Purification of Chicken Liver Seryl Transfer Ribonucleic Acid by Complex Formation with Elongation Factor EF-Tu:GTP. A General Micromethod of Aminoacyl Transfer Ribonucleic Acid Purification†

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ABSTRACT: The interaction of chicken liver AA-tRNA with *Escherichia coli* elongation factor EF-Tu–GTP was studied. Formation of the ternary complex, AA-tRNA–EF-Tu–GTP, was quantitative and the complex participated in polynucleotide-directed but not triplet-directed ribosomal binding. The complex was isolated free of nonacylated tRNA by chromatography on Sephadex G-100. Ser-tRNA isolated as a ternary complex had an average purity greater than 78% and could be directly fractionated into isoaccepting species by chromatography on benzoylated DEAE-cellulose. The purification procedure utilizes small amounts of tRNA (0.5–15 nmol of AA-tRNA) and is rapid, quantitative, and reproducible. Results with Leu-, Lys-, and Phe-tRNA indicate that the procedure may be used with other AA-tRNAs.

The availability of procedures for the purification of specific tRNAs from the limited amount of total tRNA that can be isolated from metazoan organs would greatly facilitate study of the properties of individual tRNA species. The chemical derivatization procedure of Gillam *et al.* (1968) has been widely used to isolate specific isoaccepting tRNA species, but requires relatively large amounts of tRNA and does not work well with the acceptors for certain amino acids such as threonine (Gillam *et al.*, 1968) and serine (Roy and Söll, 1970). tRNA from specific organs of metazoans has been purified by a variety of procedures (Fink *et al.*, 1968; Müller *et al.*, 1971; Pearson *et al.*, 1973; Petrissant *et al.*, 1971), usually repeated column chromatography, but in each instance a large amount of starting material was required.

Ser-tRNA is of interest to us because of the specific increase in serine acceptance induced in rooster liver tRNA by estrogen administration (Mäenpää and Bernfield, 1969). Therefore, to study several aspects of this system, we developed a purification procedure capable of isolating $tRNA^{Ser}$ from small quantities of unfractionated tRNA. The method uses the *Escherichia coli* elongation factor EF-Tu, which as EF-Tu–GTP reacts specifically with AA-tRNA to form a ternary complex (AA-tRNA–EF-Tu–GTP) which can be separated from unreacted tRNA by gel filtration on Sephadex G-100 (Ravel *et al.*, 1968; Skoultchi *et al.*, 1968). The procedure is rapid, reproducible, and quantitative, and results in the isolation of nanomole quantities of isoaccepting sets of AA-tRNAs in high purity. The purification procedure is described in this communication, and its use in the study of $tRNA^{ser}$ synthesis is presented in the accompanying paper (Klyde and Bernfield, 1973).

Materials and Methods

Radioisotopes. [^{14}C]- and [^{3}H]amino acids were obtained from New England Nuclear and Amersham/Searle. [^{3}H]GTP (1.38 Ci/mmol) was purchased from Schwarz/Mann.

Preparation of tRNA. The purification procedure requires that the unfractionated tRNA be free of higher molecular weight polynucleotides and be deacylated as completely as possible. The following tRNA preparation procedure was found to be adequate. Livers from 10-day-old white Leghorn chicks were the primary source of tRNA; experiments using tRNA from rooster liver are noted in the figure legends. Animals were decapitated and livers were quickly removed, chilled in cold saline, and weighed. The tissue was disrupted (Sorvall Omnimixer; setting of 8) at 4° for 2 min in a mixture of one volume (ml/g) of pH 5.0 buffer containing 0.05 M NaCl, 0.01 M EDTA, 0.1 M sodium acetate, 10 USP units/ml of heparin, 5 mg/ml of purified bentonite (Fraenkel-Conrat *et al.*, 1961), and two volumes of water-saturated redistilled phenol. The mixture was centrifuged (20,000*g*, 10 min, 0°) and the aqueous layer was further extracted in the Omnimixer with an equal volume of phenol at 0°. After recovery, the aqueous phase was extracted with an equal volume of $CHCl_3$, and the tRNA was precipitated by addition of 0.1 volume of 2 M potassium acetate (pH 5.0) and 2.2 volumes of 95% ethanol. After 1 hr at −20°, the tRNA was collected by centrifugation and deacylated for 90 min with the procedure of Sarin and Zamecnik (1964). The tRNA was precipitated as above and redissolved in 0.2 M potassium acetate (pH 5.0), and the solution was layered over 5% sucrose in the same buffer. After centrifugation (105,000*g*, 2° for 2 hr) to pellet glycogen, the tRNA in the supernatant was precipitated as above. The precipitate was dissolved in a solution of 0.1 M Tris-HCl and 0.02 M $CuSO_4$ (pH 7.4), and incubated at 37° for 30 min to deacylate the tRNA a second time (Schofield and Zamecnik, 1968). The tRNA was recovered by ethanol precipitation (potassium acetate was omitted) and freed of cupric ion by three consecutive ethanol precipitations from 1 mM EDTA. The tRNA was then dissolved in 0.5 M NaCl, 0.01 M $MgCl_2$, and 0.005 M sodium acetate (pH 4.5) and passed through a column of Sephadex G-100 (2.5 × 100 cm, flow rate 30–35 ml/hr) in the same buffer at room temperature. The major A_{254}-absorbing peak, corresponding to tRNA, was precipitated with ethanol, redissolved, and passed through the

