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PURIFICATION AND PROPERTIES
OF PHENYLALANINE TRANSFER RNA FROM
BACILLUS LICHENIFORMIS AND BACILLUS STEAROTHERMOPHILUS

by

Cynthia R. Y. Yang

A Dissertation
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Doctor of Philosophy

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April 1972

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INTRODUCTION

Most organisms carry on their normal physiological processes in a moderate range of temperatures (about 20 to 45°). These organisms are known as mesophiles. Some organisms, however, grow at much higher temperatures (about 55 to 80°). These organisms are known as thermophiles.

Three main theories have been advanced to explain the phenomenon of thermophily. One theory (1, 2) considers thermophily to be a special type of metabolic state, involving high rates of breakdown and synthesis of cellular constituents. A second theory relates thermal stability to the protective action of lipids and attempts to correlate heat stability with the melting point of the cellular lipids (3). A third interpretation ascribes thermophily to physical and chemical differences of macromolecules from thermophiles as compared to similar macromolecules from mesophiles.

Most of the evidence accumulated so far supports the latter theory. Such evidence has come mostly from studies of proteins and nucleic acids. Koffler (4) showed that cytoplasmic proteins from thermophilic bacteria are more heat stable than those from mesophilic bacteria. Stenesh and Koffler (5) demonstrated a striking difference in heat stability for flagella from these two types of bacteria. Campell (6, 7) showed that crystalline α -amylase isolated from cultures of Bacillus coagulans grown at 55° was more heat stable than the same enzyme isolated from cultures

grown at 37°.

Early studies of nucleic acids yielded ambiguous results. Thus, for example, DNA (8) and transfer RNA (9, 10) from thermophiles showed no unusual heat stability. In most of these studies components from thermophilic strains of Bacillus were compared to similar components of Escherichia coli. Since such comparisons do not exclude intergeneric differences, a systematic study of mesophilic and thermophilic strains of one genus, namely Bacillus, was initiated in our laboratory.

Stenesh and Holazo (11) showed that the ribosomal RNA from thermophilic strains of Bacillus was more heat stable than the ribosomal RNA from mesophilic strains of the same genus. The RNA from the thermophiles melted out at a higher temperature and had a higher guanine plus cytosine content than that from the mesophiles. Similar results were obtained in studies of the DNA isolated from the above strains of Bacillus (12). An in vitro protein synthesizing system from a thermophilic strain had a higher optimum temperature than one from a mesophile (13). Some differences were also found in the fatty acid distribution of mesophilic and thermophilic strains of Bacillus (14).

The present work is an extension of the above comparative studies of mesophilic and thermophilic strains of the genus Bacillus. Specifically, the research project involved the isolation and characterization of phenylalanine transfer RNA (Phe-tRNA) from the mesophile B. licheniformis and the thermophile B. stearothermophilus 10.

Transfer RNA's are low-molecular weight RNA molecules (molecular weight $2.2-2.6 \times 10^4$), which constitute about 10-20% of the total cellular RNA. The tRNA molecule contains about 70-80 nucleotides and most of the tRNA molecules have the unique sequence, C-G-A, at their 3'-terminal. The function of a tRNA molecules is to bind covalently the proper amino acid and then transfer the amino acid to the site of protein synthesis (the ribosome) where the amino acids are assembled into polypeptides.

The binding of an amino acid to the tRNA molecule (known as amino acid activation) requires the presence of a specific enzyme - aminoacyl tRNA synthetase. For each amino acid there is at least one specific tRNA and one specific aminoacyl tRNA synthetase.

In the present study several approaches were used to prepare crude tRNA and to fractionate the latter in order to isolate phenylalanine specific tRNA. Various properties of purified phenylalanine tRNA were then determined. In addition, the characteristics of the phenylalanine activation reaction were studied using purified Phe-tRNA and crude aminoacyl tRNA synthetase isolated from the same species.

MATERIALS AND METHODS

Organisms and Growth Conditions

One mesophilic and one thermophilic strain of the genus Bacillus were used. The former was B. licheniformis (NRS 243). The latter was B. stearothermophilus 10.

Due to the large quantities of bacterial cells required in this work, it was necessary to grow the bacteria in a fermentor much larger than the one available in our laboratory. The Upjohn Company was kind enough to grow the cells for us starting from slants which contained 2% Bacto agar (Difco), 1% Trypticase (BBL), and 0.2% yeast extract (Difco). The cells were grown in 100 l. fermentors using a medium which consisted of 1% Trypticase, 0.2% yeast extract, and 4 ml of antifoam (SAG-471, Union Carbide Corp.). Cells of B. licheniformis were grown at 37° for 8 hours, and those of B. stearothermophilus were grown at 55° for 6 hours. In both case, cells were harvested at the late log phase and then stored at -20°. For the isolation of crude transfer RNA about 4.0 kg (wet weight) of B. licheniformis and about 2.3 kg of B. stearothermophilus were used.

Apparatus

All spectrophotometric measurements were made in a Zeiss model PMQ-II or Beckmann model DU-2 spectrophotometer. For thermal denaturation studies, a Haake model F constant temperature

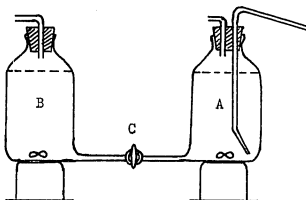
circulator was used in conjunction with the Zeiss spectrophotometer.

A Sorvall model RC-2 refrigerated centrifuge was used for low speed centrifugations (below 15,000 r.p.m.), and a Spinco model L preparative ultracentrifuge was used for high speed centrifugations (above 15,000 r.p.m.).

An ISCO model 326 fraction collector was used for collecting fractions from column chromatography. A single beam ultraviolet monitor, ISCO model UA-2, was used to record the absorbance of the column eluate at 254 nm. A polystaltic pump (Buchler model 2-6100) was used at the outlet of the column to control the flow rate.

Eppendorf pipets (Brinkmann) were used in all assays. Paper chromatography was carried out in a Chromatocab chamber (Research Specialties model 125). A Burrell shaker was used for eluting free bases from paper chromatograms.

An apparatus for preparing solutions which had a linear concentration gradient was prepared by connecting two one liter polyethylene bottles with spouts by means of a small tube having a stopcock. Each bottle was placed on a magnetic stirrer. The set-up is sketched below. A volume of water or dilute solution was



placed in bottle A, and an equal volume of a more concentrated solution was placed in bottle B. The siphon in A was first started with the help of a pump and stopcock C was then opened. The solution in both A and B were stirred continually. Hence the solution obtained through the siphon in A had a linear concentration gradient.

Analytical Determinations

I. Protein

Protein was determined by the method of Lowry et al. (15), using bovine serum albumin as a standard.

One hundred ml of 2% Na_2CO_3 in 0.1 N NaOH were mixed with 1 ml of 1% $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ and 1 ml of 2% sodium potassium tartrate. To 1 ml of sample were added 5 ml of this reagent. After at least 10 minutes, 0.5 ml of 1 N Folin-Ciocalteu reagent was added, and the solution mixed immediately using a vortex stirrer. After at least 30 minutes at room temperature, the absorbance of each solution was measured at 750 nm.

