Modifications of Ribonucleic Acid by Chemical Carcinogens. I. *In Vitro* Modification of Transfer Ribonucleic Acid by *N*-Acetoxy-2-acetylaminofluorene*

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ABSTRACT: *Escherichii coli* transfer ribonucleic acid was reacted *in vitro* with the carcinogen *N*-acetoxy-2-acetylaminofluorene. Evidence is presented that covalent attachment of the acetylaminofluorene residues to transfer ribonucleic acids produces specific modifications in their amino acid acceptance capacities, codon recognition and ribosomal binding, and chromatographic behavior on DEAE-Sephadex and benzoylated DEAE-cellulose columns.

An hypothesis is presented that attributes the functional modifications in nucleic acids produced by this carcinogen to a change in nucleoside conformation from *anti* to *syn*.

Previous studies from this and other laboratories suggest that tRNA may be a critical target during chemical carcinogenesis (Axel *et al.*, 1967; Weinstein, 1968, 1969; Novelli, 1969). In addition, evidence is accumulating that the tRNA population of certain tumor cells may differ qualitatively from that of normal mammalian cells (Bergquist and Matthews, 1962; Tsutsui *et al.*, 1966; Holland *et al.*, 1967; Baliga *et al.*, 1969; Goldman *et al.*, 1969). *In vivo* modification of liver RNA has been described with the hepatic carcinogen ethionine (Farber *et al.*, 1967; Axel *et al.*, 1967; Rosen, 1968; Ortwerth and Novelli, 1969). Acetylaminofluorene (AAF)[1] is also a potent hepatic carcinogen which is known to bind to liver tRNA when administered *in vivo* (Henshaw and Hiatt, 1963; Irving *et al.*, 1967; Agarwal and Weinstein, 1970). The drug also binds *in vivo* to liver rRNA, DNA, and protein (Marroquin and Farber, 1965; Henshaw and Hiatt, 1963; Kriek, 1968; Miller and Miller, 1967; Kriek *et al.*, 1967). This carcinogen, and certain other aromatic amides, require metabolic activation as a prerequisite for combination with macromolecules (Miller, 1968; King and Phillips, 1968). The primary step in this activation is N hydroxylation (Miller, 1968). The final metabolite, or proximate carcinogen, has not been identified *in vivo*. It is thought that this may be an ester of *N-OH*-AAF since a synthetically prepared ester, *N*-acetoxy-AAF, complexes directly with RNA and DNA at neutral pH *in vitro*. Hydrolysis of these nucleic acids indicated that the major nucleoside derivative is 8-(*N*-2-fluorenylacetamido)-guanosine (Miller *et al.*, 1966; Kriek *et al.*, 1967).

In the present study we have examined the effects of *in vitro* modification of *E. coli* tRNA by *N*-acetoxy-AAF with respect to amino acid acceptance capacity, ribosomal binding, and chromatographic behavior. Evidence is presented that in this model system the carcinogen selectivity modifies the function and chromatographic behavior of specific types of tRNA. Preliminary reports of some of these results have been presented elsewhere (Fink *et al.*, 1968; Weinstein, 1969).

Materials

N-Acetoxy-AAF and [9-^{14}C]*N*-acetoxy-AAF (960 dpm/μg) were generously supplied by Dr. James Miller of the University of Wisconsin. A stock solution of 0.005 M drug in ethanol was prepared just prior to use. *E. coli* B tRNA and *E. coli* B tRNA^{Met} were prepared as previously described (Nishimura *et al.*, 1967). DEAE-Sephadex A-50 (capacity 3.5 ± 0.5 mequiv/g and particle size 40–120 μ) was purchased from Pharmacia Fine Chemicals. Benzoylated DEAE-cellulose (BD-cellulose), which had been prepared by the method of Gillam *et al.* (1967), was kindly supplied by Dr. Dieter Söll of Yale University. BD-cellulose purchased from Schwarz BioResearch gave similar results. Liquified phenol was analytical reagent grade (Mallinckrodt). Na_2ATP was purchased from P-L Biochemicals, Inc. Poly U, poly A, poly (U,G) (5:1), poly (A,G) (3:1), poly (U,A) (5:1), and poly (U,G,A) (10:3:1) were purchased from Miles Laboratories. Poly (U,C,A) (1:1:1), was synthesized as previously described by Ishikura and Nishimura (1968). Randomly labeled [^{14}C]-amino acids were purchased from Schwarz BioResearch and had the following specific activities in millicuries per millimole: tyrosine (355), valine (160), serine (112), phenylalanine (355), methionine (110), glycine (67), lysine (240), leucine (170) isoleucine (158), proline (130), arginine (150),

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[1] Abbreviations used are: AAF, *N*-2-acetylaminofluorene; *N*-acetoxy-AAF, *N*-acetoxy-2-acetylaminofluorene; AAF-tRNA, tRNA previously reacted with *N*-acetoxy-AAF; BD-cellulose, benzoylated DEAE-cellulose; A_{260} unit, an amount of material which has an absorbance of 1.0 when dissolved in 1 ml of water and measured with a 1-cm path width.

histidine (305), threonine (120), and aspartic acid (110). [^{3}H]Methionine (3 Ci/mmole) was also purchased from Schwarz BioResearch.

