The Coding Properties of Lysine-accepting Transfer Ribonucleic Acids from Black-eyed Peas

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ABSTRACT

Lysine-accepting transfer RNA from ungerminated and germinated embryo axes of black-eyed peas (Vigna sinensis L. Savi) was fractionated on benzoylated diethylaminoethyl cellulose in the presence of two similar lysyl transfer RNA fractions in each tissue. Ribosome binding studies revealed that the larger of the two fractions in each case is specific for the AAG codon, while the smaller one recognizes AAA and AAG. Possible implications of differences in quantities of isoacceptors in translation of genetic information are discussed.

It has been suggested that protein synthesis may be regulated at translation by the particular complement of tRNAs and aminoacyl-tRNA synthetases present in the cell (2, 18, 20). According to several recent reports, plant tissues exhibit chromatographically detectable changes in isoaccepting species of tRNA during growth and differentiation, and it is inferred that these changes may be important in the growth process (3, 6, 22, 25). Anderson and Cherry (3) have shown quantitative differences among leucine isoacceptors in soybean cotyledon and hypocotyl tissue, respectively. Also, a synthetase preparation from the hypocotyl tissue was relatively inactive in acylation of two leucine tRNA fractions that occur in appreciable quantity only in the cotyledon. Vold and Sypherd (25) found differences in elution profiles of several acylated tRNAs when preparations from dry and germinated wheat were chromatographed. And recently, Vanderhoef and Key (22) have reported quantitative differences in tyrosine isoaccepting species prepared from different regions of roots of peas.

If different tissues or tissues in various stages of growth contain isoaccepting tRNAs with different anticodons, protein synthesis could be modulated by the particular population of adaptor molecules present. It is possible by means of codon recognition studies to ascertain whether or not an anticodon difference exists. Also, it is possible to determine at which sites in a protein molecule a particular isoaccepting tRNA inserts its amino acid. For example, Sekiya, Takeishi, and Ukita (19) have reported the isolation of four isoaccepting species of glutamic acid tRNA from yeast. One of the isoacceptors bound to ribosomes specifically in the presence of the GAG code word, while two species recognized both codons, GAA and GAG. The fourth isoacceptor bound only in the presence of GAA and showed a similar specificity for insertion of glutamic acid into hemoglobin. This last result is unexpected in view of Crick’s wobble hypothesis which predicts that a tRNA species recognizing a codon with A in the third position (GAA) will be unable to distinguish this codon from one with G in position three (GAG). Recently, Yoshida, Takeishi, and Ukita (28) have reported that the tRNA isoacceptor recognizing only GAA has a modified base (derivative of 2-thiouridine) in the first position of the anticodon. This modification apparently renders this isoaccepting tRNA species specific for the GAA code word. The possible significance of this coding modification in regulation of protein synthesis remains to be assessed.

These findings suggest two questions: (a) Do chromatographically detectable changes in isoaccepting tRNA species occur generally during growth and differentiation in higher plants? (b) Do changes in codon recognition among isoaccepting species occur during the same period?

We chose to investigate these questions in a germinating seed system. Vold and Sypherd (25) have previously reported that chromatography on methylated albumin kieselguhr of tRNA extracted from dry and germinated Pawnee wheat seeds revealed changes in certain isoacceptors. Resolution of isoaccepting tRNAs into separate fractions was not possible on these columns, but pronounced shifting of elution profiles occurred when lysyl-tRNAs were compared. On the basis of these observations, and the fact that the codon assignments for lysine (AAA, AAG) predict the same kind of ambivalent codon-anticodon interaction as those of glutamic acid, we decided to concentrate on tRNA isoacceptors of this amino acid. Since Pawnee wheat is a hexaploid plant (5) and may, therefore, be abnormally redundant in isoaccepting tRNAs owing to trivial differences in an allopolyploidic genome, we chose to investigate lysyl-tRNA changes in the diploid black-eyed pea plant (9).

Below we report experiments designed: (a) to seek changes in lysyl-tRNAs during germination by use of recently developed methods of tRNA fractionation, and (b) to characterize the codon recognition of these isoacceptors by ribosome binding studies.