II. DNA

DNA was determined by the method of Burton (16). Diphenylamine reagent was prepared by dissolving 1.5 g of diphenylamine in 100 ml of glacial acetic acid and adding 1.5 ml of concentrated H_2SO_4 . Just before use, 0.1 ml of 1.6% aqueous acet-aldehyde were added per 20 ml of reagent. To a certain volume of nucleic acid solution, an equal volume of 1 N HClO_4 was

added so that the final solution contained between 0.02-0.25 micromole of DNA-phosphate per ml. One volume of this solution was then mixed with 2 volumes of diphenylamine reagent. Deoxyadenosine was used as a standard. The tubes were incubated at 25-30° for 15-17 hours, and the absorbance was then measured at 600 nm.

III. Phosphorus

A. Color Reaction

Phosphorus was determined by the method of Fiske and SubbaRow (17) using KH_2PO_4 as a standard. To 1 ml sample in H_2O , which contained 0-30 ug phosphorus, 1 ml of 5 N H_2SO_4 was added. The mixture was heated until white fumes filled the test tube. The content was left to cool before the addition of 1 drop of 1 N HNO_3 . The mixture was again heated until white fumes filled the tube. It was left to cool and 1 ml of H_2O was then added. The solution was mixed vigorously using a vortex stirrer and was then placed in boiling water for 5 minutes. Marbles were used to prevent evaporation. After cooling, 1 ml of 2.5% ammonium molybdate was added and the solution was mixed again. Water was added (7.5 ml), the solution was mixed, and the absorbance was measured at 660 nm.

B. Ultraviolet Absorbance

The phosphorus content of a tRNA sample was also estimated from ultraviolet absorbance measurements of the sample at 260 and 290 nm. The concentration of RNA was

calculated from the following formula (18):

$$49.265 \times (A_{260} - A_{290}) = \text{ug RNA/ml}$$

where A_{260} and A_{290} are the absorbance at 260 and 290 nm, respectively. The concentration of phosphorus is equal to 0.96 times the concentration of RNA.

Assay of Transfer RNA

The tRNA was assayed by "charging" it with ^{14}C -labelled amino acids. The standard assay for this "amino acid acceptor activity" followed the procedure of Kelmers et al. (19) with some modifications.

The reaction mixture (0.2 ml) contained the following (in micromoles unless otherwise stated), in the order of addition; sufficient homologous crude aminoacyl-tRNA synthetase (about 0.05 mg) to obtain maximal formation of aminoacyl-tRNA; tris-HCl buffer (pH 7.4), 20; magnesium acetate, 2; ATP, 0.4; KCl, 1; ^{14}C -labelled L-amino acid, 0.2 (specific activity approximately 300-400 mCurie per mmole); tRNA, 0.02 to 2.0 absorbance units at 260 nm. The reaction mixture was incubated at 37° for 10 minutes. The reaction was stopped by the addition of 3 ml of cold 10% trichloroacetic acid. The mixtures were left to stand in the cold room for at least one hour before the precipitate was collected on millipore filters and washed 10 times with 5 ml of cold 5% trichloroacetic acid. The filter papers were then dried and either glued to planchets and counted in a Nuclear Chicago model 4388 planchet counter or suspended in 10 ml of scintillation fluid and counted

in a Nuclear Chicago Mark II liquid scintillation counter. In the latter case, the CPM were converted to DPM using the channels ratio method and ^{14}C -quenched standard.

Preparation of Aminoacyl-tRNA Synthetase

Crude aminoacyl-tRNA synthetase was prepared according to the procedure of Kelmers et al. (19). All steps were carried out at 4° unless otherwise specified. Bacterial cells (30 g) were thawed, dispersed in 60 ml of buffer (0.01 M tris-HCl, pH 7.4, containing 0.01 M magnesium acetate and 0.001 M glutathione), and broken in a French pressure cell at 10,000 to 12,000 psi. Four volumes of buffer were then added and the mixture was centrifuged at $35,000 \times g$ for 40 minutes and then at $78,500 \times g$ for 3 hours to remove particulate matter. The supernatant solution was dialyzed overnight against 2 liters of buffer. Streptomycin sulfate (0.1 volume of a 10% solution) was then added to the dialyzed solution to precipitate out the nucleic acids. After stirring for 3 hours, the precipitate was removed by centrifugation at $16,000 \times g$ for 10 minutes. The pH of the supernatant was then adjusted to 7.5 with 0.1 M KOH and maintained at that pH during the addition of solid ammonium sulfate to 65% saturation. The mixture was stirred for 30 minutes and the precipitate recovered by centrifugation at $16,000 \times g$ for 10 minutes. The precipitate was dissolved in 100 ml of buffer and dialyzed overnight against 6 liters of buffer. Glycerol (0.25 volume) was added to the enzyme solution which was then stored at -20° . Crude aminoacyl-tRNA synthetase prepared in

this manner was stable for at least one year.

Base Composition of Transfer RNA

Base composition of the tRNA was determined according to the procedure of Bendich (20). To 5 mg of tRNA in a small glass-stoppered tube was added 0.1 ml of 70% HClO_4 . The tubes were tightly stoppered and placed in boiling water for 1 hour with occasional agitation. The mixture was ground with a glass rod to produce a homogeneous suspension. It was then centrifuged for 10 minutes in a clinical centrifuge. About 40 μl of the clear supernatant were then spotted on Whatman No. 1 paper. Standard solutions of purines and pyrimidines in 0.1 N HCl were spotted along with the sample. The solvent for development of the chromatograms was prepared by addition of 16.7 ml concentrated HCl to 65 ml of peroxide free absolute isopropanol and addition of 18.3 ml water after mixing. Descending paper chromatography was carried out in a chromatocab for 18 hours. The spots were visualized using short wavelength ultraviolet light and the R_f values were calculated. Each spot was cut into small pieces and the sample eluted with 5 ml of 0.1 N HCl while shaking on a shaker for 2 hours. The fibers were removed by centrifugation and the absorbance of the supernatant was then measured at two wavelengths. Blanks were prepared similarly by cutting out spots at corresponding positions from a blank lane. The base composition was calculated using the formulas of Bendich (20).

Thermal Denaturation of Transfer RNA

The tRNA was dissolved in 0.01 M tris-HCl buffer (pH 7.2) containing 0.01 M MgCl_2 and 0.001 M $\text{Na}_2\text{S}_2\text{O}_3$. The thermal denaturation profile of the tRNA was obtained by measuring the absorbance of the solution at 260 nm at various temperatures from 25° to 90°. The solution was kept for 15 minutes at each temperature before the absorbance was measured in order to allow for temperature equilibration. The observed absorbance was corrected for the thermal expansion of the solution. The temperature in the cuvette was obtained from the temperature of the circulating water bath by reference to a calibration curve. The calibration curve represents a plot of cuvette temperature versus bath temperature and is required because of unavoidable heat loss between the water bath and the cuvette in the spectrophotometer.

RESULTS AND DISCUSSION

Isolation of Crude Transfer RNA

Crude transfer RNA was first isolated in pilot experiments (using 100-400 g of cells) according to the three different procedures described below. These methods had been designed originally for cells of Escherichia coli and difficulties were encountered with the first two methods when adapting them to cells of Bacillus. The third method, using dimethyl sulfoxide, gave very satisfactory results. Hence the entire batches of cells (4.0 kg for B. licheniformis and 2.3 kg for B. stearothermophilus) were processed according to this procedure, using about 230 g of cells for each separate isolation. The final product for each strain of cells was then pooled.

