed for 2 hr against H_2O to remove reagents and salts. The tRNA solution was then lyophilized. The dried tRNA was dissolved in 100 μl of H_2O. One-half of the solution was deposited on a filter disk to determine the blank value. The remaining solution was analyzed in the following reagent mixture: 0.1 A_{260} of benzoylated tRNA^{Glu}, 0.125 mg of the crude *E. coli* aminoacyl synthetases, 25 μmol of Tris-HCl at pH 7.5, 30 μmol of $MgCl_2$, 2.5 μmol of ATP and CTP, 0.5 μmol of EDTA, and 0.125 μCi of L-[^{14}C]glutamic acid (50 Ci/mol) in a total volume of 250 μl. The incubation was for 10 min at 37°. Samples of 100 μl were deposited on filter disks which were washed as described above.

DEAE-cellulose Chromatography. A DEAE-cellulose column (0.4 × 100 cm) was washed with 1.0 M ammonium carbonate and then equilibrated with 0.01 M $(NH_4)_2CO_3$. A concave carbonate gradient was established by filling five solvent chambers with 100 ml each of 0.01, 0.15, 0.01, 0.60, and 1.0 M ammonium carbonate, respectively. A RNA digest, prepared from 100 μg of acylated tRNA^{Glu} treated for 2 hr at 37° with 50 μg of pancreatic ribonuclease A in 0.01 M Tris-HCl buffer (pH 7.5) containing 0.001 M $MgCl_2$, was applied to the column, and the column was eluted with the carbonate gradient at the rate of 30 ml/hr.

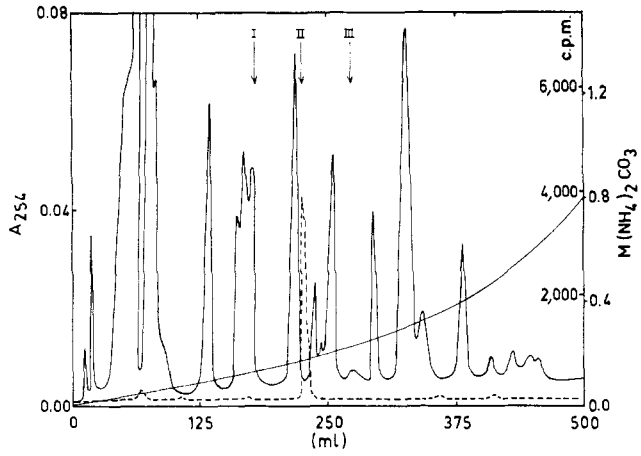


FIGURE 1: DEAE-cellulose chromatography. A digest of 100 μg of benzoylated tRNA^{Glu} was deposited on a DEAE-cellulose column (0.4 × 100 cm) and eluted with a concave ammonium carbonate gradient as described in Methods. Elution was performed at 30 ml/hr. The symbols represent the position of the radioactive peak after acylation with acetylimidazole (I), benzoic anhydride (II) and phthalic anhydride (III). A_{254}, —; radioactivity, ----.

Thin-Layer Chromatography. Samples of benzoylated tRNA^{Glu} were treated with a mixture of T_1, T_2, and pancreatic ribonucleases in a solution of 0.05 M ammonium acetate (pH 4.5). This digestion was carried on at 37° for 2 hr using a substrate/enzyme ratio of 10:1. The resulting digest was deposited as a streak on 20 × 20 cm commercial cellulose thin-layer plates and developed in ethanol–water (7:3). Radioactive compounds were detected by autoradiography. These compounds were isolated by scraping and collecting the radioactive band and finally by eluting them from the cellulose by water. After concentration this substrate was then treated for 2 hr with alkaline phosphatase (substrate/enzyme ratio of 10:1) in 0.05 M Tris-HCl (pH 8.0) containing 0.025 M $MgCl_2$.

Alternatively, benzoylated tRNA^{Glu} was treated with a mixture of alkaline phosphatase and snake venom phosphodiesterase and pancreatic ribonuclease in 0.02 M Tris-HCl (pH 7.8) containing 0.01 M $MgCl_2$. This digestion was performed at 37° for 6 hr using a substrate/enzyme ratio of 10:1. Subsequent chromatographies were performed with commercial cellulose plates in either 1-butanol–ethanol–water (50:17:35) or 1-butanol–methanol–water (50:20:50).