Methods

Spectrophotometric measurements were made on a Zeiss spectrophotometer, Model PMQ II. Absorption spectra were determined on a Cary recording spectrophotometer Model No. 14.

Reaction of tRNA with N-Acetoxy-AAF in Vitro. *N*-Acetoxy-AAF was reacted with tRNA by a modification of the procedure described by Miller *et al.* (1966). *N*-Acetoxy-AAF, at a final concentration of 0.3–30 $\times$ 10^{-3} M (see legends for concentrations used in a given experiment), was reacted with 1.3 mg/ml of *E. coli B* tRNA in 0.1 M Tris-HCl (pH 7.2)–0.005 M $MgCl_2$ and 33% ethanol at 37° under N_2 for 3.5 hr. The modified tRNA was chilled to 4° and was twice extracted with an equal volume of ether. The aqueous phase was adjusted to 0.1 M sodium acetate (pH 4.3). The tRNA was twice precipitated in ethanol at −20° and resuspended in 0.1 M Tris-HCl (pH 7.2)–0.005 M $MgCl_2$. This material is subsequently referred to as AAF-tRNA. Control *E. coli* tRNA was incubated under identical conditions in the absence of *N*-acetoxy-AAF and extracted in the same manner. Both control and AAF-tRNA stocks were then diluted to equivalent concentrations on an A_{260} basis. Assays with the orcinol reaction (Schneider, 1945) indicated that these solutions contained equivalent amounts (±5%) of RNA.

Assay of Amino Acid Acceptor Activity of tRNA. A crude mixture of *E. coli* AA-tRNA synthetases was prepared as previously described (Nishimura *et al.*, 1967). The assay system for determining the amino acid acceptor activity of tRNAs is also essentially the same as that previously described by Nishimura *et al.* (1967). The reaction mixture contained either unfractionated tRNA (0.12–1.2 A_{260} units) or 0.02–0.05 ml of the column fractions, 10 μmoles of Tris-HCl (pH 7.5), 1 μmole of magnesium acetate, 1 μmole of KCl, 0.2 μmole of ATP, 0.01–0.02 μCi of ^{14}C-labeled amino acid or 10 μCi of [^{3}H]methionine, and 0.04 ml (approximately 1.4 mg of protein) of the AA-tRNA synthetase, in a total volume of 0.1 ml. The reaction mixture was incubated at 37° for 10 min. Aliquots (0.08 ml) were then applied to Whatman No. 3MM filter paper disks (diameter 24 mm). The disks were washed in cold 5% trichloroacetic acid, ethanol–ether (1:1), and ether, then dried, and assayed for radioactivity (counting efficiency 57%) in a Packard Tri-Carb liquid scintillation counter (Model 3375).

To prepare [^{14}C]AA-tRNAs to be used in the ribosomal binding assay, the reaction system was the same as that described above, but was scaled up to a total volume of 1 ml. After charging with a [^{14}C]amino acid at 37° for 10 min, an equal volume (1 ml) of water-saturated phenol was added to the reaction system, the mixture was shaken at room temperature for 3 min, and then centrifuged for 5 min at 1500 rpm. The supernatant fluid was removed, mixed with 0.03 ml of 2 M sodium acetate buffer (pH 4.3), dialyzed against at least 300 volumes of 0.05 M sodium acetate buffer (pH 5.0)–0.002 M $MgCl_2$ at 4° overnight, then dialyzed against 300 volumes of 0.002 M $MgCl_2$ for 5 hr, and then stored at −20° until needed.

Assay for Ribosomal Binding. The ribosomal binding assay procedure described by Nirenberg and Leder (1964) was used. The reaction mixture (0.05 ml) contained 0.1 M Tris-HCl (pH 7.5), 0.05 M KCl, 1–2 A_{260} units of ribosomes, 0.02 M magnesium acetate, [^{14}C]AA-tRNA, and polynucleotides as specified in the legends. Incubation was carried out at 25° for 15 min, and samples were processed as previously described (Nirenberg and Leder, 1964).

Column Chromatography. The preparation of DEAE-Sephadex and BD-cellulose columns has been described previously (Nishimura *et al.*, 1967; Gillam *et al.*, 1967) and conditions for each chromatographic procedure are given in the legends.