MATERIALS AND METHODS

Plant Material. Black-eyed pea seeds (Vigna sinensis (L.) Savi) were obtained from local supermarkets. Embryo axes were prepared in bulk from the dry seeds by a modification of the method of Johnston and Stern (12). Whole seeds were ground in a blender for three intervals of about 3 sec each. Broken seeds were screened, and the material passed by a 2-mm pore diameter screen and retained by a 1-mm pore screen was stirred into a mixture of carbon tetrachloride and cyclohexane (25:10). Embryo axes floated to the top and were skimmed off.
After drying, residual seed coat material was removed with forceps. As has been previously shown for wheat seed (10), this procedure does not affect viability. Growth of the intact pea axes over a 4-day period was qualitatively the same with bulk-isolated axes as with hand-dissected ones.

Seeds were germinated in 1-pound lots on circular screens attached around their circumference to Lucite rings. Four of these 2-inch high cylinders were stacked in a plastic bucket with drainage holes at the bottom. Tap water at about 25°C was sprayed over the top layer of seeds, and this trickled down through the underlying layers. After 3 to 4 days of growth in the dark, cotyledons were removed from the seedlings by hand, and the axes were immediately frozen in liquid nitrogen and subsequently lyophilized.

Transfer RNA Preparation. Embryo axes were ground in 95% ethanol and extracted with ethanol and ether according to Vold and Sypherd (24). RNA was extracted from the resultant dried powder by the phenol method of Kirby (13). The powder was stirred for 4 hr in equal parts of (a) a buffer mixture: 10 mM tris-Cl, pH 7.0; 10 mM magnesium acetate; 50 mM NaCl; 0.5% naphthalenedisulfonate (w/v) and (b) redistilled phenol, pH 7.0, saturated with 10 mM tris-Cl and containing 7.1% cresol (v/v) and 0.1% hydroxyquinoline (w/v). After extraction and centrifugation at 10,000 g for 10 min, the buffer phase was removed, re-extracted with fresh phenol solution, and centrifuged again. RNA was precipitated from the final buffer phase by adding 2.5 volumes of ethanol at −20°C and storing for at least 4 hr at −20°C. Ethanol was removed by decanting after centrifugation; the pellet was dried under reduced pressure and extracted with a solution of 1.0 M NaCl and 10 mM MgCl₂.

After standing for 4 hr, centrifuging, and drying further, the RNA pellet was dissolved in 10 mM sodium acetate, pH 4.5, containing 10 mM MgCl₂ and 0.30 M NaCl and applied to a preparative B-D cellulose column (4 × 10 cm). The column was washed with the same buffer until the A₂₆₀ fell to 0.1 or below, and RNA was removed by elution with 1.2 M NaCl containing 10% ethanol (v/v). RNA was precipitated from the eluate by addition of 2 volumes of ethanol at −20°C. After centrifuging and drying, the final pellet was dissolved in 1.2 M MgCl₂ and stored at −70°C.

Aminoacyl Synthetase Extraction. Synthetases were extracted from ungerminated and germinated lyophilized axes by a modification of the method of Yang and Novelli (27). Tissue was ground at liquid nitrogen temperature with mortar and pestle until powdered and then extracted with a solution of 10 mM tris-Cl, pH 7.4: 5 mM MgCl₂; 5 mM dithiothreitol; and 10% glycerol (v/v). The extraction medium also contained insoluble polyvinylpyrrolidone (Polyvinyl, General Aniline and Film Corp., New York, N. Y.), 0.1 g/g of tissue (15). The mixture was stirred gently by hand for 15 min and then filtered through several thicknesses of cheesecloth and centrifuged at 15,000 g for 15 min. The lipid pellicle was removed, and the supernatant liquid was further centrifuged at 198,000 g for 1.5 hr in a Beckman model L2-65 B ultracentrifuge. Protein in this supernatant fraction was concentrated by precipitating with 4 parts of saturated ammonium sulfate, pH 7.0, and centrifuging at 10,000 g. The protein pellet was dissolved in a minimal volume of 10 mM potassium phosphate buffer, pH 7.5. 0.5 mM dithiothreitol; 5 mM KCl; and 10% glycerol (v/v) and applied to a Sephadex G-100 column. Synthesis purification was continued according to Yang and Novelli (27). After elution of protein containing synthetase activity from DEAE-cellulose, the active fractions were pooled and glycerol concentration was increased to 40% (v/v) by addition of glycerol at −20°C. This preparation was stored in small aliquots at −20°C. There was no appreciable loss of activity over a period of 3 months.

