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Modification of Ribonucleic Acid by Vitamin B_6. 1. Specific Interaction of Pyridoxal 5′-Phosphate with Transfer Ribonucleic Acid†

Levy Kopelovich* and Gloria Wolfe

ABSTRACT: Whole tRNA preparation obtained from a human cell line (HT-29) of colon carcinoma and purified specific *Escherichia coli* tRNA were reacted with pyridoxal 5′-phosphate, reduced by sodium borohydride and digested with RNase A and snake venom phosphodiesterase. Two-dimensional chromatography of the pyridoxal 5′-phosphate treated tRNA digest showed that pyridoxal 5′-phosphate binds specifically to GMP, presumably in the form of a Schiff base with the exocyclic amino group of the purine. The reaction of pyridoxal 5′-phosphate with whole tRNA was competitively inhibited by *N*-acetoxy-2-acetylaminofluorene. This suggests that binding occurred primarily to the G_{20} base residue at the unpaired region of the dihydrouridine loop (Fujimura et al., 1972). The modification of tRNA by pyridoxal 5′-phosphate resulted in the inhibition, to varying extent (10–80%), of amino acid acceptance in the aminoacyl-tRNA synthetase reaction. Defects in codon recognition by pyridoxal 5′-phosphate modified amino acid acylated tRNAs in the presence of the corresponding guanine-containing polynucleotide triplets were observed by the ribosomal binding assay.

The extensive modification of tRNA is probably concerned primarily with its function in translation (Miller, 1970; Chambers, 1971; Cramer, 1971; Quigley et al., 1975; Kim, 1976; Sussman and Kim, 1976). It has also been suggested that tRNA may present a critical target causal of cancer development (Farber, 1968; Miller, 1970; Weinstein and Grunberger, 1971, 1974). Although knowledge of the primary and secondary structures of tRNA and its role in protein synthesis is rather extensive (Chambers, 1971; Cramer, 1971; Quigley et al., 1975; Kim, 1976), interaction of higher orders of the tRNA structure in respect of specific biological processes remains to be elucidated. Chemical modifications, those by physiologic compounds in particular, could provide insight into the structural and functional requirements of tRNA.

This report concerns the interaction of vitamin B_6, in the form of pyridoxal 5′-phosphate,[1] with tRNA, a nucleic acid which presents a secondary structure of distinctive open loops and base paired regions. It shows that PLP binds specifically to guanine at position 20 of the dihydrouridine loop and that ligation may involve a conformational change of the tRNA molecule, resulting in the inhibition of amino acid acceptance and ribosomal binding. The results suggest that translation may be modulated, in part, by interaction of PLP with tRNA. A preliminary report of some of these findings has appeared elsewhere (Kopelovich et al., 1976a,b).

Materials and Methods

Isolation of Whole tRNA from a Human Cell Line of Colon Carcinoma. Whole tRNA was obtained from a human cell line of colon carcinoma (HT-29) propagated in this laboratory and purified specific *E. coli* tRNA from Boehringer. Whole tRNA was extracted from cells essentially as described by Fink et al. (1970). Cells were homogenized in 0.01 M Tris-HCl, pH 7.0, 0.2% bentonite, 0.3 M sucrose, and water saturated phenol containing 0.1% 8-hydroxyquinoline. Following centrifugation, 13 000*g* for 25 min at 4 °C, the aqueous layer was reextracted three times with water-saturated phenol containing 0.1% sodium dodecyl sulfate and 0.1% 8-hydroxyquinoline. This was followed by precipitation with 2 volumes of 95% ethanol and 0.1 volume of 2 M sodium acetate, pH 5.0, at −20 °C, solubilization in 1 M NaCl, and an additional precipitation with 95% ethanol at −20 °C. To remove polysaccharides and other low molecular weight contaminants, the tRNA was further purified by DEAE-cellulose chromatography. A DEAE-cellulose column (1.0 × 10 cm) was equilibrated with 0.1 M Tris-HCl (pH 7.2)–0.01 M $MgCl_2$–0.2 M NaCl. A sample of tRNA (about 10 mg) was applied to the column and the column washed three times with the equilibrating buffer. The tRNA was then eluted with 0.1 M Tris-HCl (pH 7.2)–0.8 M NaCl–0.001 M $MgCl_2$ and recovered by ethanol precipitation at −20 °C. The tRNA was resuspended in distilled water containing 0.01 M $MgCl_2$. Stripping of the isolated tRNA (deaminoacylation) was carried out under mild conditions in

