Isolation and Structure Determination of the Fluorescent Base from Bovine Liver Phenylalanine Transfer Ribonucleic Acid†

Steven H. Blobstein,‡ Dezider Grunberger,*,§ I. Bernard Weinstein, and Koji Nakanishi

ABSTRACT: Calf and beef liver $tRNA^{Phe}$ were obtained by fractionation of calf and beef tRNA on BD-cellulose columns and selected regions were further purified by hydroxylapatite chromatography. The $tRNA^{Phe}$ was then incubated at pH 2.9 to liberate the strongly fluorescent base of this tRNA, which was purified by silica gel thin-layer chromatography. The structure of the new base obtained was determined by comparing its absorption, fluorescent, and high-resolution mass spectra to that of the similar base of $tRNA^{Phe}_{yeast}$ (originally termed "Y" base). Results of this analysis indicate that the new base of bovine liver $tRNA^{Phe}$ ("peroxy base") differs from the one from yeast only by the presence of a unique hydroperoxide group on the β carbon of the side chain. To exclude the possibility that the peroxy base is an artifact formed during the isolation procedure, the following studies were performed: (1) incubation of synthetic *d,l* base of yeast and yeast $tRNA^{Phe}$ with a postribosomal supernatant fraction from liver; (2) addition of yeast tRNA to the liver homogenate and subsequent isolation of the $tRNA^{Phe}$; (3) isolation of calf liver $tRNA^{Phe}$ in the presence of sodium azide. The fluorescent bases isolated from these experiments remained unchanged. Therefore, the hydroperoxide moiety in the side chain of the peroxy base is not merely an artifact of isolation.

Phenylalanine tRNAs,[1] isolated from many eukaryotic cells, such as yeast (RajBhandary and Chang, 1968), wheat germ (Dudock *et al.*, 1968), beef (Yoshikami *et al.*, 1968; Zimmerman *et al.*, 1970), and rat liver (Fink *et al.*, 1968, 1971)

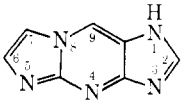
† From the Institute of Cancer Research and Departments of Medicine and Biochemistry, Columbia University College of Physicians and Surgeons, and the Department of Chemistry of Columbia University, New York, New York 10032. *Received August 22, 1972.* This research was supported in part by National Institutes of Health Grants CA-11572 and CA-02332, and by the Eli Lilly and Co. and Merck, Sharpe and Dohme Co.

‡ National Institutes of Health Predoctoral Fellow (GM 50055).

§ Scholar of Leukemia Society of America.

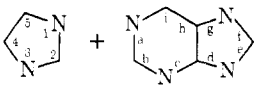
[1] Abbreviations used are: $tRNA^{Phe}$, phenylalanine specific, tRNA; $tRNA^{Phe}_{calf}$, $tRNA^{Phe}$ from calf liver; $tRNA^{Phe}_{beef}$, $tRNA^{Phe}_{rat}$, $tRNA^{Phe}_{chicken}$, and $tRNA^{Phe}_{yeast}$, are the corresponding materials from beef liver, rat liver, chicken liver, and baker's yeast, respectively; Y-imGua, the fluorescent base found in baker's yeast $tRNA^{Phe}$ and originally termed "Y"; HO_2-Y-imGua, the major fluorescent base found in $tRNA^{Phe}_{beef}$; BD-cellulose, benzoylated diethylaminoethyl-cellulose.

A Note on Nomenclature. i. The systematic nomenclature according to Dr. K. Loening, Nomenclature Director, Chemical Abstract Service, for the tricyclic nucleus of these compounds is as follows:

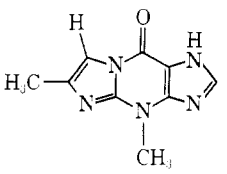


1*H*-imidazo[1,2-*a*]purine

The fusion locants are derived by fusing imidazole to purine.

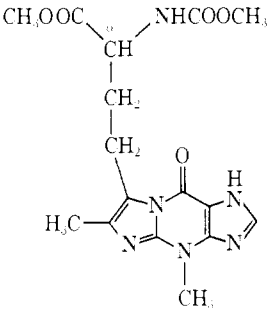


Therefore, the fluorescent bases isolated from brewer's (Kasai *et al.*, 1971) and baker's (Nakanishi *et al.*, 1970) yeast phenylalanine tRNAs are designated in structures A and B, respectively, as



structure A

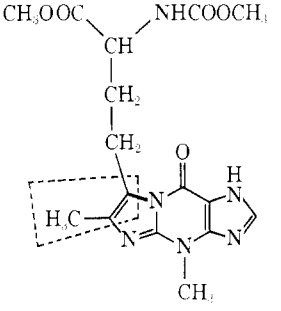
4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine



structure B (I)

α-(carboxyamino)-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]-purine-7-butyric acid dimethyl ester.