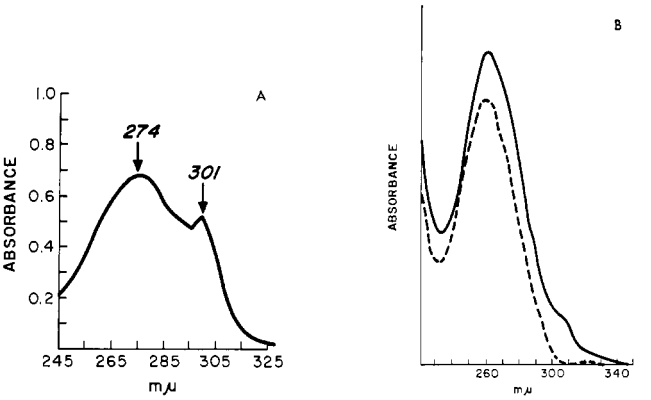

FIGURE 1: Absorption studies. (A) The absorption spectrum of *N*-acetoxy-AAF in water. (B) Absorption spectra of *E. coli* tRNA before (- - -) and after (——) reaction with 3 $\times$ 10^{-2} M *N*-acetoxy-AAF. Reaction conditions were similar to those described by Miller *et al.* (1966).

Results

Incorporation of AAF into tRNA. The absorption spectrum of *N*-acetoxy-AAF in water indicates maxima at 274 and 301 mμ (Figure 1A). The tRNA previously reacted with *N*-acetoxy-AAF revealed absorption in the 300–310-mμ region reflecting the presence of bound AAF (Figure 1B). A similar change in the absorption spectrum of nucleic acids, after reaction with *N*-acetoxy-AAF, has been described by Miller *et al.* (1966). With increasing concentrations of *N*-acetoxy-AAF in the reaction mixture there was an increase in the A_{310}:A_{260} (ratio of absorbance at 310 mμ to that at 260 mμ) of the reisolated tRNA, indicating increasing amounts of bound AAF (Figure 2). More quantitative studies employing [^{14}C]*N*-acetoxy-AAF indicated that at 1.5 $\times$ 10^{-3} M *N*-acetoxy-AAF there was approximately 1 mole of AAF bound per mole of tRNA and at 3.0 $\times$ 10^{-3} M *N*-acetoxy-AAF there were approximately 3 moles of AAF/mole of tRNA. These calculations assume a uniform distribution of AAF among the tRNAs. Subsequent studies (see below), however, indicate that this is probably not the case. Time course studies with 1.5 $\times$ 10^{-3} M *N*-acetoxy-AAF indicated that maximum binding of AAF to tRNA occurred after incubation for 3 hr. A 3.5-hr incubation period was, therefore, chosen for the preparation of all subsequent batches of AAF-tRNA.

At the present time, we do not know whether the limited substitution of tRNA with AAF residues reflects the known instability of *N*-acetoxy-AAF in aqueous media, and/or

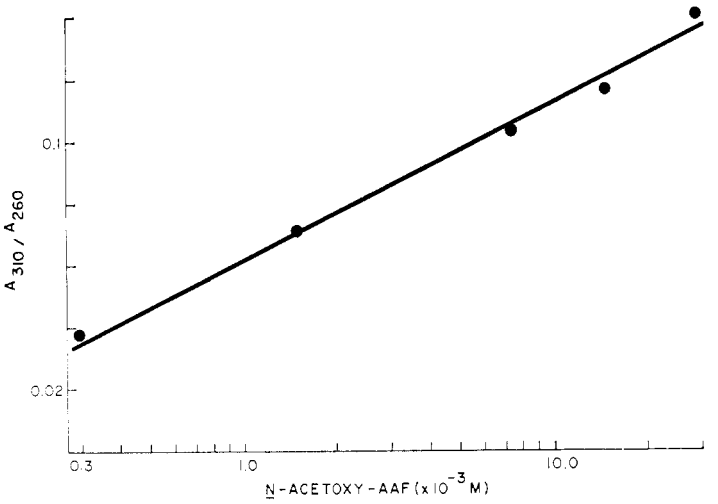

FIGURE 2: Binding of AAF to tRNA with increasing concentrations of *N*-acetoxy-AAF. The abscissa indicates the concentration of *N*-acetoxy-AAF in the reaction mixture used to modify the tRNA and the ordinate indicates the $A_{310}:A_{260}$ ratio of the modified tRNA. The reaction conditions and the procedures for reisolating the tRNAs are described under Methods and Results.

restrictions imposed by the conformation of tRNA (see Discussion).

Amino Acid Acceptance Capacity of AAF-tRNA. The first functional property of the modified tRNA which we tested was its amino acid acceptance capacity in the AA-tRNA synthetase reaction. To be certain that we were measuring *total* acceptance capacity, these studies were done in the range of limiting tRNA concentration, after establishing that the response to increasing amounts of tRNA was approximately linear in the range of 0.12–1.2 A_{260}/0.1-ml assay system. In addition, samples were incubated for 10 min at 37° since time course studies indicated that by this time the charging reaction had reached a plateau, with both control and AAF-tRNA.