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G-100 column a second time to insure that no 5S RNA or tRNA aggregates remained. The tRNA peak from the second column was precipitated, dissolved in water, and stored at −20°. Several early preparations included a DNase step to eliminate DNA, but the gel filtration step was found to make this unnecessary.

Preparation of AA-tRNA Synthetases and Acylation of tRNA. Preparation of synthetases and tRNA acylation were carried out as previously described (Mäenpää and Bernfield, 1969) except that the synthetase preparations were passed through a Sephadex G-25 column immediately before use to remove free amino acids. One milliliter of synthetase was passed through a column of Sephadex G-25 (1.8 × 5.0 cm, flow rate 20 ml/hr, 4°) in the buffer used in preparing the synthetase. Assays of the enzyme eluted in the void volume determined the amount of synthetase to be used in preparative acylations; an enzyme concentration at which tRNA was limiting was used in each instance. Acylation reactions were stopped by the addition of 0.1 volume of 2 M potassium acetate (pH 5.0) and 2.2 volumes of 95% ethanol. After 1–4 hr at −20° the precipitated acylated tRNA was collected by centrifugation and dissolved in 1 ml of 0.5 mM potassium cacodylate (pH 5), and insoluble protein was removed by centrifugation. Acylated tRNA prepared in this way was found to react as well with EF-Tu–GTP as acylated tRNA which had been deproteinized by two phenol extractions.

Purification of EF-Tu. EF-Tu was prepared from *E. coli* using the procedure of Miller and Weissbach (1970) through the Sephadex G-100 step. Identical results were obtained with a EF-Tu preparation kindly provided by Dr. H. Weissbach. The purified EF-Tu was stored as EF-Tu–GDP in aliquots under liquid nitrogen in a buffer containing 0.05 M Tris-HCl (pH 7.8), 0.01 M $MgCl_2$, and 0.001 M dithiothreitol. Each aliquot was thawed once, adjusted to a concentration of 0.005 M dithiothreitol, and stored at 0°. The thawed EF-Tu showed no appreciable loss of [^{3}H]GTP exchange activity for at least 3 weeks. EF-Tu concentrations were determined by the [^{3}H]GTP exchange assay (Weissbach *et al.*, 1970). An EF-Tu preparation is available commercially (Miles Laboratories, Inc.), but has not been assessed.

Determination of Purity of Isolated AA-tRNA. tRNA purity was assessed by comparing the pmoles of amino acid, determined by measuring amino acid radioactivity, with the amount of tRNA, established by organic phosphate analysis. Samples for organic phosphate analysis received 100 μg of bovine serum albumin, were made 10% in trichloroacetic acid, chilled on ice for 20 min, and centrifuged (10 min 30,000*g*) to precipitate the tRNA and carrier protein. The precipitate was washed with 10% trichloroacetic acid, suspended in 0.1 ml of water and analyzed for organic phosphate using the method of Ames (1966). GTP in the ternary complex was shown to be removed during trichloroacetic acid precipitation and washing. tRNA concentrations were expressed in A_{260} units[1] and were determined by organic phosphate analysis of each purified AA-tRNA preparation in parallel with tRNA solutions of known concentration in A_{260} units.

Ribosomal Binding of Ternary Complex. E. coli ribosomes were prepared by the method of Brot *et al.* (1970). Ternary complex was prepared and reacted with ribosomes by the procedure of Weissbach *et al.* (1971). The tRNA used for ribosomal binding was isolated from rat livers using the method of Mäenpää and Bernfield (1969) which included only a 0.1 M, pH 9.0, Tris-HCl deacylation step. Poly(U), poly(U,C), poly(A,G,U), and UpUpU were obtained from Miles. ApGpC was prepared from GpC (Miles Laboratories) with polynucleotide phosphorylase (Leder *et al.*, 1965).

BD-cellulose Chromatography. Chromatography of $tRNA^{Ser}$ species on BD-cellulose was performed as previously described (Mäenpää and Bernfield, 1969).

TABLE I: Ternary Complex Formation With Different AA-tRNAs, as Measured by the Nitrocellulose Filter Assay.