ii. The abbreviated nomenclature is based on a recommendation by Dr. W. Cohn, Director, Office of Biochemical Nomenclature, National Research Council. Guanine is taken as a root, to which a three-carbon



isopropeno group has been attached at positions 1 and N^2. This leads to the following abbreviations: structure A: imGua (for 1,N^2-isopropeno-3-methylguanine); structure B: Y-imGua (it so happens that the branched butyrate side chain looks like the letter Y, and hence the original letter can be retained as a modifier). The respective nucleosides become imG and Y-imG while the peroxy compound, II, is represented by HO_2-Y-imGua.

contain a highly modified hydrophobic fluorescent base which has been termed "Y" by its discoverers. In both yeast (RajBhandary and Chang, 1968) and wheat germ (Dudock *et al.*, 1968) tRNA^{Phe}, this base is adjacent to the 3′ end of the anticodon. Presumably, in the tRNA^{Phe} from liver it is located in the same position of the molecule (Fink *et al.*, 1971). Those from yeast and wheat germ tRNA^{Phe} possess a low order of cytokinin activity (Hecht *et al.*, 1970).

The structure of the base from the tRNA^{Phe} of baker's yeast, I, proposed by Nakanishi and coworkers (1970) and supported by Thiebe *et al.* (1971) is the most modified purine so far identified in any nucleic acid. The structure has been confirmed by synthesis, and an L configuration has been established for the single asymmetric center in the side chain (Funamizu *et al.*, 1971). The one isolated from brewer's yeast tRNA^{Phe} has recently been assigned a structure which corresponds to the I nucleus lacking the side chain (imGua) (Kasai *et al.*, 1971). Yoshikami and Keller (1971) using chemical modifications suggested that the base from wheat germ and beef liver tRNA^{Phe} probably differ from Y-imGua of yeast in the structure of the side chain, but no structural evidence was presented. Fink *et al.* (1971) found that the fluorescent and uv spectra of the material from rat liver are similar to those of yeast but the former material has a lower R_F on tlc.

This study was undertaken to elucidate the structure of the fluorescent base isolated from bovine liver tRNA^{Phe}. A preliminary report of some of our results has been published elsewhere (Nakanishi *et al.*, 1971).

Materials

Benozylated DEAE-cellulose (20–50 and 50–100 mesh) was purchased from Schwarz/Mann. Hydroxylapatite was obtained from Bio-Rad Laboratories. Silica gel plates (type G-254, E. Merck, Darmstadt, Germany) were used for thin-layer chromatography. [^{14}C]Phenylalanine was obtained from Schwarz/Mann (specific activity 455 Ci/mol) or from Nuclear Dynamics (specific activity 315 Ci/mol). Phenol was analytical reagent grade from Mallinckrodt or Schwarz/Mann, and was freshly distilled prior to use. Sodium dodecyl sulfate was purchased from Sigma Co.; 8-hydroxyquinoline was from Fisher Scientific. Bentonite (Fisher) was washed by the method of Fraenkel-Conrat *et al.* (1961). Baker's yeast tRNA was purchased from Plenum Research Scientific, Inc., Hackensack, N. J. Calf and beef liver from freshly slaughtered animals was immediately chilled and was obtained from S. Schweid, Paterson, N. J.

Methods

Spectra absorption measurements were determined on a Zeiss spectrophotometer (Model PMQ II) and a Cary recording spectrophotometer (Model 14). Fluorescence measurements were conducted on an Aminco-Bowman or a Perkin-Elmer (Model MPF-2A) spectrophotofluorometer, fitted with a xenon lamp and 1-cm light path. One A_{260} unit is that amount of material which has an absorbance of 1 when dissolved in 1 ml of water and measured at 260 nm with a 1-cm light path. The theoretical acceptance capacity of pure tRNA was assumed to be 1.67 nmol of amino acid/A_{260} unit (Hoskinson and Khorana, 1965). The mass spectra were obtained using an Associated Electronics Industry MS-9 double-focusing high-resolution mass spectrophotometer ($m/\Delta m$ = 10,000).

Preparation of tRNA. Beef and calf liver tRNA were prepared from 500-g batches of frozen liver by the method of Fink *et al.* (1971), the only modification being that 0.6 M sucrose was used in the homogenizing step to reduce DNA contamination of the tRNA. The yield for bovine liver was 0.5 mg of tRNA/g wet weight of liver.