Results

Acylation of tRNA^{Glu}. The reaction of *E. coli* tRNA^{Glu} with various acylating reagents was first characterized by pancreatic ribonuclease digests of acylated tRNA. Figure 1 shows the chromatogram resulting from the digestion of [^{14}C]benzoylated tRNA^{Glu}. As can be noted, a single large radioactive peak (II) elutes approximately in the dinucleotide region. Arrows I and III in the chromatogram indicate the position of the major radioactive peaks after acylation with *N*-[^{14}C]acetylimidazole and [^{14}C]phthalic anhydride, respectively. Particularly in the case of *N*-acetylimidazole, other minor radioactive peaks were noted.

The acylation data for all of the acylating reagents used in this study are summarized in Table I. Using benzoic anhydride more than 1 mol of reagent is incorporated per mol of tRNA. Benzoic and phthalic anhydrides give only one major peak of radioactivity when the pancreatic RNase digest of acrylated tRNA is chromatographed. The difference in acylation ability of benzoic and phthalic anhydrides should

TABLE I: Incorporation and Distribution of Acyl Groups in $tRNA^{Glu}$

Reagent	Acyl Incorp[a] as mol of Reagent/mol of $tRNA^{Glu}$	% Radioactivity Incorporated[b]		
		Dead Vol	Mono- and Dinucleotides	Oligonucleotide
Phthalic anhydride	0.32	2	88	10
Benzoic anhydride	1.10	3	90	7
N-Benzoylimidazole	0.26	40	35	25
N-Acetylimidazole	0.20	45	45	10

[a] These values were obtained by dividing the number of moles of the reagent calculated from its specific activity by the moles of tRNA from the aminoacyl accepting activity of the sample. [b] The percentage of the total radioactivity incorporated into $tRNA^{Glu}$ as a function of the region of the pancreatic RNase chromatogram.

not be considered as definitive, since the latter experiments, under maximum yield conditions, were performed only with benzoic anhydride.

The aminoacyl acceptance assay performed on a sample of benzoylated $tRNA^{Glu}$ shows the modified $tRNA^{Glu}$ to accept 290 pmol of glutamate/A_{260}. This value is approximately 25% of the glutamate acceptance of unmodified $tRNA^{Glu}$, which assays at 1210 pmol/A_{260}.

The two imidazole derivatives are much less selective in their action, although one of the major peaks of radioactivity is analogous to the major peak obtained using the anhydrides. As Table I indicates much of the radioactivity incorporated during reaction with acylimidazoles is collected at the void volume. Since the tRNA was subjected to dialysis before enzymatic digestion to remove excess reagent it is felt that this radioactivity is the result of O-acylation of the 3′-terminal nucleoside. It may be significant to note that acylimidazoles

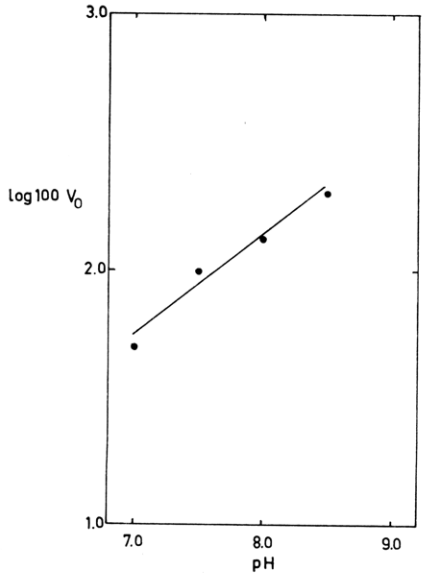


FIGURE 2: Logarithms of initial velocities. A sample of 100 μg of $tRNA^{Glu}$ was acylated by 500 μg of benzoic anhydride. Samples of 0.1 ml were taken at time intervals and acid-precipitable counts were determined.