Aminoacylation. Charging (aminoacylation) assays were carried out in 50-μl volumes by a filter paper disk technique. The reaction mixture contained 0.40 A₂₆₀ unit of tRNA and 0.05 A₂₆₀ unit of synthetase protein in a solution of 5 mM HEPES, pH 7.5; 10 mM magnesium acetate; 50 μM ATP; 10 μM CTP; and 50 mM "C-lysine (25 μCi/μmole). After 25 min of incubation at 31°C, a 40-μl aliquot was withdrawn and spotted onto a Whatman No. 1, 24-mm filter disk. This was dropped into beaker containing 10% trichloroacetic acid at 4°C. After two subsequent washings in fresh trichloroacetic acid the disk was twice washed in ethanol at −10°C, dried, and placed in vials containing 5 ml of scintillation fluid (0.5% PPO in toluene). Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer (model 3375).

For acylation of larger quantities of tRNA for chromatography, the procedure above was scaled up. After the 25-min incubation an equal volume of 10 mM sodium acetate, pH 4.5, was added to the incubation tube, and protein was precipitated by the addition of an equal volume of phenol. After centrifugation the buffer phase was applied to a Sephadex G-25 column (2 × 40 cm) and tRNA was eluted at the first 254 nm absorbing peak. An aliquot was removed for counting, and the remainder was loaded immediately onto analytical columns (B-D cellulose or reverse phase Freon) or was precipitated with ethanol at −20°C for use later.

B-D Cellulose and Reverse Phase Freon Chromatography. Benzoylated DEAE-cellulose was prepared according to Gilham et al. (10). For fractionation of 50 or more A₂₆₀ units of tRNA a 1.5 × 60-cm column was used; for smaller amounts of RNA a 0.5 × 35-cm column sufficed. (In estimating quantities of tRNA, it was assumed that the A₂₆₀ of 1 mg of tRNA = 24). Transfer RNA was eluted with a linear gradient of 0.3 to 1.2 M NaCl containing 10 mM sodium acetate, pH 4.5, and 10 mM MgCl₂. At the end of the gradient the elution the column was washed with 1.2 M NaCl containing 10% ethanol (v/v). Absorbance at 254 nm was monitored with an Isco model UA-2 ultraviolet analyzer, and all fractions containing ultraviolet-absorbing material were prepared for counting. Suitable aliquots (usually 0.5 ml) were added to vials containing 10 ml of scintillation fluid of the following composition: 5.5 g of PPO, 0.1 g of POPOP, 333 ml of purified Triton X-100 (Packard Instrument Co.), and 667 ml of toluene. The vials were chilled, and radioactivity was determined in the liquid scintillation spectrometer. Carbon-14 was counted at 28% efficiency and tritium at 27%. Counting data were converted to disintegrations per minute by external standardization.

Unfractionated tRNA or lysyl-tRNA from B-D cellulose columns was also chromatographed on reverse phase Freon columns (Freon-214; E. I. DuPont de Nemours and Co.). The resin was prepared according to Weiss and Kelmers (26) and packed into a 0.9 × 50-cm column under gravity flow. A linear gradient of 0.25 to 0.65 M NaCl containing 10 mM sodium acetate, pH 4.5, and 10 mM MgCl₂ was used to elute the tRNA.

Ribosome Binding Studies. Trinucleotides, AAA and AAG, and polynucleotides, polyadenylic acid and polyuridylic acid, were purchased from Miles Laboratories, Elkhart, Indiana. Copolyadenylicguanylic acid (A: G = 1:1.5) was purchased from P-L Biochemicals, Milwaukee, Wisconsin.

The binding studies were modeled closely after those originally described by Nirenberg and Leder (18). The 50-μl incubation solution of 0.1 M tris-Cl, pH 7.2: 15 or 20 mM MgCl₂; and 50 mM KCl contained 1.0 A₂₆₀ unit of Escherichia coli ribo-
lysyl-tRNA (75 unfractionated some, 0.21 A_{260} unit of trinucleotides or 0.35 A_{260} unit of poly-
nucleotides, and tRNA as indicated in Table I. Pea tRNA was
acylated with "C-lysine (100 μc/μmole) or "H-lysine (500 μc/
μmole) by the homologous enzyme system. E. coli tRNAs, "C-
lysyl-tRNA (75 μc/μmole), and "C-phenylalanyl-tRNA (167 μc/
μmole), were gifts from Dr. John R. Menninger.