I. Isopropanol Method

Crude tRNA was prepared according to the procedure of Kelmers et al. (19). Frozen cells (400 g) were thawed and 800 ml of 0.001 M tris-HCl buffer (pH 7.4) containing 0.01 M magnesium acetate were added. The suspension was extracted with 688 ml of 88% phenol for one hour at room temperature. The aqueous phase was recovered by centrifugation at 8,500 rpm (Sorvall, GSA rotor) for one hour at 4°. All subsequent steps were carried out at 4° unless otherwise indicated. The aqueous phase was adjusted to 2% in potassium acetate, and two volumes of 95% ethanol were added to precipitate the nucleic acids.

After one hour, or longer, the precipitate was recovered by centrifugation at 8,500 r.p.m. (Sorvall, GSA rotor) for 10 minutes. The precipitate was dissolved in 400 ml of 0.3 M sodium acetate at 20°. One-half volume of isopropanol was then added and the mixture was stirred at 20° for one hour. The precipitate (the bulk of the DNA and high molecular weight RNA) was removed by centrifugation at 11,700 x g for 10 minutes at 20°. The supernatant was cooled to 4°, and an additional 0.5 volume of isopropanol was added slowly while stirring at the same time to precipitate the RNA. Stirring was continued for one hour and the solution was then centrifuged at 11,000 x g for 10 minutes. The precipitate was dissolved in 500 ml of 0.05 M NaCl, and loaded onto a DEAE-cellulose column (4 x 20 cm). After washing with two liters of 0.2 M NaCl, the tRNA was eluted with one liter of 0.65 M NaCl. The crude tRNA was recovered from the eluate by precipitation with two volumes of 95% ethanol. After four hours, the precipitate was collected by centrifugation at 11,000 x g for 10 minutes. The crude tRNA was dissolved in 50 ml of 0.2 M glycine buffer at pH 10.3 and incubated at 37° for two hours to discharge any bound amino acids. This results in "stripped" tRNA. The solution was cooled at 4°, and 4 ml of 5 M NaCl and two volumes of 95% ethanol were added. The precipitate was recovered after two hours by centrifugation at 11,000 x g for 10 minutes, dissolved in 18 ml of doubly distilled water overnight, lyophilized, and stored at -20°.

The major difficulty with this method involved the second isopropanol treatment which should precipitate out the tRNA. Little or no precipitate was obtained with the cells from the two Bacillus species. A report (21) which appeared after the publication of the procedure by Kelmers et al., stated that successful isopropanol precipitation of tRNA depends critically on the concentration of the material dissolved in 0.3 M sodium acetate. The concentration must be between 0.3 and 3.5 mg per ml. It is possible that the ineffectiveness of the procedure in our systems was due to improper concentration of the material dissolved in 0.3 M sodium acetate. Alternatively, there may be compounds in Bacillus species which interfere with isopropanol precipitation. It is of interest that another isopropanol precipitation method which is widely used for the precipitation of bacterial DNA, was found in our laboratory to be likewise ineffective for the isolation of DNA from Bacillus species (12).

II. Lithium Chloride Method

Crude transfer RNA was also isolated according to the procedure of Avital and Elson (22).

A. Phenol Extraction of Cells

Cell paste (100 g) was washed with 200 ml of 0.9% NaCl, and then suspended in 100 ml of 0.9% NaCl. A volume of phenol equal to that of the cell suspension was added and the mixture was stirred for 15 minutes. After transfer to an ice or ice-salt bath, stirring was continued until the temperature dropped to 0-2°. A low speed centrifugation

separated the mixture into two phases. The upper (aqueous) phase was collected and recentrifuged. Cold 95% ethanol (-20° , 2.5 volumes, containing 2% potassium acetate) was added to this phase. After at least one hour, the precipitate was collected by low speed centrifugation. The precipitate was suspended in a small volume of cold 95% ethanol, and centrifuged in prechilled tubes. The supernatant was discarded and the tubes were air dried.

B. Removal of Bound Amino Acids

The precipitate from the previous step was dissolved in 15 ml of 2 M tris-HCl (pH 8.0) and incubated for 90 minutes at 37° . After chilling, 2.5 volumes of cold 95% ethanol, containing 2% potassium acetate, were added. The mixture was kept at -20° for at least one hour and the precipitate was collected by low speed centrifugation. The supernatant was discarded and the tubes were air dried.

C. Removal of Ribosomal RNA with LiCl

To the precipitate obtained in the previous step were added 34 ml of 2 M LiCl-0.1 M potassium acetate (pH 5.0) and the mixture was stirred for 20 minutes. It was then transferred to centrifuge tubes, chilled in ice for 5 minutes, and centrifuged. The pellet may be extracted a second time with 17 ml of LiCl-potassium acetate.

D. Precipitation with $(\text{NH}_4)_2\text{SO}_4$

The supernatant obtained in the previous step was dialyzed for 2.5 hours against 1×10^{-6} M magnesium acetate

in order to remove most of the LiCl. Solid ammonium sulfate (0.315 g per ml of supernatant) was added with stirring which was continued for 15 to 20 minutes after all of the ammonium sulfate had been added. The mixture was transferred to centrifuge tubes, chilled in ice for 5 minutes, and centrifuged. To the supernatant were added 1.1 g of ammonium sulfate for every gram used previously. Stirring, chilling and centrifugation were repeated as before. The tubes were drained and wiped dry. The tRNA pellet was taken up in 15 to 20 ml of 1×10^{-6} M magnesium acetate, dialyzed overnight against several changes of the same solution, and lyophilized.

This procedure is based on the preferential solubilization of tRNA with LiCl while ribosomal RNA is being precipitated. The difficulty with this procedure involved the LiCl treatment. No precipitate of ribosomal RNA was obtained with the Bacillus preparations. This finding was in line with previous findings in our laboratory that ribosomal RNA in purified ribosome preparations from Bacillus strains is incompletely precipitated by the LiCl method for isolation of ribosomal RNA.

III. Dimethyl Sulfoxide Method

A. Preparation of Crude tRNA

The dimethyl sulfoxide (DMSO) method for the isolation of crude tRNA was that described by Gutch (23). To 227 g of frozen cells were added 425 ml of 88% phenol. After

1.5 to 2.0 hours of stirring at room temperature, the uniform, well dispersed slurry was treated with 990 ml of deionized, doubly distilled, water. After one hour of additional stirring, the slurry was left at room temperature overnight. On the following day, 475 ml of 88% phenol was added and the mixture was stirred for 30 minutes. Deionized, doubly distilled, water (1,100 ml) was added. Stirring was continued for one hour and the suspension was again left overnight at room temperature. The top aqueous layer was removed by syphoning and was then centrifuged at $16,000 \times g$ for one hour. To the aqueous layer were added successively 0.1 volume of 20% potassium acetate (pH 5.2) and two volumes of 95% ethanol. The precipitate which contained the tRNA was removed the following day by syphoning off the supernatant and centrifuging the remaining slurry at low speed. The precipitate was washed once with cold 75% ethanol and once with cold isopropanol, and dried in vacuo at room temperature.