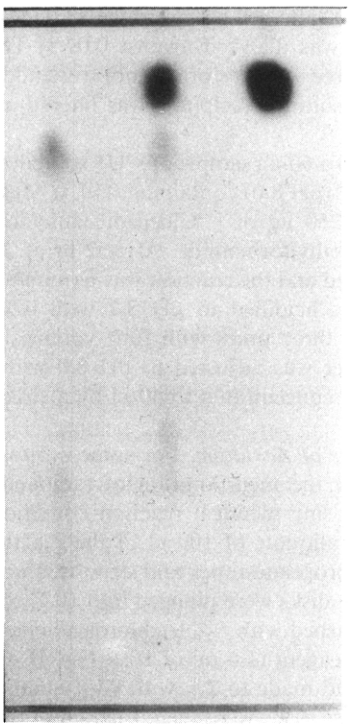


FIGURE 3: Autoradiogram of thin-layer chromatography. Samples were deposited on thin-layer cellulose sheets and chromatography was performed in 1-butanol–ethanol–water (50:17:35). Chromatograms were subjected to autoradiography. Samples are: left, benzolyated $tRNA^{Glu}$ digest; center, reaction mixture from benzoylation of s^2U^*; right, benzoic acid standard.

are known particularly for their O-acylating ability (Riordan *et al.*, 1965). Acylation in the oligonucleotide region may as well be the result of 2′-hydroxyl derivatization (Knorre *et al.*, 1965; Cedergren *et al.*, 1971).

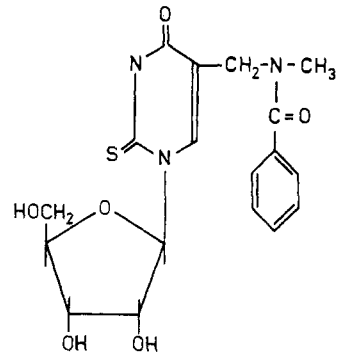
pH Dependence of the Benzoylation of $tRNA^{Glu}$. Since pancreatic ribonuclease digests indicated that acylation leads essentially to one product, it was decided to attempt a full characterization of the major product. The pH dependence of the acylation reaction of $tRNA^{Glu}$ was first examined. In these experiments [^{14}C]benzoic anhydride was the acylating reagent and the extent of acylation was inferred from the acid precipitable counts determined as described in Methods. The incorporation of radioactivity as a function of time was first used to determine the kinetics of benzoylation. From these curves the initial velocities were obtained by extrapolation. The logarithm of initial velocities is plotted in Figure 2 as a function of the pH of the reaction.

Characterization of the Product. Because the product obtained from the enzymatic digest seemed to be a small fragment (mono- or dinucleotide), the number of possible sites of acylation was very limited. Complete digests of the [^{14}C]benzoylated $tRNA^{Glu}$ were chromatographed on cellulose thin-layer plates as described in Methods. The chromatogram of a digest by T_1, T_2, and pancreatic ribonucleases followed by alkaline phosphatase is shown in Figure 3. Along with this digest is shown a chromatogram of the reaction mixture from the benzoylation of s^2U^* as well as a [^{14}C]benzoic acid standard. In another experiment the [^{14}C]benzoylated $tRNA^{Glu}$ was treated with a mixture of pancreatic ribonuclease, snake venom phosphodiesterase, and alkaline phosphatase. A summary of the R_F values of radioactive compounds from this experiment and the R_F values of the previous experiments in two solvent systems is given in Table II. The R_F value of the

TABLE II: Cellulose Thin-Layer Chromatographic Data.[a]

	Bz-s^{2}U*		Benzoate	
	I	II	I	II
T_1, T_2, A, AP	0.79	0.83	0.88	0.95
SVP, AP	0.78	0.85	0.92	0.96
Synthetic	0.79	0.83		

[a] Symbols used are: T_1, ribonuclease T_1; T_2, ribonuclease T_2; A, pancreatic ribonuclease; AP, alkaline phosphatase; SVP, snake venom diesterase; Bz-s^{2}U*, benzoyl-s^{2}U*. Solvent I, 1-butanol–ethanol–water (50:17:35); solvent II, 1-butanol–methanol–water (50:20:50).