Amino Acid on tRNA	pmole of Complex Formed/ pmole of AA-tRNA Added[a]
Glu	1.03
His	1.52
Leu	1.16
Lys	0.85
Ser	0.95
Tyr	1.33

[a] Amount of AA-tRNA added was calculated by adding together the unlabeled AA-tRNA, determined by EF-Tu binding prior to acylation with the labeled AA-tRNA, and labeled AA-tRNA determined by acid precipitation. The mean ratio of pmoles of complex formed to pmoles of AA-tRNA added was 1.14.

Results and Discussion

The feasibility of purifying specific, acylated metazoan tRNA species as a heterologous complex with *E. coli* EF-Tu–GTP was demonstrated by (a) quantitative complex formation as measured by the nitrocellulose filter assay, (b) polynucleotide-directed ribosomal binding of the heterologous complex which showed that the complexes were functional and not merely artifactual, and (c) chromatographic isolation of the complex.

Ternary Complex Formation with Different AA-tRNAs. The nitrocellulose filter assay of Weissbach *et al.* (1971) was used to determine if chick liver tRNA acylated with different amino acids forms ternary complexes with *E. coli* EF-Tu–GTP. The results summarized in Table I show that chick liver tRNA acylated with radioactive glutamic acid, histidine, leucine, lysine, serine, or tyrosine could participate in complex formation, and that complex formation was approximately quantitative under conditions where the acylated tRNA concentration was limiting. Further details will appear in a subsequent publication.[2]

Template-Directed Ribosomal Binding of Ternary Complex. Rat liver [^{14}C]Phe-tRNA in a ternary complex with EF-Tu–GTP was bound to ribosomes in response to poly(U) but did not bind when UpUpU was used as template (Figure 1A). Similarly, ternary complex containing [^{14}C]seryl-tRNA responded to poly(U,C) or poly(A,G,U), but did not bind to ribosomes when ApGpC was used as template (Figure 1B), suggesting that under these conditions, metazoan tRNA complexed with *E. coli* EF-Tu–GTP will bind to *E. coli* ribosomes in response to polynucleotides, but not in response to trinucleoside diphosphate codons.

[1] An A_{260} unit of RNA is defined as that quantity which, when dissolved in 1 ml of distilled water, gives a solution having an absorbance of 1 at a path length of 1 cm and a wavelength of 260 nm.

[2] E. R. Shelton and M. R. Bernfield, manuscript in preparation.

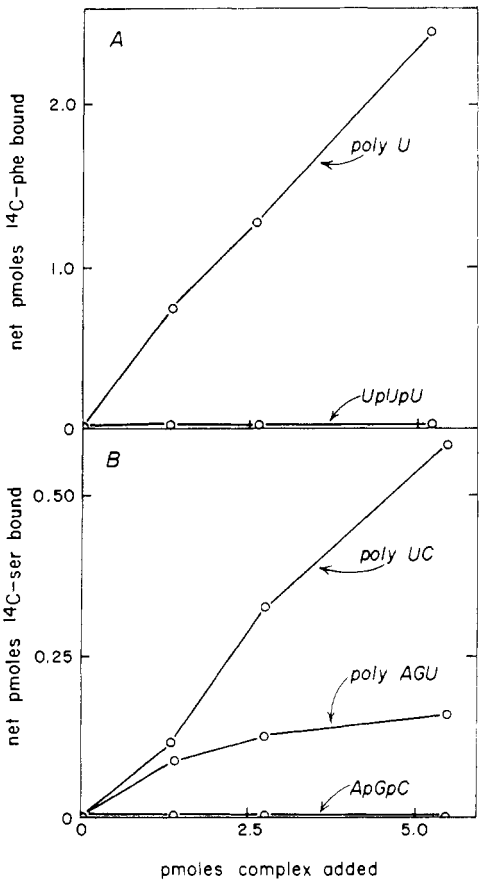

FIGURE 1: Comparison of polynucleotide and triplet-directed ribosomal binding of aminoacyl-tRNA–EF-Tu–GTP complexes. (A) Ribosomal binding of [^{14}C]Phe-tRNA ternary complex. Varying amounts of ternary complex were incubated for 7 min at 0° with 4.58 A_{260} units of *E. coli* ribosomes and either 0.160 A_{260} unit of poly(U) or 0.206 A_{260} unit of UpUpU, in a total reaction volume of 50 μl. (B) Ribosomal binding of [^{14}C]Ser-tRNA ternary complex. Incubations were carried out for 3 min at 0° with 4.58 A_{260} units of *E. coli* ribosomes and either 0.148 A_{260} unit of poly(U,C), 0.144 A_{260} unit of poly(A,G,U), or 0.138 A_{260} unit of ApGpC. Results of each experiment were corrected for ribosomal binding occurring in the absence of template, 0.006 pmol of [^{14}C]Phe-tRNA ternary complex in part A, and 0.158 pmol of [^{14}C]Ser-tRNA ternary complex in part B.