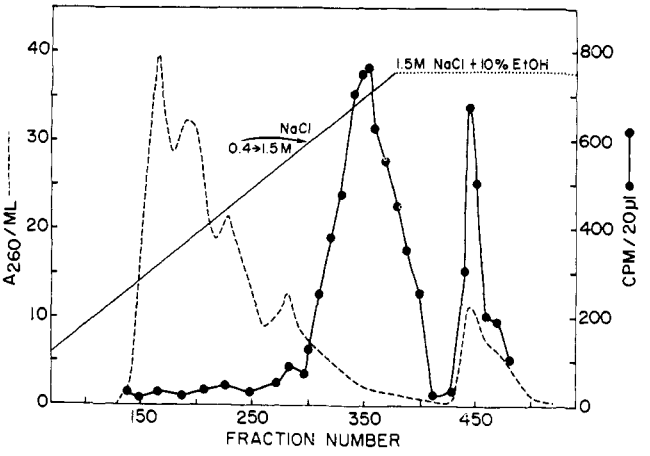


FIGURE 1: Chromatography of 6.8 g of calf liver tRNA on a BD-cellulose column. The column (5 × 85 cm) was equilibrated with 0.4 M NaCl and 0.01 M $MgCl_2$ at 4°, and the sample was applied in 500 ml of starting buffer. The tRNA was eluted with a linear gradient of 8 l. of 0.4 to 1.5 M NaCl containing 0.01 M $MgCl_2$. The remaining tRNA was eluted with 1.5 M NaCl, 0.01 M $MgCl_2$, and 10% ethanol (v/v). Fractions of 20 ml were collected at a flow rate of 1.5 ml/min and assayed for A_{260} absorbance and [^{14}C]phenylalanine acceptance capacity as described in Methods.

Assay of Amino Acid Acceptor Activity. A crude preparation of rat liver aminoacyl-tRNA synthetase was obtained as previously described (Nishimura and Weinstein, 1969). The enzyme was dialyzed against 50% glycerol overnight. When stored at −20° the enzyme lost approximately 50% of its charging capacity over a period of 8 months. Ten microliters of this crude dialyzed enzyme and twenty microliters of fractions from the BD-cellulose columns were assayed in a 0.1-ml system. Fractions from the hydroxylapatite columns were assayed with a purified beef liver phenylalanyl-tRNA synthetase prepared as described by Lanks *et al.* (1971), up to and including chromatography on Sephadex G-100. The enzyme was thus purified approximately 150-fold. This enzyme (3 µl) was used to assay 20-µl fractions of the hydroxylapatite columns. The remaining components of the assay systems, the processing of samples, and radioactivity measurements were as previously described (Nishimura and Weinstein, 1969).

Column Chromatography. Columns of BD-cellulose were prepared and run as previously described (Gillam *et al.*, 1967). The partially purified calf and beef liver tRNA^{Phe} were then rechromatographed on hydroxylapatite columns similar to those described by Zimmer and Hartman (1970).

Results

Purification of Beef and Calf Liver tRNA^{Phe}. When 6.8 g of calf liver tRNA was fractionated on a BD-cellulose column, the major amount of tRNA$^{Phe}_{calf}$ eluted during the latter part of the 0.4–1.5 M gradient of NaCl; a small amount of tRNA$^{Phe}_{calf}$ remained bound to the column and was eluted with 10% ethanol (Figure 1). A similar elution profile was obtained when 640 mg of beef tRNA was fractionated on a 1.5 × 100 cm column of BD-cellulose. The partially purified tRNA$^{Phe}_{calf}$ obtained from the latter part of the NaCl gradient (40 mg) was

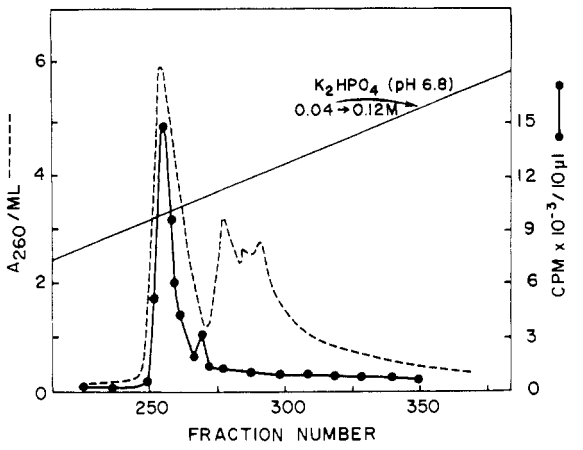


FIGURE 2: Chromatography of the partially purified $tRNA^{Phe}_{calf}$ from BD-cellulose on hydroxylapatite. The column (1.5 × 50 cm) was equilibrated with 0.04 M sodium phosphate (pH 6.8) at room temperature and 800 A_{260} units of $tRNA^{Phe}_{calf}$ was applied. The tRNA was eluted with a linear gradient of 2 l. of 0.04–0.12 M sodium phosphate (pH 6.8). The remaining tRNA was eluted with 0.15 M sodium phosphate (pH 6.8). Fractions of 2.5 ml were collected at a flow rate of 0.4 ml/min and assayed as described in Methods.