2-thio-5-(N-methylbenzamidomethyl)uridine

FIGURE 4: Proposed product of the reaction of benzoic anhydride with tRNA^{Glu}, after digestion with various enzymes.

labelled product obtained from the different systems is identical to the product of benzoylation of s^{2}U*.

Discussion

The initial observation that tRNA^{Glu} was easily acylated was indeed surprising, since previous work with yeast tRNA^{Phe} and *E. coli* tRNA^{Glu} indicated that tRNAs are not easily acylated under the conditions used (B. Larue, J. Toupin, and R. J. Cedergren, unpublished observation). Although many of the reactions here were the result of 2-hr reaction times, more recent experiments indicate that the reaction is essentially terminated after 10–15 min.

The chromatogram resulting from the pancreatic ribonuclease digest of benzoylated tRNA^{Glu} led to the deduction that the acylation reaction site was either a mono- or dinucleotide and subsequently, to the suspicion that s^{2}U* in the "wobble" position was the site of acylation. This suspicion was verified by the comparison of the product of benzoylation of tRNA^{Glu} after digestion with a mixture of enzymes and the product of the benzoylation of an authentic s^{2}U* sample.

Further characterization of the product was done by taking advantage of the different specificities of two enzymatic systems for digesting the acylated tRNA^{Glu}. If the 2′-hydroxyl of s^{2}U* was acylated, a mixture of ribonucleases (T_1, T_2, and pancreatic) followed by alkaline phosphodiesterase would give an acylated dinucleoside monophosphate. On the other hand, snake venom phosphodiesterase with alkaline phosphatase and pancreatic ribonuclease should give the acylated nucleoside. Since the products of the two digests have identical R_F values, we conclude that acylation took place not on the 2′-hydroxyl but on the base moiety. The further observation that the initial velocities of the reaction increase with pH is suggestive of the reaction profile of an amine (Pitman *et al.*, 1972). All this information is consistent with the proposed product of the reaction shown in Figure 4, 2-thio-5-(*N*-methylbenzamidomethyl)uridine.

It is of interest to compare this product of acylation with the unidentified phenoxyacetyl derivative reported by Friedman (1972). Acid hydrolysis of this unknown led to the production of phenoxyacetic acid and a second product, presumably the nucleoside moiety. The uv spectral data of this product are very similar to those of 5-methylaminomethyluridine, the dethiolated product from acid hydrolysis of s^{2}U* (Carbon *et al.*, 1968).

The benzoylation of tRNA^{Glu} leads to the loss of approximately 75% of the glutamate accepting activity.[2] Singhal (1971) as well as Saneyoshi and Nishimura (1971) have previously shown the high sensitivity of aminoacylation ability to modification of s^{2}U*. The bisulfite modification experiments described by Singhal (1971) show that at least part of the aminoacyl-tRNA synthetase recognition site is located in the anticodon of tRNA^{Glu}. In addition, cyanogen bromide modification indicated that the integrity of at least the thio group of s^{2}U* is directly related to the recognition by the synthetase (Saneyoshi and Nishimura, 1971). Thus, although the nature of these modifications is different, results from the three different experiments implicate the tRNA^{Glu} anticodon in the process of recognition by the glutamyl-tRNA synthetase.

Finally, we feel that the acylation reaction of tRNA^{Glu} herein described may be a powerful tool in the study of the interaction of this tRNA with various subcellular components. Since the reaction seems to be independent of the acylating reagent, one can easily imagine the incorporation of various reporter molecules into this tRNA. The position of this modification (anticodon loop) suggests studies not possible with other chemical modifications.