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[1] Abbreviations used: *N*-acetoxy-AAF, *N*-acetoxy-2-acetylaminofluorene; PLP, pyridoxal 5′-phosphate; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; UV, ultraviolet.

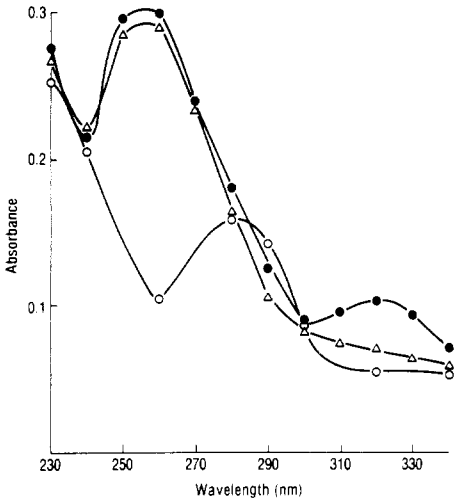

FIGURE 1: Spectral absorption studies. Whole tRNA, about 5 A_{260} units, was reacted with 0.001 M PLP in 0.04 M Tris-HCl (pH 8.0)–0.005 M $MgCl_2$ for 30 min at 37 °C. The reacted tRNA was reduced by successive additions (1 mg × 2) of solid $NaBH_4$ at pH 8.0. These were dialyzed extensively against 0.04 M Tris-HCl, pH 8.0, to remove unreacted PLP and excess $NaBH_4$. The absorption spectrum of the tRNA in Tris-HCl, pH 8.0, was determined in a Cary recording spectrophotometer Model No. 14: (△) $NaBH_4$-treated tRNA; (●) $NaBH_4$-PLP-treated tRNA; (○) free PLP.

0.5 M Tris-HCl, pH 8.8, at 37 °C for 60 min and ethanol precipitation as described by Von Ehrenstein (1967).

Reaction of PLP with tRNA and Identification of the Modified Base Residue. Whole tRNA and purified specific *E. coli* tRNA were reacted with 0.001 M PLP in the presence of 0.04 M Tris-HCl (pH 8.0)–0.005 M $MgCl_2$ for 30 min at 37 °C. Following the incubation period, samples were cooled in ice and reduced by successive additions (1 mg each × 2) of solid $NaBH_4$ at pH 8.0 (Nisselbaum and Kopelovich, 1975). These were dialyzed extensively against three changes of 0.04 M Tris-HCl, pH 8.0, to remove unreacted PLP and excess $NaBH_4$.

Five A_{260} units of the PLP-modified tRNA samples was incubated with 5 μg of RNase A (Worthington) in 0.04 M Tris-HCl, pH 7.9, for 17 h at 37 °C. This was followed by digestion with 25 μg of snake venom phosphodiesterase (Worthington) in 0.1 M Tris-HCl (pH 9.0)–0.02 M $MgCl_2$ for 2 h at 25 °C. The PLP modified tRNA digest was applied on a thin-layer polyethylenimine-cellulose MN 300 plastic plate (Brinkman). Following sample application, the plates were immersed in anhydrous reagent-grade methanol for 10 min, dried, and chromatographed in a two-dimensional discontinuous gradient system that consisted of LiCl in the first dimension and formic acid in the second dimension according to Randerath and Randerath (1964). The PLP modified base residues were identified by the UV fluorescence of this ligand. Pure ribonucleoside monophosphates were reacted with PLP in the same manner and were separated from unmodified bases on a Dowex-50 H^+ column eluted with 0.01 N HCl. These modified bases were analyzed by a two-dimensional chromatography system as described above, extracted, and rechromatographed to ascertain the purity of the reacted species. The chromatographic profiles of both unmodified and modified ribonucleoside monophosphates were compared against PLP-modified tRNA digests for identification of the modified base residues.