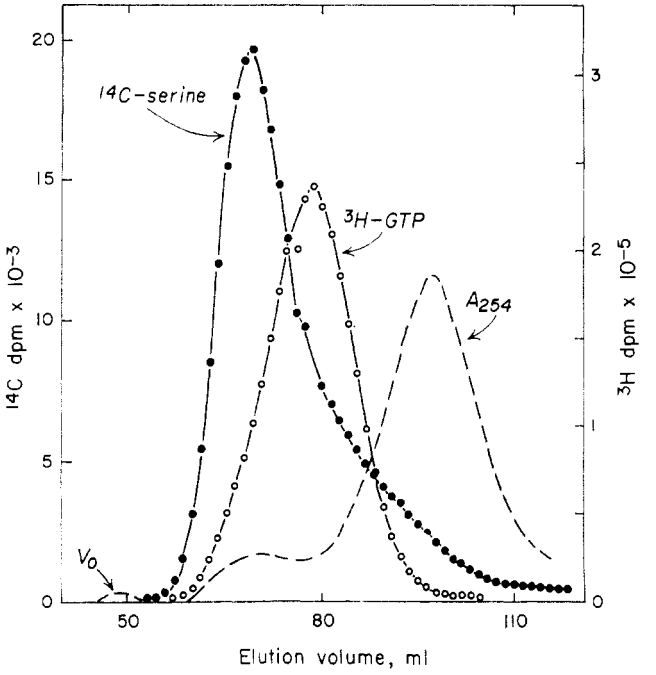

FIGURE 2: Sephadex G-100 chromatography of products of the reaction of [^{14}C]Ser-tRNA with EF-Tu–GTP. Rooster liver tRNA (166 nmol; 104 A_{260} units) containing 13.0 nmol of [^{14}C]-Ser-tRNA was reacted with 31.5 nmol of EF-Tu–[^{3}H]-GTP. The reaction mixture was applied to a 1.6 × 82 cm column of Sephadex G-100 and eluted at a flow rate of 14.6 ml/hr. Fraction size was 1.34 ml, and 0.20 ml of each fraction was counted in 10 ml of a dioxane-based scintillation fluid. Fractions between an elution volume of 59 and 76 ml were pooled, and purity of the [^{14}C]Ser-tRNA recovered in the complex was determined.

Isolation of the Ternary Complex. The purity of the isolated AA-tRNA is determined by the purity of the amino acid with which the unfractionated tRNA is acylated, since only acylated tRNA will participate in complex formation (Gordon, 1967). The presence of other amino acids, caused, for example, by inadequate deacylation during tRNA isolation or by proteolytic activity in the ligase preparation, will result in a final product with diminished purity. The usual methods for preparing tRNA yield a product which may still be more than 2% acylated. Two deacylations were therefore included in the tRNA preparation procedure, yielding tRNA less than 0.6% acylated.[2] Free amino acids were removed from synthetase preparations by Sephadex G-25 chromatography immediately before use.

Acylated tRNA was complexed with EF-Tu–GTP in a scaled-up modification of the method of Weissbach *et al.* (1971). Routinely an amount of EF-Tu 1.67-fold greater than the amount of AA-tRNA to be complexed was preincubated for 5 min at 37° in the presence of a 10-fold molar excess (over EF-Tu) of nonradioactive GTP, a 2000-to 3000-fold excess of phosphoenolpyruvate (Calbiochem) and 25–45 μg of pyruvate kinase (Sigma) in a 2-ml solution containing 0.05 M Tris-HCl, 0.05 M NH_4Cl, 0.01 M $MgCl_2$, and 0.005 M dithiothreitol at pH 7.4. The preincubation mixture was chilled on ice and added to 1 ml of a solution containing 500–15,000 pmol of AA-tRNA in 0.5 mM potassium cacodylate (pH 5). Complex formation was allowed to proceed for 5 min at 0° and was then stopped by passage of the reaction mixture through a stack of two nitrocellulose filters (0.45 μ, HAWP, Millipore) prewetted with 0.05 M Tris-HCl, 0.05 M NH_4Cl, 0.01 M $MgCl_2$, and 0.005 M dithiothreitol at pH 7.4 and the filtrate was collected on ice. EF-Tu–GTP is retained by the nitrocellulose filters, while the ternary complex and other reaction components pass through (Weissbach *et al.*, 1971).