TABLE I: Amino Acid Acceptance Capacity of *E. coli* tRNA after Treatment with *N*-Acetoxy-AAF (1.5×10^{-3} M).[a]

Amino Acid	Amino Acid Acceptance ($\mu\mu$moles/assay system)		
	Control tRNA	AAF-tRNA	% of Control[b]
Arginine	35	12	34
Lysine	17	9	52
Leucine	63	36	57
Isoleucine	15	9	60
Threonine	19	12	62
Glycine	17	11	62
Histidine	6	4	63
Phenylalanine	18	12	65
Proline	17	12	69
Aspartic	20	15	74
Tyrosine	21	17	80
Serine	9	8	88
Methionine	45	41	91
Valine	37	51	136

[a] All tRNAs were tested at a limiting concentration, *i.e.*, 0.61 A_{260} unit/0.1-ml assay system. The preparation of the AAF-tRNA was similar to that described in Figure 3. The preparation of *E. coli* AA-tRNA synthetases and additional details of the assay system are similar to those described by Nishimura *et al.* (1967). [b] Acceptance of AAF tRNA × 100/Acceptance of control tRNA.

The acceptance capacity for 14 amino acids, of control tRNA and tRNA previously reacted with 1.5×10^{-3} M *N*-acetoxy-AAF, is indicated in Table I. The activities for arginine and lysine tRNAs were inhibited to the greatest extent; there was a lesser inhibition or no inhibition for several other tRNAs, and there was actual stimulation (136%) of valine tRNA acceptance, when compared with equivalent amounts of control tRNA. The inhibition of tRNA-acceptance capacity for arginine and lysine, and the stimulation for valine, were reproducible with repeated batches of AAF-tRNA and were apparent when the tRNA concentration in the aminoacylation reaction was varied over a threefold range.

In contrast to the selective effects obtained with 1.5×10^{-3} M *N*-acetoxy-AAF, when the tRNA was reacted with high concentrations (10^{-2} M) of drug, there was extensive inactivation of the acceptance capacity of tRNA for all amino acids.

Ribosomal Binding of AAF-tRNA. The functional properties of AAF-tRNA with respect to ribosomal binding and codon response are indicated in Table II. Since control and AAF-tRNAs differed in their amino acid acceptance capacities (see Table I), they were compared at equivalent amounts of [^{14}C]AA-tRNA rather than equivalent amounts of total tRNA. The studies were performed with a low concentration of [^{14}C]AA-tRNA (and an excess of ribosomes and polyribonucleotides), so that the system would not be saturated with tRNAs which had not reacted with AAF or had not been aminoacylated.

When AAF-tRNA was aminoacylated with lysine both the poly A and the poly (A,G) stimulated ribosomal binding of this tRNA were less than 40% of that obtained with control Lys-tRNA. On the other hand, no significant difference between AAF and control tRNA was observed in the binding of Phe-tRNA stimulated by poly U, the binding of Met-tRNA stimulated by poly (U,G) or poly (U,G,A), and the binding of Tyr-tRNA stimulated by poly (U,A). A

TABLE II: Codon Response of AAF-tRNA.[a]

		AA-tRNA Bound to Ribosomes		
	Template	Control tRNA		AAF-tRNA
[^{14}C]Lys-tRNA	Poly A	1.58	Δμμmoles	0.61
	Poly (A,G) (3:1)	2.06		0.45
	None	0.28	μμmoles	0.21
[^{14}C]Phe-tRNA	Poly U	2.80	Δμμmoles	2.55
	None	0.14	μμmoles	0.27
[^{14}C]Met-tRNA	Poly (U,G) (5:1)	0.88	Δμμmoles	1.05
	Poly (U,G,A) (10:3:1)	0.94		0.98
	None	0.41	μμmoles	0.58
[^{14}C]Tyr-tRNA	Poly (U,A) (5:1)	0.86	Δμμmoles	0.70
	Poly (U,A,C) (1:1:1)	0.87		0.39
	None	0.18	μμmoles	0.59

[a] *E. coli* control tRNA and *E. coli* tRNA previously reacted with 1.5×10^{-3} *N*-acetoxy-AAF (AAF-tRNA) were charged with the indicated [^{14}C]amino acids and tested in the Nirenberg and Leder (1964) ribosomal binding assay. All reaction mixtures contained approximately 3 μμmoles of the [^{14}C]-AA-tRNA (expressed as [^{14}C]amino acid content), 1.0 A_{260} unit of *E. coli* ribosomes, and 0.1 A_{260} unit of the indicated polynucleotides as templates. Remaining details of the assay system are described by Nishimura and Weinstein (1969).