Spectral Methods. Absorption spectra of the unmodified and the PLP-modified tRNA were determined in a Cary recording spectrophotometer Model No. 14. The PLP modified tRNAs generated an absorption maximum at 325 nm, characteristics of a Schiff base formation between PLP and a primary amino group. The amount of PLP bound was estimated from the molar extinction coefficient for such linkage, 10150 M^{-1} cm^{-1} (Fischer et al., 1963).

Determination of Site and Stoichiometry of Binding of PLP to tRNA by Kinetic Analysis. *N*-Acetoxy-9-[^{14}C]AAF was prepared by the method of Miller et al. (1961) from *N*-2-hydroxy-9-[^{14}C]AAF (ICN). About 0.6 OD_{260} unit of $tRNA^{whole}$ or $tRNA^{fMet}$ was incubated with varying concentrations of PLP and *N*-acetoxy-9-[^{14}C]AAF in 0.025 M Tris-HCl (pH 7.2)–33% ethanol. Total incubation time was 3.0 h to ensure that both ligands were in equilibrium with the tRNA (Kopelovich et al., 1971, 1976a,b; Fink et al., 1970; Fujimura et al., 1972). The reaction mixtures were then chilled to 4 °C and precipitated with 0.5 mL of 10% Cl_3CCOOH containing crude carrier tRNA and filtered through Whatman glass fiber filter GF/C, 24 mm in diameter. The filters were washed with ethanol three times, dried, and assayed for radioactivity in a Nuclear-Chicago Mark III liquid scintillation counter. The amount of tRNA bound *N*-acetoxy-9-[^{14}C]AAF was plotted as a function of PLP concentration as described by Scatchard (1949).

Assay of Amino Acid Acceptor Activity of tRNA. A crude mixture of aminoacyl-tRNA synthetases free of endogenous tRNA was prepared from rat liver according to Nishimura et al. (1967). The reaction mixture contained whole or specific tRNA (0.12–1.2 A_{260} units), 0.01 M Tris-HCl (pH 7.5), 0.01 M $MgAc_2$, 0.01 M KCl, 0.002 M ATP, 0.01–0.02 μCi of ^{14}C-labeled amino acid, and 0.04 mL (about 1.4 mg of enzyme protein) of the aminoacyl-tRNA synthetase, in a total volume of 0.1 mL (Nishimura et al., 1967). The reaction mixture was incubated at 37 °C for 10 min and then precipitated with 3 mL of 10% cold Cl_3CCOOH and filtered through a Whatman glass fiber filter GF/C, 24 mm diameter, and washed three times with 3 mL of 5% Cl_3CCOOH. The radioactivity was measured in a Nuclear-Chicago Mark III scintillation counter.

Ribosomal Binding Assay. Codon recognition of a specific tRNA was assayed by aminoacylation of whole or specific tRNA with the appropriate radioactive amino acid and measuring the binding of charged tRNAs to ribosomes in the presence of the appropriate polynucleotide triplets (Miles). Charging of the specific tRNA with the appropriate ^{14}C-labeled amino acid was carried out as described by Fink et al. (1970). The ribosomal binding assay was carried out according to Nirenberg and Leder (1964). The reaction mixture consisted of 0.1 M Tris-HCl (pH 7.5)–0.05 M KCl, 1–2 A_{260} units of *E. coli* ribosomes, 0.02 M $MgAC_2$, [^{14}C]aminoacyl-tRNA, and the synthetic codons. These assay mixtures were incubated at 25 °C for 15 min and samples processed as described by Nirenberg and Leder (1964).