B. First DMSO Purification

The crude tRNA was dissolved in 0.05 M potassium acetate to give an absorbance of 360 at 260 nm. The pH was then raised to 8.8-9.0 by the addition of 5 M NH_4OH . The suspension was incubated at 37° for one hour to strip off any bound amino acids. The pH was then lowered to 7.0-7.2 by the addition of 5 M acetic acid at 15 to 20° . Dimethyl sulfoxide (0.7 volume) was slowly added with stirring and

chilling so that the temperature of the suspension did not exceed 35° . A solution of 3 M NaCl (0.25 volume of the initial aqueous phase) was then added at $30-32^{\circ}$ over a period of 30 minutes. The mixture was then centrifuged and the precipitate was washed with a cold mixture of H_2O : DMSO : 3 M NaCl (1 : 1 : 0.25, v/v, 0.25-0.33 volume of the initial aqueous phase). The suspension was centrifuged, and the supernatants from the two centrifugations were combined. Ethanol (95%, 0.5 volume) was then added at $5-10^{\circ}$. The precipitate was recovered by centrifugation, washed with cold 75% ethanol and then with cold isopropanol, and dried in vacuo at room temperature.

C. Second DMSO purification

The tRNA was dissolved in water to give an absorbance of 640 at 260 nm. An equal volume of DMSO was added slowly with stirring and chilling so that the temperature did not exceed 30° . A solution of 3 M NaCl (0.2 volume of the initial aqueous phase) was added, and the suspension was cooled to $20-23^{\circ}$. After 15 minutes, the suspension was centrifuged and the residue was washed as above with a cold mixture of water-DMSO-3 M NaCl (1 : 1 : 0.2, v/v). To the combined supernatants, 0.33 volume of ethanol was added at $5-10^{\circ}$. After one hour, the precipitate was recovered by centrifugation and washed with cold 75% ethanol and cold isopropanol. The tRNA was then dried in vacuo at room temperature and stored at -20° . The yield of tRNA obtained

in this procedure was similar to that reported by Gutcho (23) for E. coli (2.7 g tRNA/kg cells) and varied only slightly from one batch of cells to another. The yield and the activity of crude tRNA, when assayed by the charging reaction as described under Methods, are summarized in Table I.

It can be seen from Table I that the specific activity of B. stearothermophilus tRNA was higher but its yield lower than that of B. licheniformis tRNA. As a result, the total activity per kg of cell paste was the same for both organisms.

The activity of the crude tRNA obtained from DMSO method was compared to that of commercially available B. subtilis tRNA using homologous and heterologous systems of tRNA and aminoacyl-tRNA synthetase. The results are shown in Table II. The activity of B. stearothermophilus tRNA is of the same order of magnitude as that of B. subtilis, but the B. licheniformis tRNA is somewhat less active. Furthermore, it can be seen that the tRNA's and the aminoacyl-tRNA synthetase are interchangeable.

Fractionation of Crude Transfer RNA

Three different methods for fractionating crude tRNA were used.

I. Reversed Phase Column Chromatography

Fractionation of crude tRNA by this method was carried out

TABLE I
Yield and Phenylalanine Acceptor Activity of Crude tRNA

Organism	Yield (g tRNA/kg cells)	Specific Activity (CPM/g tRNA)	Total Activity (CPM/kg cells)
<u>B. licheniformis</u>	4.5	2.2×10^7	1.8×10^8
<u>B. stearothermophilus</u>	1.4	8.2×10^7	1.2×10^8

TABLE II
Phenylalanine Acceptor Activity of Various Crude tRNA's^a

Aminoacyl-tRNA synthetase	tRNA		
	<u>B. subtilis</u> ^b	<u>B. licheni-</u> <u>formis</u>	<u>B. stearo-</u> <u>thermophilus</u>
<u>B. subtilis</u>	7.86	3.38	5.71
<u>B. licheniformis</u>	8.37	3.04	5.49
<u>B. stearothermophilus</u>	11.55	2.77	6.52

^a¹⁴C-Phenylalanine binding (CPM x 10⁴/mg tRNA).

^bCommercial product of General Biochemicals Co.

according to the procedure of Kelmers (24). In reversed phase chromatography, the organic phase is stationary and the aqueous phase is the moving phase.

A. Reversed Phase Chromatographic Columns

The organic phase consisted of 4% (w/v) dimethyldilaurylammonium chloride (Aliquat 204) in isoamyl acetate. The commercial grade of dimethyldilaurylammonium chloride was recrystallized three times from acetone by cooling to -20° . The organic phase was washed successively with three volumes of 1 M NaOH, 1 M HCl, and 0.3 M NaCl to remove soluble contaminants. The packing material consisted of one part organic phase thoroughly mixed with two parts (w/w) of hydrophobic diatomaceous earth (chromosorb W). Jacketed columns, thermostated at 37° , were used throughout. For preliminary experiments, columns of 1 x 120 cm were used. Full scale experiments involved columns of 2.5 x 240 cm. A 50 or 100 mg sample of tRNA was dissolved in 2-4 ml of the initial eluting solution and applied to the column. Elution from the column was carried out by means of a linear sodium chloride gradient (0.45-0.95 M) at a flow rate of 1 ml per minute. In addition to NaCl, the eluting solutions contained 0.01 M $MgCl_2$ and 0.01 M sodium acetate buffer, pH 4.5. All solutions were saturated with isoamyl acetate at 37° to prevent depletion of the isoamyl acetate on the column. All eluting solutions were prewarmed to column temperature before passage through the column. The

column eluate was passed through an ultraviolet monitor (254 nm) and 10 ml fractions were collected. The fractions were assayed for amino acid acceptor activity (generally every second or third fraction was used) and appropriate fractions were pooled for further use.

B. Bio-Gel P-2 Desalting Columns

Bio-Gel P-2 polyacrylamide gel columns were employed to remove NaCl and other salts from the sample of partially purified Phe-tRNA. Samples of 250 to 300 ml were desalted on a column (2.5 x 200 cm) maintained at approximately 5° (cold room). The tRNA sample was eluted from the column with distilled water at a flow rate of 2.5 ml per minute and 10 ml fractions of eluate were collected. The fractions were tested for the presence of chloride ions and the absorbance of each fraction was measured at 260 nm. The column can be regenerated by passing approximately one column volume of 0.10 M tris-HCl (pH 8.0), containing 0.4 M NaCl, through the column, followed by several column volumes of water.

C. Concentration on Flash Evaporator

Desalted samples of partially purified Phe-tRNA were concentrated in a Buchler flash evaporator. The bath was maintained at 37° and the sample was concentrated to several milliliter.

D. Bio-Gel P-100 Gel Filtration Columns

Bio-Gel P-100 polyacrylamide gel columns were used for

the final purification of Phe-tRNA. Desalted samples were made up to a solution which was 0.40 M in NaCl and 0.01 M in MgCl_2 with a final volume of 4 ml. The sample was applied to a column (1 x 120 cm or 1 x 240 cm) and eluted at a flow rate of 0.5 ml per minute with a solution containing 0.40 M NaCl, 0.01 M MgCl_2 , and 0.05 M tris-HCl buffer, pH 7.0. The column was maintained at approximately 10° (jacketed columns and circulating coolers were used at this stage). The column eluate was collected in 4 ml fractions, and the absorbance at 260 nm and the phenylalanine acceptor activity of the fractions were measured.