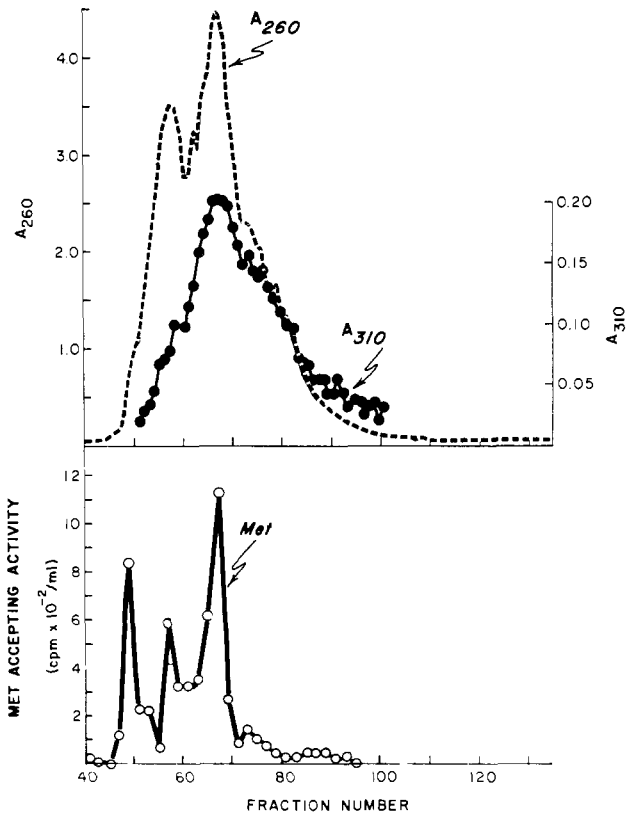

FIGURE 3: Chromatography of *E. coli* AAF-tRNA on a DEAE-Sephadex A-50 column. The column (0.5 × 100 cm) was prepared as described by Nishimura *et al.* (1967), 220 A_{260} of *E. coli* tRNA, previously reacted with 1.5×10^{-3} M *N*-acetoxy-AAF, was diluted to 10 ml with initial buffer and loaded on the column. A linear gradient elution was carried out using 250 ml of 0.525 M NaCl–0.016 M $MgCl_2$–0.02 M Tris-HCl (pH 7.5) in the reservoir and 250 ml of 0.375 M NaCl–0.0075 M $MgCl_2$–0.02 M Tris-HCl (pH 7.5) in the mixing chamber. The flow rate was 10 ml/hr. Each fraction contained 2 ml of eluate. The recovery was 85%. Aliquots (0.02 ml) of each fraction were assayed for [^{14}C]methionine acceptance using *E. coli* AA-tRNA synthetase.

significant decrease in the binding of the AAF-tRNA was observed, however, with Tyr-tRNA and poly (U,A,C) (Table II).

In these experiments, the tRNA was first modified with AAF, then enzymatically aminoacylated, and then tested for binding. This sequence was employed since it seemed unlikely that *in vivo* the drug attacks primarily those tRNAs which have already been aminoacylated. Our results, therefore, describe only the function of those tRNAs which retained their ability to be aminoacylated. Experiments in which tRNAs are first aminoacylated and then derivatized with AAF might bring out additional defects in codon recognition. Other codons and tRNAs also remain to be tested.

Chromatographic Behavior of AAF-tRNA. When AAF-tRNA was chromatographed on DEAE-Sephadex there was a discordance between the 260- and 310-mμ elution profile suggesting that molecules of tRNA carrying AAF were delayed in their elution from the column (Figure 3). Previous studies by Nishimura *et al.* (1967) indicated that *E. coli* $tRNA^{Met}$ (this includes a mixture of formylmethionine and methionine tRNAs) normally elutes from this column as a single peak at the beginning of the A_{260} profile. Figure 3 indicates that following AAF modification we observed three methionine tRNA peaks. The first is eluted at a position which is identical with that of control methionine tRNA (Nishimura *et al.*, 1967). Peaks 2 and 3 are eluted later and in the region of 310-mμ absorption. These results suggested that peaks 2 and 3 might be derived from peak 1 by AAF modification, or that AAF had induced modifications of other tRNAs which permitted them to accept methionine. To distinguish these two possibilities, previously purified methionine tRNA (Nishimura *et al.*, 1967) was reacted with *N*-acetoxy-AAF and then chromatographed on DEAE-Sephadex. Again three methionine tRNA peaks were obtained (Figure 4), indicating that peaks 2 and 3 are derived from peak 1 as a result of AAF modification. Consistent with this was an increase in A_{310}:A_{260} ratio of peaks 2 and 3 when compared with peak 1.