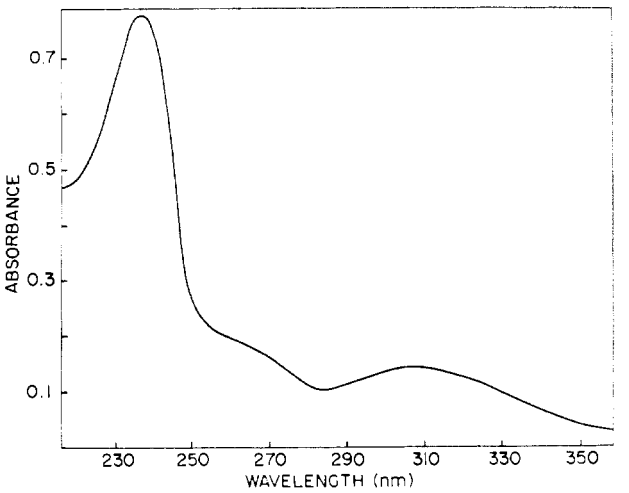


FIGURE 3: Absorption spectrum of the fluorescent base (II) obtained from $tRNA^{Phe}_{calf}$; measured in water.

TABLE I: R_F Values of Isolated Fluorescent Bases.[a]

$tRNA^{Phe}$ Source	R_F	
Baker's yeast[b]	0.48	
Calf liver[c]	0.50	0.34
Beef liver		0.34
Rat liver[b,c]	0.48	0.35
Chicken liver[b]		0.35

[a] Silica gel thin-layer plates, developed using upper phase of an ethyl acetate–1-propanol–water (4:1:2) mixture. [b] Data from Nakanishi *et al.* (1971). [c] Two spots were obtained from $tRNA^{Phe}_{rat}$ and $tRNA^{Phe}_{calf}$.

applied to a hydroxylapatite column and eluted with a gradient of 0.04–0.12 M sodium phosphate. A sharp optical density peak was coincident with phenylalanine acceptance activity (Figure 2). The fluorescence emission maximum of the material was at 435 nm when excited at 310 nm,[2] and the fractions at the peak accepted 1.52 nmol of [^{14}C]phenylalanine/A_{260} unit, corresponding to a purity of 90%. The $tRNA^{Phe}_{beef}$ was similarly purified on hydroxylapatite. We have found that our best resolution was obtained on small columns of hydroxylapatite using relatively fast flow rates. Larger columns with slow rates resulted in poorer separation and partial loss of acceptance activity. Prior to precipitation, the $tRNA^{Phe}$ from the hydroxylapatite column was dialyzed against water overnight, to avoid coprecipitation of inorganic phosphate.

Isolation and Structure Determination of the Peroxy Base. About 100 A_{260} units of the $tRNA^{Phe}$ was dissolved in 2–3 ml of 0.1 M potassium phosphate adjusted to pH 2.9 with phosphoric acid, and incubated at 37° for 3–5 hr. The solution was then neutralized with dilute NaOH and the bases excised were extracted with ethyl acetate, concentrated, and applied to silica gel tlc plates. The R_F values of the fluorescent bases obtained from calf and beef liver (Table I) are identical with those from chicken and rat liver, all being lower than that of the yeast.

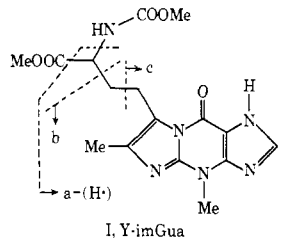
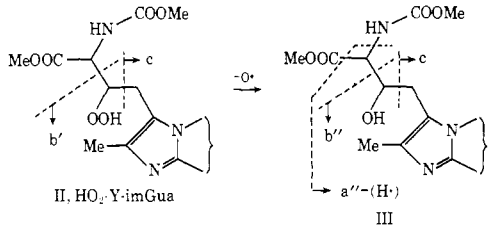
When 300 A_{260} units of $tRNA^{Phe}_{calf}$ was used as starting material two fluorescent spots were observed on thin-layer chromatography (tlc). The major component (more than 90%) had an R_F of 0.34 corresponding to the peroxy base, and the minor component appeared as a faint spot whose R_F was identical with that of Y-imGua, I. The possible significance of the minor component is discussed below. The strongly fluorescent materials were eluted with the developing solvent.

Figure 3 shows the absorption spectrum of the calf liver peroxy base (R_F 0.34), having absorption maxima at 308, 260, and 236 nm. The fluorescence spectra of this base are given in Figure 4, indicating excitation maxima at 247, 273, and 322 nm and an emission maximum at 445 nm. These values are almost identical with those reported by Fink *et al.* (1971), for the rat liver fluorescent base.