Results

Reaction of PLP with tRNA. The absorption spectra of PLP in solution at pH 8.0 indicate a maximum at 280 nm (Figure 1). The absorption spectrum of the PLP-modified tRNA shows a maximum at 325 nm generally observed upon a Schiff base formation between PLP and a primary amino group following reduction with $NaBH_4$ (Figure 1). The binding of PLP occurs, presumably, through an aldimine linkage of the 4′ aldehyde moiety of PLP with the primary exocyclic amino group of the nucleotide(s) within the tRNA molecule. The amount of adduct formed was estimated at about 3.9 mol of PLP per 1 mol of tRNA. This result was calculated on the assumption that 1 A_{260} unit of tRNA is equal to 1.66 nmol of tRNA and a value

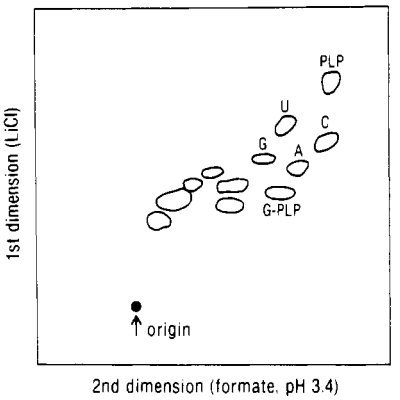

FIGURE 2: Two-dimensional chromatography of PLP modified tRNA digest on thin-layer polyethylenimine-cellulose. Five A_{260} units of tRNA modified with PLP was incubated with 5 μg of RNase A (Worthington) in 0.04 M Tris-HCl, pH 7.9 for 17 h at 37 °C. This was followed by incubation with 25 μg of snake venom phosphodiesterase (Worthington) in 0.1 M Tris-HCl (pH 9.0)-0.02 M $MgCl_2$ for 2 h at 25 °C. The digest was applied on thin-layer polyethylenimine-cellulose MN 300 plastic plates. Plates were chromatographed in a two-dimensional discontinuous gradient system that consisted of 0.2, 1.0, and 1.6 M LiCl in the first dimension, and 0.5, 2.0, and 4.0 M formic acid, pH 3.4, in the second dimension. A, AMP; C, CMP; G, GMP; U, UMP; G-PLP, GMP-PLP adduct.

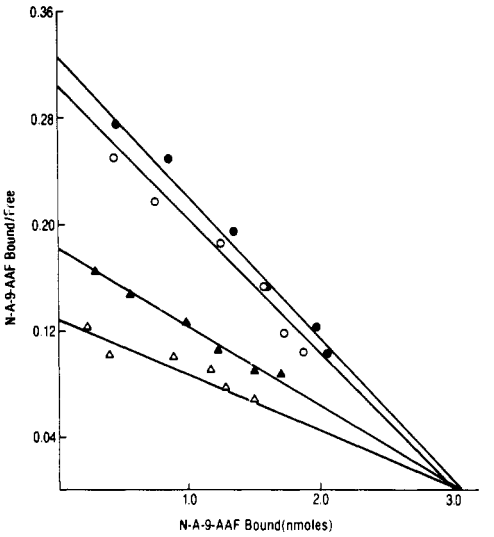

FIGURE 3: Scatchard analysis on the binding of AAF and PLP to tRNA. The reaction mixture contained in a final volume of 0.1 mL: tRNA (0.6 A_{260}); 0.025 M Tris-HCl, pH 7.2; 33% ethanol; PLP and [^{14}C]-*N*-acetoxy-AAF (specific activity = 0.502 μCi/μmol) at the amounts indicated. Samples were incubated for 3 h at 37 °C, then chilled to 4 °C, and precipitated with 0.5 mL of 10% Cl_3CCOOH and filtered through Whatman glass filters GF/C (24 mm in diameter). The filters were washed with ethanol three times, dried, and assayed for radioactivity. The concentrations of *N*-acetoxy-AAF were: 0.208 mM; 0.416 mM; 0.832 mM; 1.248 mM; 1.664 mM; and 2.080 mM. Those of PLP were: (●) zero; (○) 0.5 mM; (▲) 1.0 mM; (△) 1.87 mM.

of 0.02 ΔA_{325} between the unreacted and PLP-reacted tRNA (Figure 1).