The fact that BD-cellulose has a strong affinity for lipophilic residues (Gillam *et al.*, 1967) suggested that it might give even better resolution between AAF-tRNA and control tRNA. When control tRNA was chromatographed on BD-cellulose, 95% of the A_{260} material was eluted during the

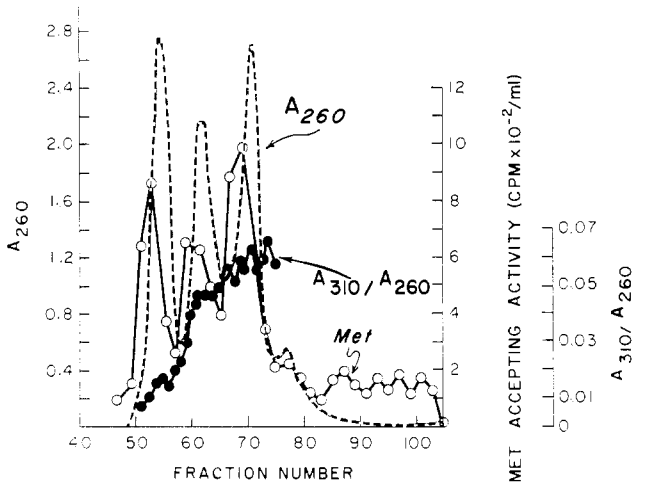

FIGURE 4: Fractionation of purified *E. coli* methionine tRNA, which had been previously reacted with 1.5×10^{-3} M *N*-acetoxy-AAF, on a DEAE-Sephadex A-50 column. The column was prepared and run as described in Figure 3. The tRNA (83 A_{260}) was applied in 3 ml of the initial buffer. The recovery was 70%, fraction aliquots of 0.02 ml were assayed for [^{14}C]methionine acceptance. The ratio $A_{310}:A_{260}$ indicates the ratio of absorbance of each fraction measured at 310 and 260 mμ and is an indication of the amount of bound AAF.

NaCl gradient and the remaining 5% was eluted with 10% ethanol; methionine tRNA eluted as a single peak in the early portion of the salt gradient (Figure 5). Figure 6 indicates the results obtained when purified methionine tRNA was reacted with [^{14}C]*N*-acetoxy-AAF, chromatographed on BD-cellulose, and the fractions assayed for methionine acceptance with [^{3}H]methionine. Three major A_{260} and methionine tRNA peaks were observed. The first peak eluted during the NaCl gradient, similar to control methionine tRNA (Figure 5), and contained no [^{14}C]AAF, the second peak eluted with 10% ethanol and contained [^{14}C]AAF, and the third peak eluted with 20% ethanol and also contained [^{14}C]AAF. The specific activities of peaks two and three indicated that both contained approximately 1 mole of bound AAF/mole of tRNA.

It appears, therefore, that under the conditions employed, approximately one-third of *E. coli* methionine tRNA does not react with *N*-acetoxy-AAF and the remaining two-thirds forms at least two derivatives. These results probably reflect the heterogeneity of our starting material (see Discussion).

Discussion

The above studies indicate that a low concentration of the carcinogen *N*-acetoxy-AAF, at neutral pH *in vitro*, produces specific modifications in tRNA. The attachment of AAF to tRNA impairs the capacity of certain tRNAs to accept amino acids and also impairs the function of specific tRNAs during ribosomal binding and codon recognition.

Previous studies indicate that the major product obtained in hydrolysates of RNA previously reacted with *N*-acetoxy-AAF is 8-(*N*-2-fluorenylacetamido)guanosine (Miller *et al.*, 1966; Kriek *et al.*, 1967). This suggests that the sensitivity of a given tRNA to modification by this drug depends on the number of guanosine residues present, the degree to which secondary and tertiary structure of the tRNA influences the reactivity of these residues, and whether they occupy sites in the tRNA which are critical for its function. The multiple reaction products obtained with methionine tRNA probably reflect the heterogeneity of our starting material. Since the completion of these studies, it has become apparent that it contains three components: $tRNA^{Met_1}$, $tRNA^{Met_2}$, and $tRNA^{fMet}$ (Seno *et al.*, 1968; Cory *et al.*, 1968). In preliminary studies we have found that $tRNA^{fMet}$ reacts with *N*-acetoxy-AAF to a much lesser extent than $tRNA^{Met_1}$ and $tRNA^{Met_2}$. This may reflect the tighter secondary structure of $tRNA^{fMet}$ (Seno *et al.*, 1968).

The fact that AAF modification impaired both the amino acid acceptance and the codon recognition of lysine tRNA suggests that the drug reacts with multiple sites in lysine tRNA or causes a gross change in the conformation of this tRNA. *E. coli* lysine tRNA is also unusually sensitive to inactivation by bromine, semicarbazide, hydroxylamine, and ultraviolet light (Yu and Zamecnik, 1963; Miura, 1967) and it is possible that the presence of a sulfur-containing nucleotide (Lipsett, 1965; Carbon and David, 1968) in *E. coli* lysine tRNA, sensitizes it to chemical modification. Possible interaction between AAF and sulfur-containing nucleotides, warrants further investigation in view of the known interaction of derivatives of AAF with the sulfur residue of methionine (Miller, 1968).