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Comparison of Tyrosyl Transfer Ribonucleic Acid and Brome Mosaic Virus Tyrosyl Ribonucleic Acid as Amino Acid Donors in Protein Synthesis†

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ABSTRACT: Several plant viruses possess RNA which can be aminoacylated in a tRNA-like manner at the 3′ terminus; for example, the RNA components of brome mosaic virus specifically bind tyrosine. The possibility that tyrosine, bound to the viral RNA, could be donated to nascent peptides was investigated using a cell-free system from wheat, a natural host for brome mosaic virus. This system was found to be efficient at mediating the transfer of tyrosine from tyrosyl-tRNA to peptides when programmed with a viral messenger RNA, a plant RNA fraction, and the synthetic heteronucleotide poly(A,C,U). However, only a very small amount of tyrosine was transferred by this system to peptidyl material when brome mosaic virus tyrosyl-RNA was supplied as the amino acid substrate. It appears unlikely, therefore, that the ability of brome mosaic virus to bind tyrosine is directly involved in peptide chain elongation.

The RNA from plant viruses belonging to three different groups has recently been found to be capable of accepting amino acids in a tRNA-like manner, the specific amino acid bound varying from one virus group to another. Thus, the RNA from TYMV[1] (Yot *et al.*, 1970), eggplant mosaic virus, and okra mosaic virus (Pinck *et al.*, 1972) binds valine. Histidine binds to TMV RNA (Öberg and Philipson, 1972) and the RNA from BMV and cowpea chlorotic mottle virus binds tyrosine (Hall *et al.*, 1972; T. C. Hall, unpublished observations).

No explanation of the biological function of this property is available, but the conditions for esterification, the specificity for a single amino acid, the ability for elongation factor binding to acylated *vs.* the absence of binding for deacylated RNA (Litvak *et al.*, 1973), and the resistance to RNase degradation of ribosome-bound RNA (Haenni *et al.*, 1973) all suggest that a tRNA-like function exists. Valine, bound to TYMV RNA, has been shown to be donated to nascent peptidyl material in a cell-free system from *Escherichia coli*, but much less efficiently than from valyl-tRNA, and with kinetics which suggested that partial cleavage of the viral RNA preceded the amino acid transfer (Haenni *et al.*, 1973).

In this article we describe experiments conducted to determine if BMV RNA could function as a tyrosine donor in protein synthesis and to compare its efficiency with that of tyrosyl-tRNA. Three physiological possibilities were examined. One was that the tyrosine-charged BMV RNA might function as a virus-specific tRNA and preferentially insert tyrosine into viral coat, or some other, protein. An alternative possibility was that tyrosine might be inserted into plant proteins in incorrect locations, the viral RNA functioning as a missense adaptor. Another possibility was that regulatory systems might be operating which precluded tyrosine transfer from charged viral RNA when the cell-free system was programmed with either viral or plant messenger, and for this reason a third set of experiments was carried out using copoly(A,C,U) as messenger.

Methods and Materials

Preparation of Synthetase Enzyme Fraction. Aminoacyl-tRNA synthetases were extracted from maturing (11–15 mm long) seeds of French Bean (*Phaseolus vulgaris* L. *cv.* Tendergreen) plants which had been cultured in an aerated liquid nutrient medium under a controlled environmental regime in the Madison Biotron. All extraction steps were at 0–4°. Freshly excised cotyledons (50 g) were ground for 90 sec, using a VirTis 45 homogenizer, into 100 ml of extraction buffer containing 0.1 M imidazole-Cl (pH 7.3), 5 mM dithiothreitol, 1 mM GSH, 10 mM $MgCl_2$, 0.3 M KCl, 10 g of insoluble polyvinylpyrrolidone, and 2 g of Dowex 50-X8 (Na^+ form). The grinding flask stood in crushed ice and N_2 was continually flushed through from a side arm. The crude extract was squeezed through acetate taffeta cloth and then centrifuged at 10,000 rpm (15 min) in a JA 20 rotor on a Beckman J 21 centrifuge. A lipid pellicle was removed from the surface; then the light green supernatant was applied (in batches of 15 ml) to a DEAE-Sephadex A-25 column (3 × 40 cm) which had been previously equilibrated with 0.1 M imidazole-Cl (pH 7.3) containing 10 mM $MgCl_2$, 0.3 M KCl, 5 mM dithiothreitol, and 1 mM GSH. For elution the buffer was brought to 1 M

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[1] Abbreviations used are: BMV, brome mosaic virus; TMV, tobacco mosaic virus; TYMV, turnip yellow mosaic virus.