The Base Specificity of PLP Binding to tRNA. The tRNA was reacted with excess PLP at pH 8.0, reduced with $NaBH_4$, and digested with ribonuclease A and snake venom phosphodiesterase. Two-dimensional chromatography of the PLP-DNA digest on thin-layer polyethylenimine-cellulose (Figure 2) showed that PLP binds to GMP but not to AMP, CMP, UMP, or any other base residues present in a whole tRNA digest. Binding of PLP to other species of RNA from various sources was identical with that found in tRNA. Identification of the modified base(s) was carried out by comparing elution profiles of the tRNA digest with those of pure ribonucleoside monophosphates treated in the same manner. Spots other than those identified (Figure 2) constituted about 5% of the total UV absorbing material present on the chromatogram. These consisted presumably of single methylated bases primarily, as was deduced from their slower mobility in the direction of formate, pH 3.4, and on a Dowex -50H$^+$ column. Further, the lack of PLP binding in this region and elution profiles apparently suggest the absence of undegraded oligonucleotides. These results indicate that modification of the tRNA by PLP did not change the specificity of enzymes degrading tRNA and that a specific PLP-guanine adduct was formed.

Site Localization and Stoichiometry of PLP Binding to tRNA. Figure 3 shows a Scatchard plot of *N*-acetoxy-9-[^{14}C]AAF binding to whole tRNA in the presence of varying concentrations of PLP. This experiment was based on the observation by Fujimura et al. (1972), who have demonstrated the specific binding of *N*-acetoxy-AAF primarily to the unpaired G_{20} base residue at the dihydrouridine loop of $tRNA^{fMet}$. It should be noted that AAF binds to the 8th position (Miller et al., 1966; Kriek et al., 1967) and possibly to the 2nd position of guanine as well (Kriek and Reitsema, 1974), while PLP binds to the 2nd position of guanine only (Kopelovich and Wolfe, 1975; Kopelovich et al., 1976a). The results in Figure 3 show that, although two distinct portions of the guanine moiety may account, in part, for the respective binding of these two ligands, our kinetic analysis cannot discriminate between these two and indicates one such primary target site only. The data further show that, under our experimental conditions, AAF and PLP each bind to tRNA at a ratio of about 3 mol of ligand per 1 mol of tRNA. This result can be calculated from the intercept B_0 on the horizontal axis (Figure 3), where B_0 denotes the maximal number of sites available and the amount of tRNA present, i.e., 0.6 A_{260} or 1.0 nmol. This is similar to the results obtained by spectral analysis (Figure 1). Thus, PLP competes with AAF for the G_{20} base residue at the dihydrouridine loop and possibly two additional G residues as well. In this respect, Fujimura et al. (1972) have suggested that AAF is binding specifically to one or at most a few guanine bases in $tRNA^{fMet}$.