The reversed phase chromatographic procedure was developed at the Oak Ridge National Laboratory where it has been used for fractionation on a large scale. Hence it appeared to be a promising technique for our studies in spite of the fact that column chromatography with such large columns is very laborious and time consuming. In the case of E. coli, Phe-tRNA is well separated by this technique from other tRNA's (19, 24). In the case of B. licheniformis, unfortunately, entirely different results were obtained. Phe-tRNA activity was observed over the entire range of fractions obtained from the Bio-Gel P-100 column rather than being concentrated in a well defined peak. Furthermore, the reproducibility between different runs was very poor so that different absorbance and activity profiles were obtained for duplicate experiments. Lastly, the flow

rates obtainable for Bio-Gel P-100 columns were so low that a fractionation of the entire samples of crude tRNA from B. licheniformis and B. stearothermophilus would have been a prohibitive undertaking in terms of the time required. For these reasons, this procedure was abandoned.

II. Solvent Extraction

The procedure of Edgan et al. (25) was followed with some modifications. The organic phase contained 0.1 M Adogen 464 (methyl trialkyl ammonium chloride with an equivalent weight of 487) in Freon 113 (1, 1, 2-trichloro-1, 2, 2-trifluoro-ethane). The organic phase was washed successively, twice each time, with one half the volume of 1 M Na_2CO_3 , 0.5 M HCl, and 0.5 M NaCl. The tRNA was dissolved in 0.05 M tris-HCl buffer (pH 7.2) containing 0.01 M MgCl_2 and 0.2 M NaCl to a concentration of 1 mg tRNA per ml. This tRNA solution was extracted at room temperature, in a separatory funnel, with one volume of the organic phase, which had been saturated with the aqueous phase used to dissolve the tRNA. The organic phase was then drawn off, and was washed six times, with one volume of the aqueous phase. The tRNA was then extracted from the organic phase by a buffer solution of higher salt concentration (0.05 M tris-HCl, pH 7.2, 0.01 M MgCl_2 , and 0.4 M NaCl). The tRNA containing solution was dialyzed at 4° against doubly distilled water until no chloride could be detected. The absorbance at 260 nm and the amino acid acceptor activity of the tRNA solution were then measured. The tRNA was precipitated at 4° from the solution by

the addition of one volume of 1.0 M NaCl, followed by four volumes of 95% ethanol. After the tRNA had settled out in the cold room, the top portion of the supernatant was syphoned off and the rest of the solution was centrifuged at $11,000 \times g$ for 10 minutes. The tRNA in the centrifuge tubes was dried in vacuo overnight at room temperature. The dried residue from different batches was combined, dissolved in deionized, doubly distilled, water, and dialyzed against the same water. The solution was lyophilized and the final product was stored in a desiccator at -20° .

The original procedure called for Freon 214. However, at the time that our experiments were conducted, Du Pont had ceased to manufacture this compound. Upon consultation, they recommended the use of Freon 113 which was thought to be closest in solubility properties to Freon 214. While we have shown here that Freon 113 can be used for tRNA extraction, it is possible that purification of Phe-tRNA with Freon 214 might have been more extensive than that obtained with Freon 113.

Using the latter compound, an approximately 3-5 fold purification of Phe-tRNA was obtained. The conclusion is based on measurements of phenylalanine acceptor activity per absorbance unit at 260 nm. The recovery of the Phe-tRNA was approximately 60-80% and varied somewhat from batch to batch since the starting material itself consisted of batches with somewhat different consistency. Fluctuation in room temperature would also lead to some variation in the results, since extraction

coefficients have been shown to depend on the temperature of the organic phase extractant (25). The extraction coefficient also depends on the concentration of $MgCl_2$ and NaCl in the aqueous phase (25). It has been suggested that the tRNA's that are readily extracted into the organic phase have a more open structure and are thus more accessible to the organic phase extractant (26).

The specific activity of B. licheniformis Phe-tRNA, purified by solvent extraction (Table III), was lower than that of B. stearothermophilus Phe-tRNA, much as was the case for the crude tRNA (Table I). Apparently some other types of tRNA from B. licheniformis are extracted along with Phe-tRNA.

III. Benzoylated DEAE-cellulose Column Chromatography

Benzoylated DEAE-cellulose (BD-cellulose) column chromatography was first introduced by Gillam et al. (27) in the fractionation of tRNA's. The procedure used in this work was that of Dudok (28). The commercially available BD-cellulose was washed six times with 2.0 N NaCl containing 10% ethanol and was then suspended in deionized, doubly distilled, water. The suspension was passed through a 100-mesh sieve in order to separate the fine particles from the coarse ones. Only the particles which did not pass through the sieve were used for column-packing. The column (0.9 x 30 cm) was packed with the help of occasional shaking by means of a vibrator. The column was washed with standard buffer (0.01 M tris-HCl, pH 8.0, 0.01 M $MgCl_2$ and 0.001 M $Na_2S_2O_3$), which contained additionally 0.38 M

TABLE III
Yield and Activity of Partially Purified Phenylalanine tRNA

Organism	Solvent extraction		BD-cellulose column	
	Yield (%)	Activity (DPM x 10 ⁵ per unit absorbance)	Yield (%)	Activity (DPM x 10 ⁵ per unit absorbance)
<u>B. licheniformis</u>	70 ^a	0.24	72	0.9
<u>B. stearothermophilus</u>	73 ^b	0.63	70	2.3

^a Average of 65-76% from different batches of preparation.

^b Average of 61-78% from different batches of preparation.

NaCl. The tRNA (50 mg) was dissolved in the same buffer, and was applied to the column. The flow rate was adjusted to 0.7 ml per minute with a polystaltic pump, and the absorbance of the eluate was measured with a uv monitor, at 254 nm, using a 5 mm flow cell. Elution was first carried out with 200 ml of a linear concentration gradient (0.38-0.57 M NaCl in standard buffer), and fractions were collected at five minute intervals. Elution was next performed with 200 ml of a second linear concentration gradient (0.57-2.0 M NaCl in standard buffer). Final elution was with 100 ml of 2.0 M NaCl in standard buffer containing 10% ethanol. Every fifth or tenth fraction was assayed for Phe-, Tyr-, and Leu-tRNA activity. The fractions which had high Phe-tRNA activity (Nos. 60-100 for B. licheniformis and Nos. 29-90 for B. stearothermophilus; see Figures 1 and 2) were pooled and this solution was dialyzed against deionized, doubly distilled, water until no chloride could be detected. It was then lyophilized to give the final product of purified Phe-tRNA.

BD-cellulose chromatography resulted in a further 3-5 fold purification of Phe-tRNA (Table III). Thus this partially purified Phe-tRNA represents approximately a 9-25 fold purification with respect to the crude tRNA. The yield of the BD-cellulose step was again about 70%.

Portions of the absorbance and activity profiles obtained from BD-cellulose column chromatography are shown in Figures 1 and 2. It can be seen that the Phe-tRNA from B. stearothermophilus was eluted at a slightly lower salt concentration than

FIGURE 1

Chromatography of B. Licheniformis Transfer RNA
on Benzoylated DEAE-cellulose

- : Absorbance at 260 nm (A_{260}).
- : Phenylalanine charging activity (DPM per 0.1 ml eluate).
- △ : Leucine charging activity (DPM per 0.1 ml eluate).
- : Tyrosine charging activity (DPM per 0.1 ml eluate).
- : NaCl concentration (M).