Preliminary work indicated that preincubation of adenine-
rich nucleotides with the ribosomes prior to the addition of
magnesium ions eliminated a tendency of the polynucleotides
to precipitate in the presence of the cations. Therefore, the fol-
lowing sequence of steps was standardized for all assays: ribo-
somes and nucleotides were added together, mixed, and incu-
bated for 10 min in a 20 C waterbath. Next, salts and labeled
aminoacyl-tRNA were added; the components were again mixed
briefly and then incubated for 30 min at 20 C.

After incubation the reaction was stopped by dilution with 5
ml of ice-cold incubation buffer, and tubes were placed on ice.
Diluted reaction mixtures were filtered through nitrocellulose
membranes (Bac-T-Flex, B-6, Schleicher and Schuell) and
washed with three 5-ml portions of cold buffer solution. Filters
were dried under an infrared lamp and placed in vials contain-
ing 5 ml of toluene-PPO scintillation fluid for determination of
radioactivity. Counting efficiencies of 92% for carbon-14 and
24% for tritium were determined by internal standardization.

RESULTS

Charging assays revealed that acylation of tRNA from dry
and germinated embryos with their respective synthetases is es-
entially complete in 25 min. There was no detectable loss of
trichloroacetic acid-precipitable radioactivity on longer incu-
bation. It was calculated from the number of picomoles of la-
beled amino acid bound to tRNA that approximately 2% of
our unfractionated preparations consist of lysine-accepting
species.

Acylation with labeled lysine is usually 10 to 15% greater
when the synthetase preparation from the dry seed is used in
the assay, whether the source of the tRNA is dry or germinated
tissue. Preliminary studies show that the —CCA phosphorylase
that adds the terminal nucleotides to tRNA is highly active in
the dry seed synthetase preparation but almost inactive in
the germinated embryo preparation. It is possible that this enzy-
me in the dry seed preparation completes termini of some tRNA
chains rendering them competent for acylation, and that these
remain uncharged when the germinated enzyme preparation is
used. This has not yet been clarified.

For chromatography each tRNA was acylated with the ho-

Fig. 1. Lysyl-tRNA elution patterns of preparations fraction-
ated on B-D cellulose. Fifty A_{260} units each of ungerminated and
germinated tRNA charged with "C-lysine (100 μc/μmole) and "H-
lysine (300 μc/μmole), respectively, by the homologous synthetase
preparation.

Fig. 2. Lysyl-tRNA elution patterns of two ungerminated
preparations fractionated on B-D cellulose. One preparation
was acylated with "C-lysine and the other with "H-lysine by the same
enzyme preparation.

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308 and acylated germinated peaks I, cellulose columns. It tRNA of quantity radioactive contamination on were rechromatographed on B-D cellulose and reverse phase column, respectively, by the homologous synthetase preparation.

Each of the lysine tRNA fractions from B-D cellulose was acylated and rechromatographed on reverse phase Freon columns. Figure 3 shows the pattern of elution of dry and germinated peaks I, when they were acylated with $^{3}C$-lysine and $^{1}H$-lysine, respectively, and cochromatographed by reverse phase. These two peaks elute as a single fraction and appear to be free of contaminating lysyl-tRNA species. Therefore, the B-D cellulose lysine tRNA peaks I were used in recognition studies without further purification.

Figure 4 illustrates comparable reverse phase chromatography of B-D cellulose peaks II. In this case two radioactive fractions were observed. The first fraction, from its position in the salt gradient and the fact that only two fractions of lysyl-tRNA are found when unfraccionated tRNA is chromatographed on reverse phase columns (Fig. 5), appears to represent contamination of the B-D cellulose peaks II with peaks I material. It was much easier to prepare peak I from B-D cellulose free of peak II because of the relatively greater quantity of material under peak I. Therefore, in preparing peak II fractions for codon recognition studies, each was labeled with radioactive lysine and separately chromatographed on reverse phase columns. Peak II fractions were then precipitated free of peak I.

Peak III of germinated lysyl-tRNA from B-D cellulose was rechromatographed on Freon columns with unfraccionated, germinated tRNA as a marker. Results of this experiment are shown in Figure 6. Peak III acylated with $^{3}H$-lysine shows the same elution pattern as the unfraccionated tRNA charged with $^{3}C$-lysine. The gradient on this column was carried out to 1.0 m NaCl to ensure that any RNA that might elute at high salt concentrations would be removed.

To define this situation further, we precipitated the radioactive material from the Freon column (Fig. 6) and rechromatographed it on B-D cellulose. Figure 7 shows that the elution pattern of the original B-D peak III (tritium curve) is very similar to the profile of the unfraccionated tRNA after this procedure. That is, the former peak III appears to contain lysyl-tRNA that now elutes in peak I and peak II positions only.