The structure of the calf liver peroxy base (R_F 0.34) was determined as II by comparing its high-resolution mass spectra cleavage pattern to that of the yeast base (Table II). The parent ion of the peroxy compound is at 408.1395 (calcd for $C_{16}H_{20}N_6O_7$, 408.1393) indicating the presence of two additional oxygen atoms when compared to the yeast base. Both I and II have peaks corresponding to the loss of the elements of methanol in the high mass region. Intense peaks at *m/e* 216 (cleavage c in I and II) indicate, together with the similar absorption and fluorescent spectra, that the aromatic nucleus and α-methylene group are present in both of these bases. Cleavage b in Y-imGua (I) yielded an ion at *m/e* 230; however, for II the analogous cleavage gives peaks at *m/e* 262 and 246. This indicates that both additional oxygen atoms in the peroxy base are located on the β carbon, either as a hydroperoxide or a *gem*-diol. The possibility of a *gem*-diol grouping is unlikely because a loss of 17 or 18 mass units would be observed, which is not the case. On the other hand, hydroperoxides are known to lose oxygen atoms in the mass spectrometer (van Lier and Smith, 1971). The mass spectral behavior of II in the molecular ion region, *i.e*, loss of oxygen atoms leading to *m/e* 392, and the presence of a set of peaks differing by 16 mass units (*m/e* 376 and 360; *m/e* 262 and 246) indicates that the compound contains a hydroperoxide group.

[2] In some experiments the fluorescence spectra varied from the above values for unknown reasons.

TABLE II:[a] Pertinent Ions Observed in the Mass Spectra of Y-imGua (I) and HO_2-Y-imGua (II).

Ion	Elemental Composition[b]	m/e	Rel Abundance (%)[c]
 I, Y-imGua			
I M$^+$	$C_{16}H_{20}N_6O_5$	376	37
I − (MeOH)	$C_{15}H_{16}N_6O_4$	344	5
a − (H·)	$C_{12}H_{12}N_5O$	242	3
b	$C_{11}H_{12}N_5O$	230	32
c	$C_{10}H_{10}N_5O$	216	100
 II, HO_2-Y-imGua III			
II M$^+$	$C_{16}H_{20}N_6O_7$	408	2
III [II − (O·)]	$C_{16}H_{20}N_6O_6$	392	5
II − (MeOH)	$C_{15}H_{16}N_6O_6$	376	6
III − (MeOH)	$C_{15}H_{16}N_6O_5$	360	2
b′	$C_{11}H_{12}N_5O_3$	262	5
a″ − (H·)	$C_{12}H_{12}N_5O_2$	258	19
b″	$C_{11}H_{12}N_5O_2$	246	6
c	$C_{10}H_{10}N_5O$	216	89

[a] Ions correspond to those indicated in structures I–III. [b] All compositions are based on exact mass measurements determined on MS-9, 70 eV, PFK standard, $m/\Delta m$ = 10,000. [c] Based on low-resolution spectra.

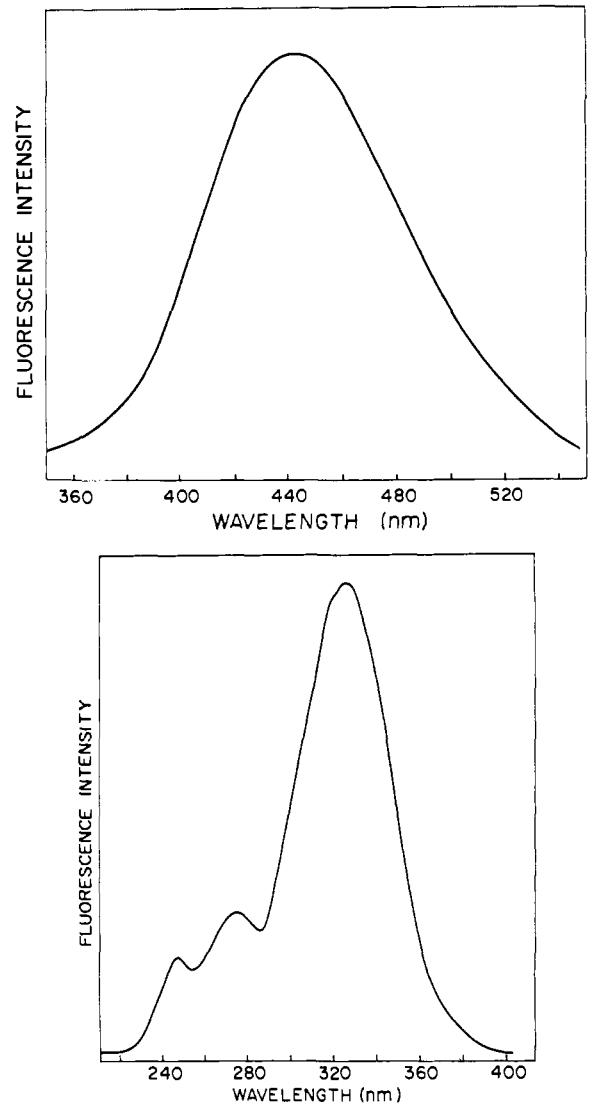


FIGURE 4: Uncorrected fluorescence emission (A, excitation at 310 nm) and excitation (B, emission at 420 nm) spectra of the fluorescent base (II) obtained from tRNA$^{Phe}_{calf}$ measured in water.