Amino Acid Acceptance Capacity and Codon Recognition of PLP-tRNA. To assess the functional significance of a PLP-modified tRNA on the translational process, amino acid acceptance and ribosomal binding capacities of the PLP-modified tRNAs were determined. Experiments on the effects of PLP-modified whole tRNA on amino acid acceptance capacity with crude rat liver aminoacyl-tRNA synthetase were carried out in the range of limiting tRNA concentration to ascertain that total acceptance capacity was measured (Fink et al., 1970). Treatment of control tRNA with $NaBH_4$ gave results identical with those of untreated tRNA. Table I shows that modification of whole tRNA by PLP resulted in the inhibition, to varying extent, of amino acid acceptance in the aminoacyl-tRNA synthetase assay (about 10-80%); that of lysine tRNA was essentially complete. On the other hand, the activity of glycine, histidine, and leucine-tRNAs was slightly stimulated. In experiments where purified specific *E. coli* tRNAs were used, a similar pattern was obtained (Table II). It is of interest that the results with PLP-modified tRNA are essentially similar to those reported by Fink et al. (1970) with AAF-modified tRNA, although we could not demonstrate the marked stimulation by $AAF\text{-}tRNA^{Val}$ reported in their study. Litt (1971) has demonstrated that partial Kethoxalation caused approximately 50% loss in chargeability of $tRNA^{Val}$

TABLE I: Effect of PLP Modified Whole tRNA on Amino Acid Acceptance.[a]

Amino acid	Amino acid acceptance activity: Plus $NaBH_4$ no PLP[b]	Plus $NaBH_4$ plus PLP[b]	PLP treated as % of control (%)
[^{14}C]Arg	0.52	0.39	75.0
[^{14}C]Gly	3.43	3.66	106.7
[^{14}C]His	3.02	3.16	104.6
[^{14}C]Ile	6.14	5.61	91.3
[^{14}C]Leu	3.29	3.55	107.9
[^{14}C]Lys	1.34	0.24	17.9
[^{3}H]Met	0.64	0.42	65.6
[^{14}C]Phe	2.66	1.08	40.6
[^{14}C]Pro	1.97	1.87	94.9
[^{14}C]Ser	4.68	3.84	82.0
[^{14}C]Thr	5.68	5.17	91.3
[^{14}C]Tyr	2.55	2.04	80.0
[^{14}C]Val	4.45	1.46	32.8

[a] The tRNA represent unfractionated whole tRNA isolated from a human cell line of colon carcinoma. Assay was conducted in the presence of crude rat liver aminoacyl-tRNA synthetase free of endogenous tRNA as follows. The reaction system contained in a total volume of 0.1 mL: 0.1 M Tris-HCl, pH 7.5, 0.01 M magnesium acetate, 0.01 M KCl, 0.002 M ATP, 0.2 A_{260} of tRNA (limiting concentration), and 1.4 mg of aminoacyl-tRNA synthetase. The specific activity of ^{14}C-labeled amino acids and [^{3}H]methionine was about 0.31 and 3.4 Ci/mmol, respectively. The reaction mixture was incubated at 37 °C for 10 min and the radioactivity present on membrane filters (GF/C Whatman) measured. [b] In pmol/assay system.

TABLE II: Effect of PLP Modified Specific tRNA on Amino Acid Acceptance.[a]

Amino acid	Specific tRNA	Amino acid acceptance activity: Plus $NaBH_4$ no PLP[b]	Plus $NaBH_4$ plus PLP[b]	PLP treated as % of control (%)
[^{14}C]Lys	$tRNA^{Lys}$	13.39	4.95	36.96
[^{3}H]Met	$tRNA^{Met}$	0.79	0.58	72.84
[^{14}C]Val	$tRNA^{Val}$	16.58	3.82	23.03

[a] Assay was conducted in the presence of crude rat liver aminoacyl-tRNA synthetase free of endogenous tRNA. All tRNAs were tested at 0.05 A_{260} unit/0.1 mL reaction system. For assay conditions, see Table I. [b] In pmol/assay system.

assayed with phenylalanine, but not with valine.

Codon recognition of the PLP modified whole tRNA and specific *E. coli* tRNA was determined by the ribosomal binding assay (Tables III and IV). The experiments were conducted at equivalent amounts of [^{14}C]AA-tRNA since the PLP-modified tRNA showed a decreased capacity of amino acid acceptance (Tables I and II). The charged tRNA was used at low concentration in the presence of excess ribosomes and polynucleotide triplets so that the assay system would not be saturated with tRNAs which had not reacted with PLP or had not been aminoacylated (Fink et al., 1970).