In view of the variability in quantity of B-D peak III in unfraccionated, germinated tRNA and its subsequent resolution into peaks I and II on further chromatography, we think that this material may be an association of reversibly modified molecules found normally under peaks I and II. Dimers of alanine transfer RNA with acceptor activity that elute from B-D cellulose and reverse phase columns later in the salt gradient than the monomer have been described by Loeh and Keller (14). At the present time we are unable to explain the fact that we see this fraction only in the germinated preparations. We are currently studying the peak III fraction to determine the relative size of the molecules comprising it and the conditions required for its appearance and disappearance.

Chromatography of unfraccionated tRNAs charged with

![Fig. 3. Rechromatography of B-D cellulose peak 1 fractions on reverse phase column. Seven and eight-tenths $A_{260}$ units of ungerminated and 4.4 $A_{260}$ units of germinated peak 1 charged with $^{3}C$-lysine and $^{1}H$-lysine, respectively, by the homologous synthetase preparation.](image)

![Fig. 4. Rechromatography of B-D cellulose peak 2 fractions on reverse phase column. Ten $A_{260}$ units each of ungerminated and germinated peak 2 charged with $^{3}C$-lysine and $^{1}H$-lysine, respectively, by the homologous synthetase preparation.](image)

![Fig. 5. Elution patterns of lysyl-tRNA from reverse phase column. Ten $A_{260}$ units each of ungerminated and germinated tRNA were charged with $^{3}C$-lysine (100 $\mu$C/umole) and $^{1}H$-lysine (300 $\mu$C/umole), respectively, by the homologous synthetase preparation.](image)

![Fig. 6. Reverse phase cochromatography of lysyl-tRNA peak 3 fraction from B-D cellulose and unfraccionated, germinated tRNA. Three $A_{260}$ units of B-D cellulose peak 3 were acylated with $^{1}H$-lysine and 15 $A_{260}$ units of unfraccionated tRNA were acylated with $^{3}C$-lysine by a germinated synthetase preparation. Transfer RNA eluted with a 500-ml linear gradient, from 0.25 to 1.0 m NaCl. Four-milliliter fractions were collected. Unfraccionated tRNA used here was previously shown to contain no B-D peak 3 material.](image)
labeled lysine and fractionated on B-D cellulose or reverse phase Freon columns can be compared in Figures 1 and 5. The particular preparation of germinated tRNA used for the Freon chromatography (Fig. 5) was one that contained very little B-D peak III material, and only two peaks are seen on Freon chromatography. The major and minor peaks from the Freon column are in the same relative position in the salt gradient as peaks I and II from B-D cellulose. The work discussed above indicates that either resin is capable of differentiating the same two lysine isoaccepting species. In our hands, the reverse phase Freon columns gave somewhat better resolution of the isoacceptors.

The unfraccionated tRNAs, G and D (G: germinated tissue; D: ungerminated tissue) and the peaks derived chromatographically from them, two from the dry seed axis (DI and DII) and two from the germinated axis (GI and GII), were analyzed in the ribosomal binding assay (Table I). The results indicate that DI and GI recognize only the AAG codon, whereas DII and GII each recognize both lysine codons, AAG and AAA.

Controls included to test the proper functioning of the assays all gave adequate results. Binding of unfraccionated E. coli $^{14}$C-lysyl-tRNA to ribosomes was stimulated by AAA, AAG, poly A, and poly AG and was not stimulated by poly U. On the other hand, $^{14}$C-phenylalanyltRNA from E. coli showed good binding with poly U in the system. It was also observed that addition of a "nonrecognizable" polynucleotide to the binding system; i.e., poly U in a system with $^{14}$C-lysyl-tRNA, reduced the background binding. This has been previously reported by Nirenberg and Leder (18).

**DISCUSSION**

Transfer RNA may function in control of protein synthesis in a variety of ways. There are at least three functional "sites" in each tRNA molecule: (a) aminoacyl-tRNA synthetase recognition site, (b) codon recognition site, and (c) ribosome binding sites. Direct regulation of protein synthesis might be possible by modification of any or all of these sites. Gross

![Diagram](image-url)
effects on total protein synthesis or more subtle regulation affecting translation of selected messengers can be envisioned.