To isolate the ternary complex free of unreacted tRNA and other components of the reaction mixture, the filtrate was immediately applied to a column of Sephadex G-100 (80–85 × 1.6 cm) equilibrated with a buffer containing 0.05 M Tris-HCl, 0.01 M magnesium acetate, 0.10 M ammonium acetate, 0.001 M EDTA, and 0.005 M dithiothreitol at pH 6.5 and eluted at a flow rate of 20–25 ml/hr at 2–4°. The column effluent was monitored at 254 nm with an ISCO single-beam uv monitor attached to a Sargent recorder. Aliquots of column fractions were counted in 10 ml of a dioxane-based scintillation fluid in a Beckman LS-233 scintillation counter. ^{3}H and ^{14}C disintegrations were calculated by the discriminator ratio method of Okita *et al.* (1957) using an IBM 360/50 computer.

Isolation of the ternary complex was demonstrated by showing that aminoacyl-tRNA in the complex (a) elutes distinctly from unreacted EF-Tu–GTP, unacylated tRNA and unreacted AA-tRNA; (b) elutes in the same position as

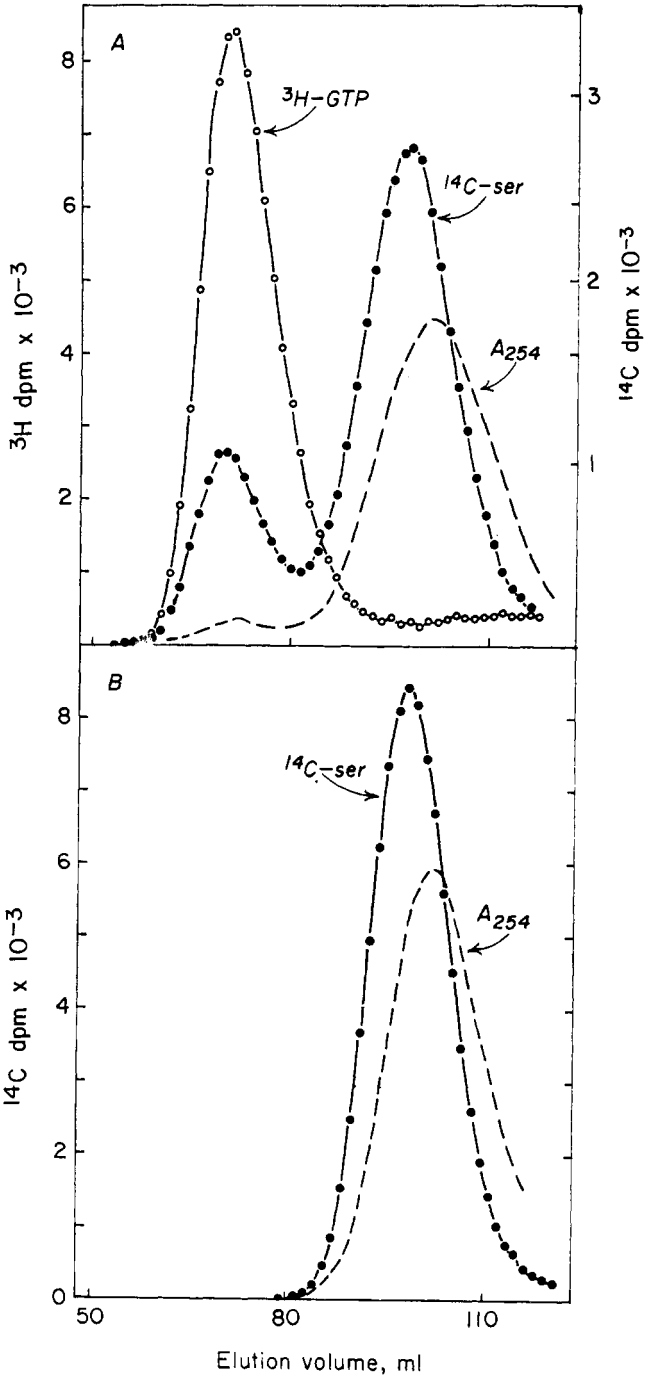

FIGURE 3: Identification of labeled serine peaks from Sephadex G-100 chromatography of products of the reaction of [^{14}C]Ser-tRNA with EF-Tu-[^{3}H]GTP. (A) Chick liver tRNA (14.7 A_{260} units) containing 1.67 nmol of [^{14}C]Ser-tRNA was reacted with 1.05 nmol of EF-Tu-[^{3}H]GTP. The reaction mixture was applied to a 1.6 × 85 cm column of Sephadex G-100 and 1.40-ml fractions were collected. Aliquots of 0.10 ml were taken from each fraction for measurement of radioactivity and fractions between an elution volume of 60 and 80 ml were pooled for determination of [^{3}H]GTP and [^{14}C]serine in the ternary complex. (B) Chick liver tRNA (4.52 A_{260} units) containing 0.573 nmol of [^{14}C]Ser-tRNA was applied to the same column used above, without prior reaction with EF-Tu-GTP, and 1.45-ml fractions were collected. Aliquots of 0.60 ml were taken for measurement of radioactivity.