AAF modification actually enhanced the acceptance capacity of tRNA for valine. Possible explanations are that: (1) AAF modification permits other tRNAs to become "mischarged" with valine; (2) AAF residues enhance the affinity of valine tRNA for its corresponding AA-tRNA synthetase; or, (3) AAF converts a portion of valine tRNA from a denatured into a renatured conformation. The latter explanation is the most likely since renaturation of tRNA has been described with magnesium (Adams *et al.*, 1967; Sueoka *et al.*, 1966) or chloroquine (Muench, 1966). Enhancement of acceptance activity of certain tRNAs has also been described as a consequence of 5-fluorouracil incorporation (Giege *et al.*, 1969; Kaiser, 1969) or as a result of *in vitro* methylation with dimethyl sulfate (Pellinger *et al.*, 1969).

How might the presence of an AAF residue on a tRNA molecule alter its functional properties? The attachment of AAF to the 8 position of guanosine does not directly interfere with hydrogen bonding. In contrast to the effects of alkylation of N-7 of guanosine (Brookes and Lawley, 1964) AAF modification does not greatly increase acidic ionization of N-1 or cause depurination due to secondary cleavage of the glycoside bond (Kriek *et al.*, 1967). Recent studies by A. N. Michelson (personal communication) indicate that the presence of a bromine residue on the 8 position of guanosine restricts, *via* steric hindrance, rotation at the glycoside bond, thus converting the nucleoside from the "*anti*" conformation, that found in double-stranded helices with Watson–Crick geometry (Donohue and Trueblood, 1960; Ward and Reich, 1968) to the "*syn*" conformation. Presence of the bulky AAF residue on the 8 position of guanosine would produce a similar conformational change at the glycoside bond and the construction of a Corey, Pauling, and Koltun (Koltun, 1965) space-filling model indicates that this is the case (Weinstein, 1969). The presence of a single AAF residue might,

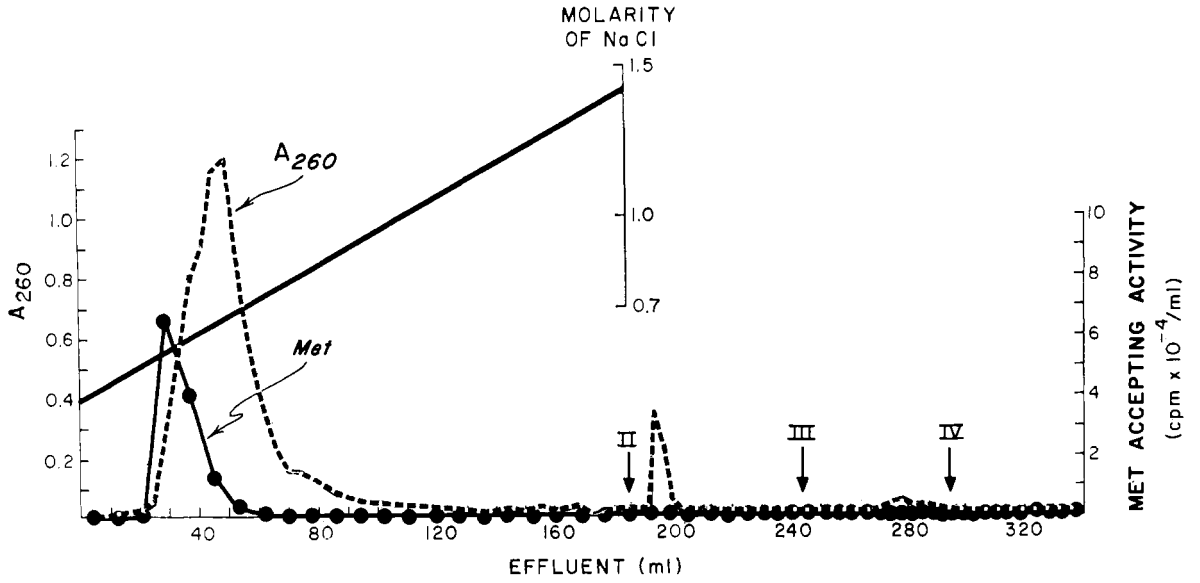

FIGURE 5: Chromatography of unfractionated *E. coli* tRNA on a BD-cellulose column (Gillam *et al.*, 1967) at 4°. The column (29 × 0.5 cm) was equilibrated with 0.4 M NaCl, 0.05 sodium acetate (pH 5.0), and 37 A_{260} of control tRNA was applied. The elution sequence was: (I) a linear gradient of 100 ml of 0.4 M NaCl–0.05 M sodium acetate (pH 5.0) in the mixing chamber and 100 ml of 1.5 M NaCl in 0.05 M sodium acetate (pH 5.0) in the reservoir; (II) 75 ml of the latter buffer but containing 10% ethanol; (III) 75 ml of the latter but containing 15% ethanol; and (IV) 75 ml of the latter containing 20% ethanol. The flow rate was 0.7 ml/min. Aliquots of 0.5 ml were assayed for [^{3}H]methionine acceptance.