Compound II gave a single tlc spot with several other solvent systems (Yoshikami and Keller, 1971), but the possibility that the compound is a mixture of II and III (Table II), although unlikely, is not rigidly precluded by these data.

Attempts to Convert Yeast Base to the Peroxy Base. The isolation of an additional fluorescent material from tRNA$^{Phe}_{rat}$ having the same R_F and mass spectrum as the yeast base I (Nakanishi *et al.*, 1971) and also the presence of a faint spot from tRNA$^{Phe}_{calf}$ with the same R_F as I (see Table I) raised the possibility that the peroxy base might be an artifact formed during the isolation procedure. Since the major difference between the isolation of the yeast base and the peroxy base is the source of the tRNA, we considered the possibility that certain enzymes present in the liver might be modifying the liver base. Therefore we conducted a series of experiments in an attempt to convert enzymatically the yeast base to peroxy base.

A postribosomal supernatant fraction from rat liver was prepared and the enzymes were freed from nucleic acids as described by Zamecnik *et al.* (1960). The reaction mixture contained in a total volume of 0.1 ml: 45 μl of water-saturated *d,l*-Y-imGua, 30 μl of rat liver extract, 0.01 M KCl, 0.01 M $MgCl_2$, and 0.1 M cacodylate buffer (pH 6.3). Incubation was carried out at room temperature overnight. No modification of the subsequently reisolated base I was observed, judging by its R_F value on tlc. Another attempt to modify the yeast base in tRNA was made by incubating 80 A_{260} units of tRNA$^{Phe}_{yeast}$, with 0.4 ml of the rat liver extract, in the presence of 0.1 M potassium phosphate (pH 7.5), 0.0025 M ATP, and 0.01 M KCl in a total volume of 0.8 ml. The mixture was incubated at 37° for 1 hr with O_2 bubbled through the system. The tRNA^{Phe}, isolated after phenol extraction of the incubation mixture, was treated at pH 2.9. Only one spot was observed on the tlc which corresponded to the authentic yeast base Y-imGua.

To determine whether any other step in our isolation procedure had brought about a modification of Y-imGua, we prepared beef tRNA in the presence of a relatively large amount of yeast tRNA. Beef liver (200 g) was homogenized in the presence of baker's yeast tRNA (800 mg) (see Methods) and a total of 456 mg of tRNA was recovered. Since our pre-

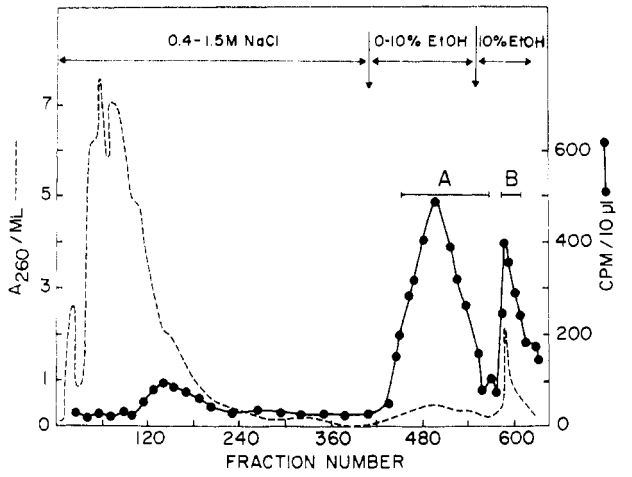
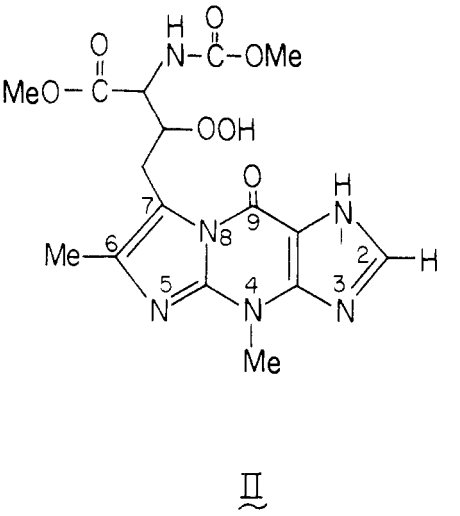