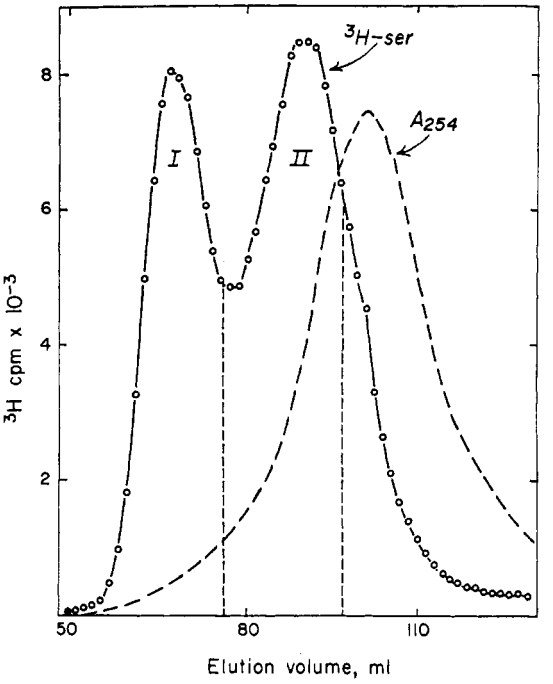

FIGURE 4: Sephadex G-100 chromatography of the products of the reaction of [^{3}H]Ser-tRNA with EF-Tu-GTP. Chick liver tRNA (51.2 A_{260} units) acylated with [^{3}H]Ser-tRNA was reacted with 20.0 nmol of EF-Tu-GTP. The reaction mixture was applied to a 1.6 × 85 cm column of Sephadex G-100 which had been used for three previous isolations of ternary complex. 1.44-ml fractions were collected and aliquots of 0.050 ml were taken for measurement of radioactivity. Peaks I and II were each subsequently applied to a column of benzoylated DEAE-cellulose (see Figure 5).

reacted EF-Tu-GTP; and (c) has a molecular weight identical with that expected for a complex of AA-tRNA-EF-Tu-GTP. To compare the elution of the complex with that of unreacted EF-Tu-GTP, a reaction mixture containing a 2.4-fold excess of EF-Tu-[^{3}H]GTP over [^{14}C]Ser-tRNA was prepared and chromatographed without prior filtration through nitrocellulose membranes. [^{14}C]Ser-tRNA eluted prior to EF-Tu-[^{3}H]GTP, as expected for the ternary complex, and well ahead of unacylated tRNA (Figure 2). The early eluting [^{14}C]Ser-tRNA peak contained greater than 76% of the 13,000 pmol of [^{14}C]Ser-tRNA chromatographed, and had 1380 pmol of serine/A_{260} unit of tRNA, a purity of 86% based on 1600 pmol/A_{260} unit for pure tRNA^{Ser}.

Ser-tRNA in the complex was shown to elute in the same position as reacted EF-Tu-GTP. A reaction mixture containing a limiting amount of EF-Tu-[^{3}H]GTP was first passed through filters to remove unreacted EF-Tu-[^{3}H]GTP and then chromatographed. Two peaks of [^{14}C]-Ser-tRNA were seen, one eluting coincident with EF-Tu-[^{3}H]-GTP and one just prior to unacylated tRNA (Figure 3A). The double-labeled peak contained 357 pmol of [^{14}C]-serine and 346 pmol of [^{3}H]GTP, consistent with an equimolar ratio of [^{14}C]Ser-tRNA and EF-Tu-[^{3}H]GTP. The later eluting peak of [^{14}C]Ser-tRNA was shown to be unreacted [^{14}C]Ser-tRNA by its elution in a position identical with that of [^{14}C]Ser-tRNA chromatographed alone without prior incubation with EF-Tu-GTP (Figure 3B).

Calculations of K_{av}, the partition coefficient between the liquid phase and the Sephadex gel phase (Work and Work, 1970, p 170) for unreacted tRNA, EF-Tu-GTP, and the ternary complex containing Ser-tRNA, gave values of 0.398 (average of 10), 0.264, and 0.148 (average of 6) respectively. The molecular weight of the ternary complex formed in these