These possibilities have led to much recent work on the tRNAs of prokaryotic and eukaryotic cells (3, 4, 11, 16, 17, 21, 22, 25, 27). Changes in isoaccepting tRNAs during growth and differentiation have been sought as possible control points of translation. Quantitative differences in isoaccepting species in different plant cells and tissues have been reported (3, 22, 25). In particular, Vold and Sypherd (25) observed that certain tRNAs (especially lysyl-tRNA) of Pawnee wheat change qualitatively or quantitatively during germination.

We studied the coding properties of lysyl-tRNA isoaccepting species of the black-eyed pea during germination and were successful in separating lysyl-tRNAs into at least two species having different recognition properties. Under the conditions of the binding assay we found that fractions D1 and G1 bound strongly in the presence of AAG, one of the two lysine codons, and not at all in the presence of the other codon, AAA. These results are mirrored in the experiments with poly A and poly AG. D11 and G11, on the other hand, both bound well in the presence of either lysine codeword. Thus, one member of the pair of lysine tRNAs in either stage of development can “see” both lysine codons, while the other recognizes only AAG. This is in agreement with Crick’s wobble hypothesis (6), which predicts a C in 3’ anticodon position will recognize only G in the 3’ codon position, while U will recognize both A and G. Of course, to discuss the coding properties of peak II fractions in terms of the wobble hypothesis presupposes that these fractions represent single species, and we have only our column data in evidence of this. Nevertheless, if D11 and G11 do represent the same single species, our binding studies indicate that ambivalent codon-anticodon interaction occurs in this species.

Since it has been demonstrated that a modification of uridine in the 3’ anticodon position of a species of glutamic acid tRNA from yeast renders this isoacceptor specific for the GAA codeword (28), it seemed possible that a similar modification of uridine in the analogous position of a lysine isoaccepting species might occur. However, none of the species that we characterized showed ribosome binding only in the presence of AAA, and we assume that uridine is not similarly modified in the 3’ position in the lysine isoacceptors of the pea plant.

We found no consistent differences in patterns of elution of dry or germinated seed lysyl-tRNA from B-D cellulose or reverse phase Freon columns. A major and a minor peak were consistently recovered from both columns, and these differed in coding properties. In a single dry tRNA preparation we observed a disproportionately large quantity of peak II. It is futile to speculate on the etiology of this, but it would seem to emphasize the need for examining several different preparations of tRNA before drawing generalizations. We are currently investigating B-D cellulose peak III, found exclusively in the germinated preparations, but we believe this to represent an aggregated state of molecular species, not a true difference between the dry and germinated tRNAs.

Although fraction I from B-D cellulose chromatography is composed of prokaryotic tRNAs that recognizes only the AAG codon, this fraction seems to constitute about three-quarters of the total lysine tRNA in these tissues. This quantitative difference is interesting for it may be a reflection of a greater number of AAG codons than AAA codons in the messenger RNAs of this organism. If this is the case, one might then ask why the cell synthesizes more of the tRNA species recognizing the AAG codon than of the species recognizing both codons. Is the relative efficiency of translation of AAG code words the same for each of the two species during protein synthesis?

These questions may be investigated in a cell-free protein-synthesizing system. Supplying the plant cell-free system with the two differentially acylated isoacceptors (H-lysine, “C-lysine) and analyzing the incorporation of lysine into trichloroacetic acid-precipitable material via each isoaccepting species would give information about the codons present in the natural messengers. Further refinement of this experiment consisting of tryptic hydrolysis of the protein synthesized in vitro and separation and analysis of radioactivity in lysine-containing peptides could yield information on the efficiency of recognition of the AAG codon by each of the two isoacceptors. Unfortunately, at the present time plant cell-free preparations are ineffective protein-synthesizing systems (7). We have recently begun development of a plant system which we hope to use for this work.

In lieu of a plant system, we are currently investigating the question of efficiency of codon recognition by these two isoaccepting species in a hemoglobin-synthesizing cell-free system (1) using the methods explained above. Similar studies are being carried out on the incorporation of lysine into hemoglobin via the rabbit reticulocyte tRNA isoacceptors (Dr. W. R. Woodward, personal communication), and, therefore, we will also be able to compare this incorporation pattern with that of the heterologous pea tRNA.

Acknowledgments—We are grateful to Dr. J. R. Menninger and Dr. W. R. Woodward for advice during this investigation and for critically reading the manuscript. We further acknowledge Dr. Menninger’s gifts of 32P-phenylalanyl-tRNA, 34C-lysyl-tRNA, and ribosomes from E. coli.

LITERATURE CITED