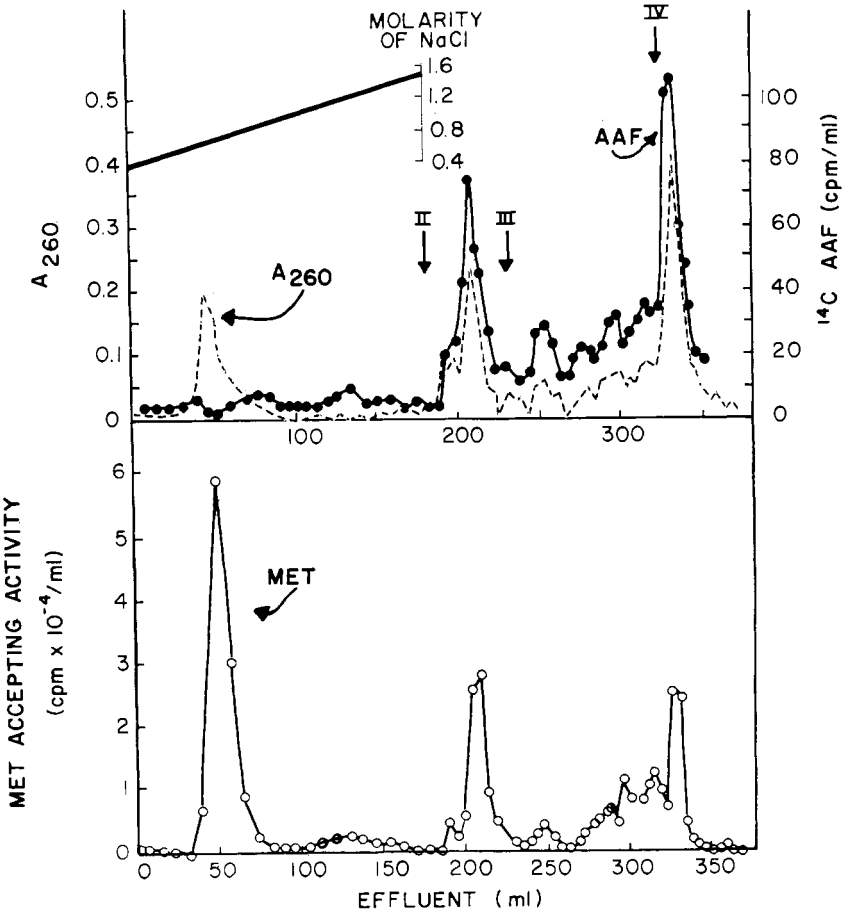

FIGURE 6: Chromatography of purified *E. coli* methionine tRNA (16 A_{260}), which had been previously reacted with 1.5×10^{-3} M [^{14}C]*N*-acetoxy-AAF, on a BD-cellulose column. The elution scheme was similar to that described in Figure 5. The A_{260} recovery was 90%; 2 ml of each fraction was counted directly in Bray's solution to determine the distribution of [^{14}C]AAF and 0.05 ml was assayed for [^{3}H]methionine-accepting activity. Increasing concentrations of NaCl and the presence of ethanol in column fractions inhibits, somewhat, the aminoacylation reaction. Therefore, methionine-accepting activity is not completely quantitative.

therefore, introduce a gross change in the conformation of that region of the tRNA, thereby altering interaction with specific activating enzymes, codons, or ribosomes. A similar disturbance in the conformation of DNA might also account for previously described effects of AAF on certain functional properties of DNA (Troll *et al.*, 1968; Maher *et al.*, 1968). This hypothesis predicts that guanosine residues contained in double-stranded regions of nucleic acids (which are in the *anti* conformation) will be less accessible to reaction with the drug than those present in single-stranded regions, since in the former configuration the 8 position of guanosine is relatively inaccessible to attack by a bulky residue. Studies designed to test this hypothesis are in progress.

The high-resolving power of the BD-cellulose column suggests that it can be used as a simple procedure for isolating and characterizing the small amounts of AAF-tRNA formed *in vivo* in liver when rats are fed the carcinogen. A related paper from this laboratory indicates that this is indeed the case (Agarwal and Weinstein, 1970). These studies may shed further light on the possible role of modifications of tRNA in tumor formation.

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