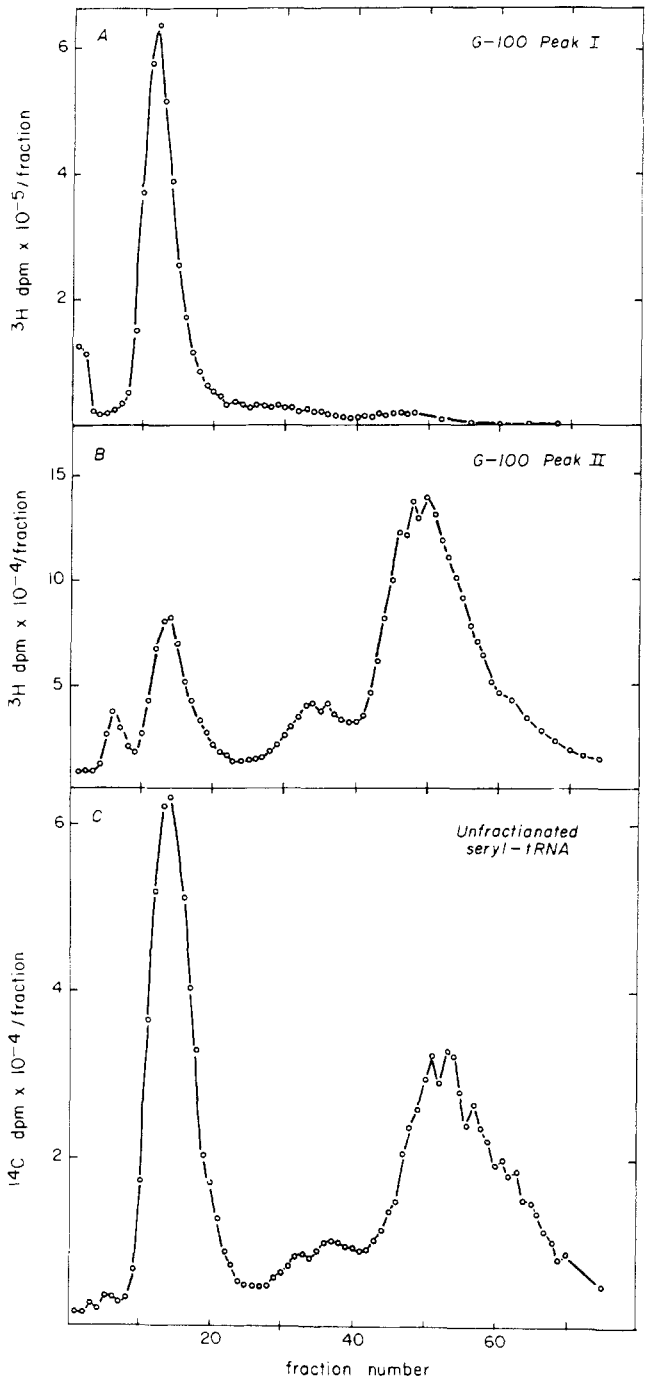

FIGURE 5: Benzoylated DEAE-cellulose chromatography of Ser-tRNA separated into two components after reaction with EF-Tu–GTP and gel filtration on Sephadex G-100. Samples were applied to a 21 × 0.9 cm column of BD-cellulose equilibrated with a buffer containing 0.45 M NaCl, 0.01 M $MgCl_2$, and 0.005 M sodium acetate (pH 4.5). Elution was with a linear gradient (400 ml, 0.60–1.0 M NaCl) in the same buffer (Mäenpää and Bernfield, 1969). (A) Fractions containing Ser-tRNA peak I (see Figure 4) were pooled and 23 A_{260} of unacylated tRNA was added as carrier. Fractions of 4.0 ml were collected, and 0.30-ml aliquots were taken for measurement of radioactivity. (B) Fractions containing Ser-tRNA peak II were pooled and 30 A_{260} of unacylated tRNA was added as carrier. Fractions of 3.9 ml were collected, and 0.10-ml aliquots were taken for determination of radioactivity. (C) Chick liver tRNA (30 A_{260}) acylated with [^{14}C]serine. Fractions of 4.0 ml were collected, and 0.60-ml aliquots were taken for measurement of radioactivity.

experiments was determined by plotting K_{av} values for unacylated tRNA and EF-Tu–GTP *vs.* log molecular weight of these species (EF-Tu, mol wt 42,000 (Miller and Weissbach, 1970); tRNA mol wt assumed to be 25,000), and extrapolating the resulting curve. The molecular weight of the Ser-tRNA ternary complex was found to be 6.60 ± 0.54 × 10^4 (mean ± SD, for six determinations, quite close to the expected size of a 1:1 complex of EF-Tu–GTP and Ser-tRNA.

AA-tRNA synthetases also form complexes with tRNA and if present as contaminants in the EF-Tu preparation might interact with tRNA, reducing the purity of the isolated AA-tRNA. However, the EF-Tu (mol wt 42,000) was purified on Sephadex G-100, likely removing most synthetases (mol wt 70,000 to 220,000; Loftfield, 1972). Further, such complexes would presumably not elute with the AA-tRNA–EF-Tu–GTP complex.