FIGURE 5: Chromatography of 340 mg of mixed yeast and beef $tRNA^{Phe}$ (approximately 80% $tRNA^{Phe}_{yeast}$) on BD-cellulose. The column (1.4 × 100 cm) was equilibrated with 0.4 M NaCl, 0.01 M $MgCl_2$, and 0.05 M sodium acetate (pH 5.0) at 4°, and the sample was applied in 75 ml of starting buffer. The tRNA was eluted with a linear gradient (1 l. each) of 0.4–1.5 M NaCl containing 0.05 M sodium acetate (pH 5.0) and 0.01 M $MgCl_2$, followed by a linear gradient of 0 to 10% ethanol (v/v), containing 1.5 M NaCl, 0.05 M sodium acetate (pH 5.0), and 0.01 M $MgCl_2$ (1-l. total volume). The remaining tRNA was eluted with 500 ml of 10% ethanol (v/v), 1.5 M NaCl, 0.05 M sodium acetate (pH 5.0), and 0.01 M $MgCl_2$. Fractions of 7.5 ml were collected at a flow rate of 0.3 ml/min and assayed for [^{14}C]phenylalanine acceptance activity. Fractions A (445–570) and B (585–610) which showed activity were pooled.



II

FIGURE 6: Structure of the peroxy base from bovine liver $tRNA^{Phe}$.

vious studies indicated that approximately 0.5 mg of tRNA is obtained from 1 g of liver, we can assume that approximately 80% of this material was yeast tRNA. This tRNA (340 mg) was fractionated on a BD-cellulose column and the two regions which showed acceptance activity for [^{14}C]-phenylalanine were pooled as described in Figure 5. The tRNAs present in these pools were harvested by ethanol precipitation, incubated at pH 2.9 for 5 hr, and after extraction into ethyl acetate the fluorescent material was spotted on silica gel tlc. Two fluorescent spots were obtained from region A. Most of the material (greater than 90%) had an R_F identical with that of the yeast base, while a trace amount of the fluorescent material had an R_F corresponding to the peroxy base. The fluorescent base obtained from $tRNA^{Phe}$ of region B had an R_F corresponding to the yeast base I, with no other fluorescent spot observed.

This experiment indicates that under conditions used to prepare beef tRNA, and during the subsequent isolation of $tRNA^{Phe}_{beef}$, no *in situ* modification of Y-imGua occurs. Although a trace of the peroxy compound was observed upon acid hydrolysis of $tRNA^{Phe}$ of region A, this presumably originates from the beef liver tRNA which, of necessity, is present during this preparation.

Since the above attempts to convert the yeast base I to the peroxy base II proved negative, we isolated beef tRNA in the presence of a potent inhibitor of several oxygenases, sodium azide. The preparation of tRNA from beef liver was carried out as described in Methods, the only exception being that 0.003 M NaN_3 was added to the homogenizing buffer. The tRNA was chromatographed on BD-cellulose in the presence of 0.001 M NaN_3. Although all the $tRNA^{Phe}_{beef}$ eluted in the ethanol region as does $tRNA^{Phe}_{yeast}$ (Gillam *et al.*, 1967), and not in the salt region as was previously observed, the R_F of the fluorescent base subsequently released by mild acid treatment was identical with that of the peroxy base.

Discussion

The present studies indicate that the fluorescent base obtained from bovine $tRNA^{Phe}$ has the same structure as I obtained from baker's yeast tRNA but for the presence of a hydroperoxide group on the β carbon of the side chain. Both compounds share the same fluorescent imidazo[1,2-*a*]purine ring system containing a methyl group on the N-4 position and a highly substituted 4-carbon side chain on the C-7 position. The systematic (IUPAC) name for the peroxy compound is α-(carboxyamino)-4,9-dihydro-β-hydroperoxy-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-butyric acid dimethyl ester (Figure 6).

We have also demonstrated the presence of the peroxy base in rat liver and chicken liver $tRNA^{Phe}$. Yoshikami and Keller (1971) have previously shown that the fluorescent base present in wheat germ $tRNA^{Phe}$ and beef $tRNA^{Phe}$ are chemically and chromatographically similar, which suggests that the wheat germ base has the same structure as the peroxy base II. It is of interest that the analogous fluorescent base present in brewer's yeast $tRNA^{Phe}$ consists only of the 4-methylimidazopurine nucleus and lacks the butyryl side chain. It is likely, therefore, that the $tRNA^{Phe}$ of all higher organisms contains the 4-methylimidazopurine base with variations occurring between species in terms of the side chain. In contrast to the situation in higher organisms, *Escherichia coli* $tRNA^{Phe}$ contains an isopentenyladenosine residue adjacent to the 3′ side of the anticodon, rather than the fluorescent base(s).