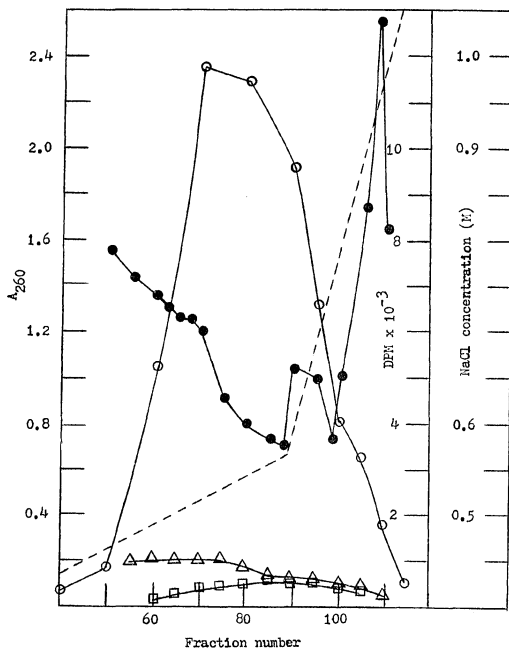
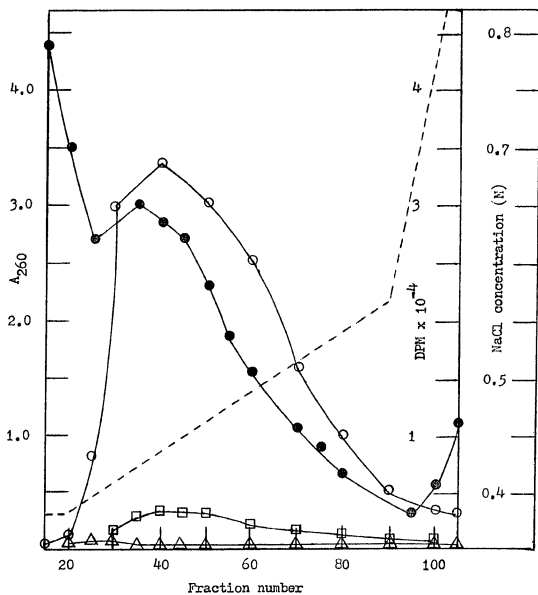


FIGURE 2

Chromatography of B. Stearothermophilus Transfer RNA
on Benzoylated DEAE-cellulose

- : Absorbance at 260 nm (A_{260}).
- : Phenylalanine charging activity (DPM per 0.1 ml eluate).
- △ : Leucine charging activity (DPM per 0.1 ml eluate).
- : Tyrosine charging activity (DPM per 0.1 ml eluate).
- : NaCl concentration (M).



that from B. licheniformis. Furthermore, the Phe-tRNA from both organisms was eluted at lower salt concentrations than the Phe-tRNA from yeast and wheat germ. These two types of Phe-tRNA are bound tightly to the BD-cellulose column and can only be eluted at high salt concentrations (> 1.0 N NaCl) in the presence of 10% ethanol or methyl-cellosolve (27, 29). The Phe-tRNA from B. licheniformis and B. stearothermophilus are, therefore, bound much less tightly to the BD-cellulose and resemble in this respect the Phe-tRNA of Escherichia coli (30) rather than that of yeast and wheat germ.

It can be seen from Figures 1 and 2 that there is a reasonable correspondence between phenylalanine charging activity and ultraviolet absorbance as would be expected for a partially purified preparation of Phe-tRNA. Furthermore, charging activity for leucine and tyrosine was essentially zero for both samples of purified Phe-tRNA. Since Leu-tRNA and Tyr-tRNA are generally the most likely contaminants of Phe-tRNA (19, 24), it is apparent that substantial purification of Phe-tRNA had been achieved. The purification of the B. licheniformis tRNA was not as good as that of B. stearothermophilus. This may be due to the presence of contaminating tRNA's (see below) or to differences in relative amounts of isoaccepting tRNA's.

Properties of Phenylalanine Transfer RNA

I. Purity

The isolated Phe-tRNA had an ultraviolet absorption spectrum

typical of nucleic acids (Figure 3). The magnitude of the absorbance depended on the purity and water content of the preparation. A tRNA solution containing 1 mg/ml had an absorbance of about 6.2 and 10.8 for B. licheniformis and B. stearothermophilus, respectively. When the spectra of the two preparations were normalized at 260 nm, the absorbances at the remaining wavelengths were essentially superimposable and indicated the absence of significant amount of protein. This was further substantiated by direct analytical determinations, the results of which are shown in Table IV. It can be seen that both samples of the Phe-tRNA were essentially free of protein and DNA, but were contaminated to a small extent by leucine and tyrosine tRNA's. The results of Table IV for protein and DNA are calculated on the basis of the dry weight of Phe-tRNA, while those for Leu- and Tyr-tRNA's were calculated from the extent of charging of the isolated tRNA with ^{14}C -Leu and ^{14}C -Tyr. It is likely that the higher contamination of B. licheniformis Phe-tRNA accounts for the fact that this tRNA, at various stages of purification, had consistently lower specific activity than the B. stearothermophilus Phe-tRNA.

II. Charging Activity

The number of Phe-tRNA molecules which have become charged with phenylalanine during a particular assay can be calculated from the counts of ^{14}C -phenylalanine precipitated out with the tRNA at the end of the reaction. If one knows the total number of RNA molecules in the sample, one can then calculate the

FIGURE 3
Ultraviolet Absorption Spectra of Phe-tRNA's

Δ : B. licheniformis.

\bigcirc : B. stearothermophilus.

Solvent: 0.01 M tris-HCl (pH 7.2), 0.01 M MgCl_2 , and 0.001 M $\text{Na}_2\text{S}_2\text{O}_3$.

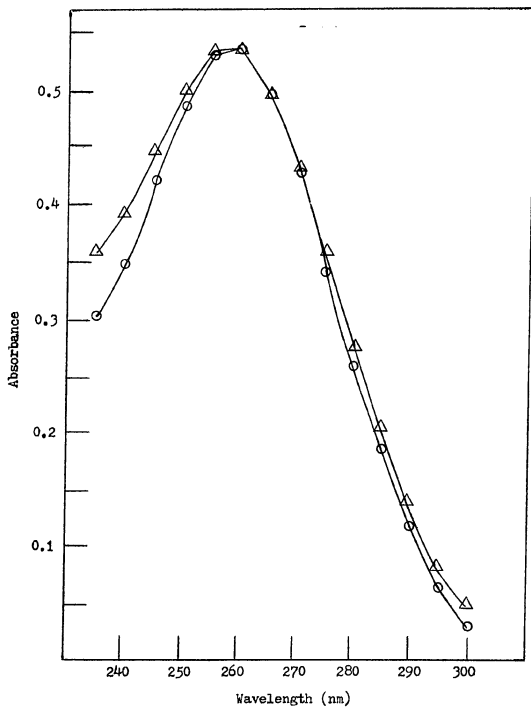


TABLE IV
Purity of Phenylalanine-tRNA

Organism	% Concentration			
	Protein ^a	DNA ^a	Leu-tRNA ^b	Tyr-tRNA ^b
<u>E. licheniformis</u>	< 0.5	< 1.0	8.9	9.3
<u>E. stearothermophilus</u>	1.0	< 0.8	1.2	5.0

^aWeight percent (see Methods).