These data demonstrate the isolation of the Ser-tRNA–EF-Tu–GTP complex and its utility for the purification of AA-tRNA species. The procedure is reproducible. Ser-tRNA from ten different isolations had an average purity greater than 78% (1260 ± 356 pmol/A_{260}, mean ± SD), quantitative; virtually all of the AA-tRNA is incorporated into the ternary complex under conditions where acylated tRNA is limiting; and rapid two 5-min incubations are required, followed by a gel filtration step in which the desired ternary complex is eluted within 3 or 4 hr.

Purification of Other AA-tRNAs by Complex Formation with EF-Tu–GTP. Chick liver Leu-tRNA, Lys-tRNA, and Phe-tRNA have also been isolated by us as ternary complexes. Wimmer *et al.* (1968) isolated yeast $tRNA^{Phe}$ by chromatography on BD-cellulose, yielding a 17-fold purification, followed by chromatography on silicic acid coated BD-cellulose, resulting in $tRNA^{Phe}$ estimated to be 97.5% pure, an overall purification of more than 25-fold. By comparison, the ternary complex formation procedure yielded Phe-tRNA with a purity of 1430 pmol/A_{260} unit, nearly 90% pure, from 20.2 A_{260} units of tRNA acylated with 25.5 pmol of Phe/A_{260} unit, a purification of 56-fold.

Separation of the AA-tRNA in the ternary complex into isoaccepting species can be accomplished by adjusting the peak containing the complex to 0.40 M NaCl and applying directly to BD-cellulose. This procedure yields results identical with that obtained by extracting twice with phenol and then precipitating the purified AA-tRNA with ethanol prior to BD-cellulose chromatography.

Variable Stabilities of Ternary Complexes of tRNA Species within an Isoaccepting Set. Following Sephadex G-100 chromatography two peaks of Ser-tRNA were sometimes seen, with about 40% of the Ser-tRNA in the ternary complex, when the column had been used more than three times or the temperature exceeded 5° (Figure 4). The additional serine peak had a K_{av} of 0.313 (average of 4) and was displaced from the position of uncomplexed Ser-tRNA (K_{av} = 0.378, average of 2). BD-cellulose chromatography of the peaks showed that the ternary complex peak contained almost exclusively $tRNA_I^{Ser}$, the species responding to the AGU and AGC codons (Figure 5A), and that the later eluting peak contained the remaining isoaccepting species and a reduced content of $tRNA_I^{Ser}$ (Figure 5B). These results suggest that the ternary complex of Ser-$tRNA_I$ is more stable than the complexes with the other isoaccepting species. The same phenomenon of differential stability has also been seen with complexes containing Leu-tRNA and Lys-tRNA. Differences in ternary complex stability could conceivably be exploited to selectively purify individual tRNA species within an isoaccepting set.

Acknowledgments

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Rate of Serine Transfer Ribonucleic Acid Synthesis During Estrogen-Induced Phosphoprotein Synthesis in Chick Liver†

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ABSTRACT: The estrogen-induced hepatic synthesis of the yolk phosphoprotein, phosvitin, in roosters is accompanied by a 25% increase in the serine acceptance of hepatic tRNA which is limited to two serine isoacceptors and not dependent on AA-tRNA synthetase (P. H. Mäenpää and M. R. Bernfield, *Biochemistry 8*, 4926 (1969). In chicks, estrogen is now shown to cause a similar increase in the concentration of hepatic Ser-tRNA as well as changes in the acylation of other tRNA species during phosvitin induction. To determine whether the change in Ser-tRNA level is due to a specific increase in synthesis, the rate of tRNA^{Ser} synthesis relative to the rate of total tRNA synthesis in hormone-treated chicks was compared to that in control chicks. The relative synthetic rate was determined by a double-label method in which nonspecific effects of the hormone were avoided. The incorporation of [^{3}H]- and [^{14}C]orotic acid, over a brief period *in vivo* or in liver slices, into purified tRNA^{Ser} from estrogen-treated and control birds was compared to the incorporation into unfractionated tRNA from these birds. No appreciable change in the relative rate of tRNA^{Ser} synthesis was found during a period when the level of Ser-tRNA continued to increase. Kinetic analysis of the data suggests that mechanisms other than increased synthesis are responsible for the increased serine acceptance of hepatic tRNA during estrogen-induced phosvitin synthesis. Several possibilities are discussed, including a decrease in degradation rate or an estrogen-evoked specific tRNA modification.

Estrogen-induced increases in different classes of RNA have often been assumed to be the result of increased gene transcription. Evidence that such increases in RNA reflect augmented synthetic rate include the estrogen-evoked increased incorporation of precursors into rRNA and tRNA of rat uteri (Billing *et al.*, 1969) and into all rooster liver RNA fractions (Coolsma and Gruber, 1968). Inhibition studies with actinomycin D (Greengard *et al.*, 1964) and DNA–RNA hybridization experiments (Hahn *et al.*, 1969) have suggested that the estrogen-induced phosphoprotein synthesis in rooster

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