Hydroperoxide-containing compounds are extremely rare as naturally occurring substances and it was, therefore, necessary to exclude the possibility that this was due to an artifact of the isolation procedure. Several types of control experiments indicate, however, that the hydroperoxide is not generated during the extraction procedure. It is likely, therefore, that the hydroperoxide residue exists as such in liver $tRNA^{Phe}$ *in vivo*. The mechanism of its biosynthesis is not known. The only analogous pathway that we are aware of is the peroxidation of unsaturated lipids. Several investigators have described a NADPH-linked rat liver microsomal enzyme that, in the presence of inorganic iron, brings about the peroxidation of

unsaturated lipids (Hochstein *et al.*, 1964; Orrenius *et al.*, 1964; Wills, 1969). It is possible that an analogous enzyme with different substrate specificity results in the peroxidation *in vivo* of Y-imGua. In addition to the peroxy base II we have observed some unoxidized base as a minor component in preparations of $tRNA^{Phe}$ from rat and calf liver and it is possible that this reflects incomplete conversion to the peroxy form.

The actual biosynthesis of Y-imGua and its peroxy derivative HO_2-Y-imGua and the functional role of these extremely complex bases in tRNA are intriguing problems which require further extensive studies.

Acknowledgments

We thank Dr. George van Lear from Lederle Laboratories for measurements of high-resolution mass spectra and Miss Naola Gersten for valuable assistance in these studies. We are also grateful to Dr. K. L. Loening, Chemical Abstracts Service, American Chemical Society, for the IUPAC nomenclature of the bases, and to Dr. W. Cohn, Office of Biochemical Nomenclature, National Research Council, for suggesting the abbreviated nomenclature.

References

Dudock, B. S., Katz, G., Taylor, E. K., and Holley, R. W. (1968), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 27*, 342.

Fink, L. M., Goto, T., Frankel, F., and Weinstein, I. B. (1968), *Biochem. Biophys. Res. Commun. 32*, 963.

Fink, L. M., Lanks, K. W., Goto, T., and Weinstein, I. B. (1971), *Biochemistry 10*, 1873.

Fraenkel-Conrat, H., Singer, B., and Tsugita, A. (1961), *Virology 14*, 54.

Funamizu, M., Terahara, A., Feinberg, A., and Nakanishi, K. (1971), *J. Amer. Chem. Soc. 93*, 6706.

Gillam, I., Millward, S., Bleu, D., von Tigerstrom, M., Wimmer, E., and Tener, G. M. (1967), *Biochemistry 6*, 3043.

Hecht, S. M., Bock, R. M., Leonard, N. J., Schmitz, R. Y., and Skoog, F. (1970), *Biochem. Biophys. Res. Commun. 41*, 435.

Hochstein, P., Nordenbrand, K., and Ernster, L. (1964), *Biochem. Biophys. Res. Commun. 14*, 323.

Hoskinson, R. M., and Khorana, H. G. (1965), *J. Biol. Chem. 240*, 2129.

Kasi, H., Goto, M., Takemura, S., Goto, T., and Matsaura, S. (1971), *Tetrahedron Lett. 29*, 2725.

Lanks, K. W., Sciscenti, J., Weinstein, I. B., and Cantor, C. R. (1971), *J. Biol. Chem. 246*, 3494.

Nakanishi, K., Blobstein, S., Funamizu, M., Furutachi, N., van Lear, G., Grunberger, D., Lanks, K. W., and Weinstein, I. B. (1971), *Nature (London) 234*, 107.

Nakanishi, K., Furutachi, N., Funamizu, M., Grunberger, D., and Weinstein, I. B. (1970), *J. Amer. Chem. Soc. 92*, 7617.

Nishimura, S., and Weinstein, I. B. (1969), *Biochemistry 8*, 832.

Orrenius, S., Dallner, G., and Ernster, L. (1964), *Biochem. Biophys. Res. Commun. 14*, 329.

RajBhandary, U. L., and Chang, S. H. (1968), *J. Biol. Chem. 243*, 598.

Thiebe, R., Zachau, H. G., Baczynskyj, L., Bieman, K., and Sonnenbichler, J. (1971), *Biochim. Biophys. Acta 240*, 163.

van Lear, J. E., and Smith, L. L. (1971), *J. Org. Chem. 36*, 1007.

Wills, E. D. (1969), *Biochem. J. 113*, 333.

Yoshikami, D., Katz, G., Keller, E. B., and Dudock, B. S., (1968), *Biochim. Biophys. Acta 166*, 714.

Yoshikami, D., and Keller, E. B. (1971), *Biochemistry 10*, 2969.

Zamecnik, P. C., Stephenson, M. L., Scott, J. F. (1960), *Proc. Nat. Acad. Sci. U. S. 46*, 811.

Zimmer, A., and Hartman, G. R. (1970), *Bull. Soc. Chim. Biol. 52*, 867.

Zimmerman, T. P., Robinson, B., and Gibbs, J. H. (1970), *Biochim. Biophys. Acta 209*, 151.