^bMole percent (calculated from amino acid incorporation data).

percent of tRNA molecules which have become charged during the reaction. The total number of tRNA molecules was calculated from both phosphorus determinations and ultraviolet absorbance (see Methods) and on the assumption that an average tRNA molecule consists of 75 nucleotides and has a molecular weight of 2.4×10^4 . This is a reasonable assumption since all tRNA molecules characterized so far contain 70-80 nucleotides and have molecular weights of the order of 2.2×10^4 to 2.6×10^4 (31). The results of these calculations are shown in Table V. If the contamination of Phe-tRNA were limited to that by Leu- and Tyr-tRNA's, one might at first expect that the percentage value of tRNA charged with phenylalanine should have been about 80-90% (see Table IV). This, however, is not the case. Even pure Phe-tRNA need not show 100% charging efficiency since this depends on the kinetics of the reaction and the degree of denaturation or degradation of the Phe-tRNA. Thus, a charging percentage of 25 does not necessarily mean a contamination by 75% of non-Phe-tRNA. The content of Phe-tRNA may well be higher but not all of the molecules were active under the conditions of the assay.

III. Thermal Denaturation Profiles

The thermal denaturation profiles of Phe-tRNA are shown in Figure 4. The ratios of the absorbance at any temperature (corrected for thermal expansion) to that at 25° are plotted versus the temperature. The T_m values (midpoint of the absorbance change) were at 71.9° and 78.9° for B. licheniformis and B.

TABLE V
Charging of Phenylalanine tRNA

Organism	Concentration (nmoles per absorbance unit)		Charging (% of tRNA charged with Phe)
	Total tRNA	Phe-tRNA	
<u>B. licheni-</u> <u>formis</u>	(a) ^a	1.76	21.1
	(b) ^b	1.51	25.0
	(c) ^c	0.372	
<u>B. stearo-</u> <u>thermophilus</u>	(a) ^a	1.91	36.3
	(b) ^b	1.64	42.2
	(c) ^c	0.693	

^aColorimetric phosphorus determination.

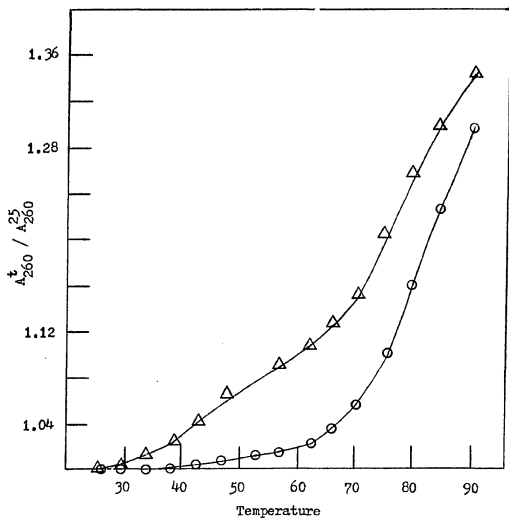
^bRNA concentration calculated from ultraviolet absorbance.

^cStandard radioactive assay for Phe-tRNA.

FIGURE 4

Thermal Denaturation Profiles of Phe-tRNA

 Δ : B. licheniformis.O : B. stearothermophilus.



stearothermophilus, respectively. The higher T_m for the thermophilic tRNA is in line with the higher thermal stability of other types of nucleic acids from thermophiles previously studied in our laboratory (11, 12). The shoulder in the profile for the mesophilic tRNA again indicates the higher degree of contamination in this preparation as already discussed in reference to Figure 1 and Table IV.

IV. Base Composition

The base composition data are summarized in Table VI. It can be seen that the base composition is essentially the same for both types of Phe-tRNA. At first glance this seems to contradict the results from thermal denaturation studies which would lead one to expect a higher (G + C) content in the thermophilic tRNA as shown previously for other thermophilic nucleic acids (11, 12). However, the base composition in Table VI refers only to the four major bases and has not taken into account the presence of minor bases which amount to about 15-20% in tRNA. These minor bases may or may not have migrated together with the four major bases. Therefore, the data in Table VI must be considered as being tentative. Furthermore, some of these minor bases form hydrogen bonds like the major bases and may, therefore, also be involved in the interpretation of thermal denaturation profiles.

V. Characteristics of the Phenylalanine Charging Reaction

A. Effect of Incubation Time

The effect of incubation time on the two types of Phe-

TABLE VI
Base Composition of Phenylalanine tRNA

	<u>B. licheniformis</u> (mole %)	<u>B. stearothermophilus</u> (mole %)
Adenine	22.9	23.9
Cytosine	17.6	20.3
Guanine	43.0	40.6
Uracil	16.5	15.2
Guanine + Cytosine	60.6	60.9

tRNA is shown in Figure 5. When the reaction mixture was incubated at 37° , B. licheniformis tRNA seemed to achieve maximum activity in 8 minutes, while B. stearothermophilus tRNA reached maximum activity within 5 minutes. Thereafter, in both cases, the activity declined slightly. B. licheniformis Phe-tRNA and B. stearothermophilus Phe-tRNA retained 88% and 79%, respectively, of their charging activity after 30 minutes of incubation.

The fact that the charging activity of B. stearothermophilus Phe-tRNA declined more than that of B. licheniformis Phe-tRNA might be due to the presence of small amounts of nucleases in the former tRNA, since it was slightly contaminated by protein (see Table IV).

B. Effect of Magnesium Concentration

The effect of Mg^{++} on phenylalanine charging is shown in Figure 6. Incubation was at 37° for 8 minutes. It can be seen that magnesium ions are an absolute requirement for phenylalanine charging in both B. licheniformis and B. stearothermophilus. When the Mg^{++} concentration dropped below 3 mM phenylalanine charging decreased drastically.

Phenylalanine charging required the same Mg^{++} concentration (6 mM) for maximum activity in systems from both B. licheniformis and B. stearothermophilus.

C. Effect of Incubation Temperature

The effect of incubation temperature on the charging reaction was studied in both homologous and heterologous

FIGURE 5

Effect of Incubation Time on Phenylalanine Charging

△ : B. licheniformis Phe-tRNA.

○ : B. stearothermophilus Phe-tRNA.

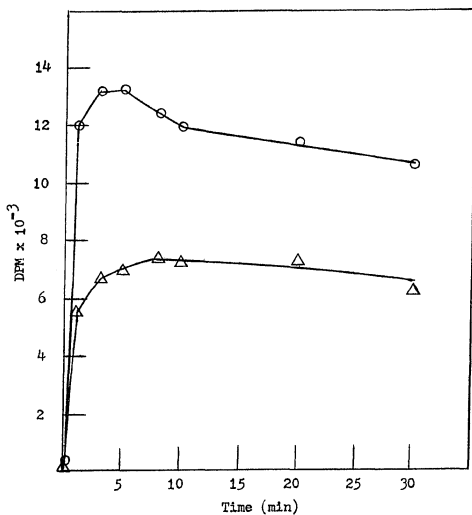
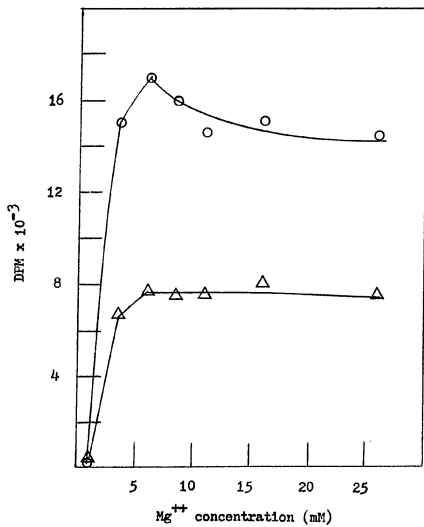


FIGURE 6
Effect of Mg^{++} on Phenylalanine Charging

- △ : B. licheniformis Phe-tRNA.
○ : B. stearothermophilus Phe-tRNA.