In the absence of added codon, $tRNA^{whole}$, specifically acylated with lysine, methionine, or valine, was not affected by PLP modification, as determined by ribosomal binding. In the presence of the corresponding codons, there was a marked stimulation in ribosomal binding by control AA-$tRNAs^{whole}$, while addition of the PLP modified AA-$tRNAs^{whole}$ resulted in a considerable inhibition (Table III). It is of interest that in experiments where purified specific *E. coli* $tRNA^{Lys}$

TABLE III: Effect of PLP-Modified Charged Whole tRNA on Codon Recognition.[a]

Polynucleotide triplets	Charged whole tRNA	Ribosomal binding: Plus $NaBH_4$ no PLP[b]	Plus $NaBH_4$ plus PLP[b]	PLP treated as % of control (%)
None	[^{14}C]Lysyl-$tRNA^{whole}$	0.560	0.561	100
AAG		1.670	0.100	6
None	[^{3}H]Methionyl-$tRNA^{whole}$	0.051	0.050	100
AUG		0.080	0.020	25
None	[^{14}C]Valyl-$tRNA^{whole}$	0.370	0.370	100
GUU		1.520	0.090	6

[a] $tRNA^{whole}$ control and $tRNA^{whole}$ previously reacted with PLP were charged with radioactive amino acids as indicated and tested for ribosomal binding (Nirenberg and Leder, 1964). All reaction mixtures contained approximately 5 pmol of the labeled AA-tRNA, 2.0 A_{260} units of *E. coli* ribosomes, and 0.1 A_{260} unit of the corresponding polynucleotide triplets as codons. The specific activity of [^{14}C]valine-$tRNA^{whole}$, [^{14}C]lysine-$tRNA^{whole}$, and [^{3}H]methionine-$tRNA^{whole}$ was 22.5, 18.9, and 12.7 pmol/1 A_{260}, respectively. [b] In pmol/assay system.

TABLE IV: Effect of PLP-Modified Charged Specific-tRNA on Codon Recognition.[a]

Polynucleotide triplets	Charged specific tRNA	Ribosomal binding: Plus $NaBH_4$ no PLP[b]	+Plus $NaBH_4$ plus PLP[b]	PLP treated as % of control (%)
None	[^{14}C]Lysyl-$tRNA^{Lys}$	0.334	0.089	26.6
AAG		0.654	0.092	14.0
None	[^{3}H]Methionyl-$tRNA^{Met}$	0.022	0.0176	20.0
AUG		0.042	0.020	52.5
None	[^{14}C]Valyl-$tRNA^{Val}$	0.487	0.139	28.6
GUU		0.890	0.134	15.0

[a] See Table III and Materials and Methods for experimental details. All reaction mixtures contained approximately 70 pmol of the labeled AA-tRNA, 2.5 A_{260} units of *E. coli* ribosomes, and 0.1 A_{260} unit of the corresponding polynucleotide triplets as codons. The specific activity of [^{14}C]valine-$tRNA^{Val}$, [^{14}C]lysine-$tRNA^{Lys}$ and [^{3}H]methionine-$tRNA^{Met}$ was 448, 415, and 235 pmol/1 A_{260}, respectively. [b] In pmol/assay system.

$tRNA^{Met}$, and $tRNA^{Val}$ were used, modification by PLP of these tRNAs resulted in a strong inhibition in the absence and the presence of the corresponding codons (Table IV).