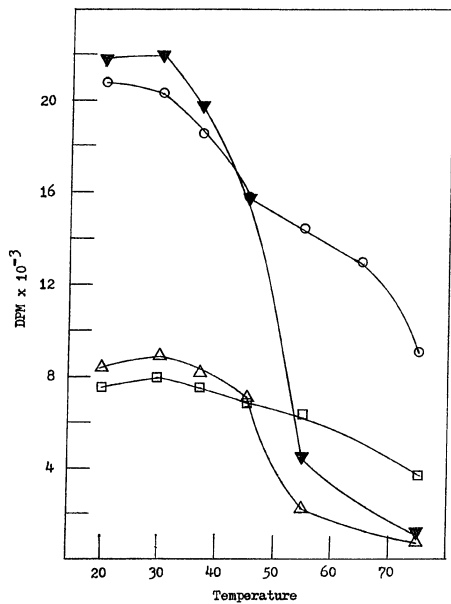
systems and the results are shown in Figure 7. Incubation was for 8 minutes.

In all cases, maximum activity was found to be around 30°. In the presence of aminoacyl-tRNA synthetase from B. licheniformis, charging activity of both B. licheniformis and B. stearothermophilus Phe-tRNA decreased greatly at higher temperatures, while with the aminoacyl-tRNA synthetase from B. stearothermophilus, both types of Phe-tRNA still retained about 50% activity at 75°. Hence, it can be concluded that Phe-tRNA from both B. licheniformis and B. stearothermophilus is quite heat stable. This agrees with the rather high T_m values of thermal denaturation (Figure 4). It can further be concluded that the synthetase from B. licheniformis is more heat sensitive than the one from B. stearothermophilus. This agrees with similar studies on other enzymes from thermophiles (32, 33).

FIGURE 7

Effect of Incubation Temperature on Phenylalanine Charging

- △ : B. licheniformis tRNA, B. licheniformis synthetase.
□ : B. licheniformis tRNA, B. stearothermophilus synthetase.
○ : B. stearothermophilus tRNA, B. stearothermophilus synthetase.
▼ : B. stearothermophilus tRNA, B. licheniformis synthetase.



SUMMARY

Phenylalanine tRNA was isolated from two strains of Bacillus. One of these was a mesophilic strain - B. licheniformis (NRS 243), and the other was a thermophilic strain - B. stearothermophilus 10.

Phe-tRNA from B. licheniformis was free of protein and DNA, but was somewhat contaminated by other tRNA's. Phe-tRNA from B. stearothermophilus was free of DNA but was slightly contaminated by protein and other tRNA's.

Both Phe-tRNA's had a similar base composition with a guanine plus cytosine content of about 60.8%. Both melted out at rather high temperatures, the one from B. stearothermophilus especially so. The T_m values were 71.9° and 78.9° for the Phe-tRNA from B. licheniformis and B. stearothermophilus, respectively.

Magnesium was required for phenylalanine charging of both Phe-tRNA's. For maximum charging a magnesium concentration of 6 mM was required. At 37° , maximum charging was achieved after eight and five minutes of incubation for the B. licheniformis and B. stearothermophilus system, respectively. While the Phe-tRNA's were rather heat stable, the aminoacyl-tRNA synthetases (especially the one from B. licheniformis) were found to be heat labile.

BIBLIOGRAPHY

1. Gaughran, E. R. L., *Bacteriol. Rev.*, 11, 189 (1947).
2. Allen, M. B., *Bacteriol. Rev.*, 17, 125 (1953).
3. Gaughran, E. R. L., *J. Bacteriol.*, 53, 506 (1947).
4. Koffler, H., Mallett, G. E., and Adye, J., *Proc. Natl. Acad. Sci. U.S.A.*, 43, 464 (1957).
5. Stenesh, J., and Koffler, H., *Federation Proc.*, 21, 406 (1962).
6. Campbell, L. L., *J. Amer. Chem. Soc.*, 76, 5256 (1954).
7. Campbell, L. L., *Arch. Biochem. Biophys.*, 54, 154 (1955).
8. Marmur, J., *Biochim. Biophys. Acta*, 38, 342 (1960).
9. Arca, M., Calvori, C., Frontali, L., and Tecce, G., *Biochem. Biophys. Res. Commun.*, 10, 117 (1963).
10. Arca, M., Calvori, C., Frontali, L., and Tecce, G., *Biochim. Biophys. Acta*, 87, 440 (1964).
11. Stenesh, J., and Holazo, A. A., *Biochim. Biophys. Acta*, 138, 286 (1967).
12. Stenesh, J., Roe, B. A., and Snyder, T. L., *Biochim. Biophys. Acta*, 161, 442 (1968).
13. Stenesh, J., Schechter, N., Shen, P. Y., and Yang, C., *Biochim. Biophys. Acta*, 228, 259 (1971).
14. Shen, P. Y., Coles, E., Foote, J. L., and Stenesh, J., *J. Bacteriol.*, 103, 479 (1970).
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, 193, 265 (1951).
16. Burton, K., in Grossman, L., and Moldave, K., *Methods in Enzymology*, vol. XII, part B, Academic Press, Inc., New York and London, 1968, p. 163-166.
17. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 66, 375 (1925).
18. Elson, D., *Biochim. Biophys. Acta*, 36, 362 (1959).

BIBLIOGRAPHY

Continued

19. Kelmers, A. D., Novelli, G. D., and Stulberg, M. P., J. Biol. Chem., 240, 3979 (1965).
20. Bendich, A., in Colowick, S. P., and Kaplan, N. O., Methods in Enzymology, vol. III, Academic Press, Inc., New York, 1957, p. 715-723.
21. Littauer, U. Z., Yankofsky, S. A., Novogrodsky, A., Galanter, Y., and Katchalski, E., Biochim. Biophys. Acta, 192, 29 (1969).
22. Avital, S., and Elson, D., Biochim. Biophys. Acta, 179, 297 (1969).
23. Gutcho, S., Biochim. Biophys. Acta, 157, 76 (1968).
24. Kelmers, A. D., J. Biol. Chem., 241, 3540 (1966).
25. Egan, B. Z., Rhear, R. W., and Kelmers, A. D., Anal. Biochem. 38, 139 (1970).
26. Egan, B. Z., Rhear, R. W., and Kelmers, A. D., Biochim. Biophys. Acta, 174, 23 (1969).
27. Gillam, I., Millward, S., Blew, D., Tigerstrom, M., Wimmer, E., and Tener, G. M., Biochem., 6, 3043 (1967).
28. Dudok, B. S., personal communication.
29. Dudok, B. S., Katz, G., Taylor, E. K., and Holley, R. W., Proc. Natl. Acad. Sci. U.S., 62, 941 (1969).
30. Roy, K. L., and Söll, D., Biochim. Biophys. Acta, 161, 572 (1968).
31. Holley, R. W., Apgar, J., Everett, G. A., Madison, J., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A., Science, 147, 1462 (1965).
32. Amelunxen, R., and Lins, M., Arch. Biochem. Biophys., 125, 765 (1968).
33. Amelunxen, R. E., Biochim. Biophys. Acta, 122, 175 (1966).

VITA

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