Discussion

Central to the problem of chemical carcinogenesis is the specific interaction between chemical carcinogens and cellular macromolecules. Elucidation of the structural and functional alterations that occur as a result of covalent binding of chemical carcinogens to nucleic acids would provide insight about the cellular consequences of these biochemical events. Natu-

rally occurring small molecular weight compounds, interacting with nucleic acids, may effect changes analogous to those observed with environmental-induced chemical carcinogens. Alternatively, the modulation by these physiologic compounds of chemical carcinogens' binding to nucleic acids could modify oncogenesis. We have previously shown that vitamin B_6, in the form of pyridoxal 5'-phosphate, reacts in a specific manner with *E. coli* DNA and isolated chromatin and that it might affect DNA metabolism (Kopelovich and Wolfe, 1975a,b). Further, incubation of intact HT-29 cells with pyridoxal resulted in the binding of 76 and 63 nmol of pyridoxal per 1 A_{260} of DNA and RNA, respectively.

The tRNA molecule is of a relatively small size and presents a secondary structure of distinct single strands and paired base regions, availing itself for studies about the mode of chemical modification of nucleic acids by various ligands. The present results show that PLP reacts with tRNA and that it binds to guanine only. Furthermore, the binding of *N*-acetoxy-2-AAF to whole tRNA was competitively inhibited by PLP, suggesting that ligation of PLP occurred primarily with the G_{18}, G_{19}, and G_{20} base residues at the unpaired region of the dihydrouridine loop (Fujimura et al., 1972; Pulkrabek et al., 1974). In this respect, Litt (1969, 1971) has shown that Kethoxal and glyoxal, which modify only guanine residues in tRNA, were both bound preferentially to the G_{20} and G_{34} base residues of yeast $tRNA^{Phe}$. The latter exists as an unpaired base in the anticodon region of this tRNA. Based on results with denatured DNA, various synthetic DNA polymers, and transcriptionally active chromatin preparations, we have suggested that binding of PLP to DNA also occurs in open loop regions. However, while PLP reacts with deoxycytosine and deoxyguanine in DNA (Kopelovich and Wolfe, 1975; Kopelovich et al., 1975), its binding to RNA is with riboguanine only.

The inhibition of amino acid acceptance by PLP ligation to tRNA would suggest a steric hindrance by the PLP moiety which may result in a conformational change, preventing the appropriate orientation of the tRNA during its binding to the aminoacyl-tRNA synthetase. The slight stimulation of glycine, histidine, and leucine PLP-modified tRNA and the considerable stimulation of valine AAF-modified tRNA amino acid acceptance (Fink et al., 1970) may suggest that some ligand-induced configurations are preferred. These could possibly alter the specificity of the enzyme with respect to its specific tRNA as well. But, no apparent changes in base stacking could be detected by circular dichroism analysis. Codon recognition would also appear to have been affected by these PLP-induced conformational changes of the tRNA molecule. In both the amino acid acceptance test and the ribosomal binding assay, ligation of PLP to tRNA has functionally affected lysine to the largest extent. Similar results were found with AAF-modified tRNA and may reflect the unusual sensitivity of this tRNA (Fink et al., 1970).

How the presence of PLP on a tRNA molecule might alter its functional properties is still a matter of conjecture. Most models of the tertiary structure of tRNA place the dihydrouridine loop at the outer surface of the molecule with certain nucleotides in this loop protected from chemical modification by base pairing (Chambers, 1971; Cramer, 1971; Quigley et al., 1975; Sussman and Kim, 1976). The attachment of PLP and AAF to the 2nd position of guanine, although in an open loop, could affect hydrogen bonding necessary to assume specific conformation essential for function. Weinstein et al. (1976) have recently shown that benzo[*a*]pyrene intermediates bind to RNA and that an adduct is formed between position 10 of the active derivatives and the 2-amino group of guanine primarily. Other explanations have been proposed regarding ligation with AAF, kethoxal, and glyoxal (Grunberger et al., 1970; Weinstein and Grunberger, 1974; Litt, 1969, 1971; Levine et al., 1974; Pulkrabek et al., 1974). The preferential binding to single strand regions shared by PLP, AAF, and kethoxal may possibly reflect a preference for base residues existing in a "syn" conformation (Grunberger et al., 1970; Fujimura et al., 1972; Weinstein and Grunberger, 1974; Levine et al., 1974).

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