Phytochemistry, 1973, Vol. 12, pp. 43 to 53. Pergamon Press. Printed in England.

ENZYMATIC BINDING OF AMINOACYL-*t*RNA AND PEPTIDE CHAIN ELONGATION BY RIBOSOMES AND RIBOSOME SUBUNITS IN PEA

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(*Revised Received* 24 *July* 1972. *Accepted* 1 *August* 1972)

Key Word Index—*Pisum sativum*; Leguminosae; pea; aminoacyl-*t*RNA; protein synthesis; peptide chain elongation; ribosome subunits.

Abstract—*In vitro* aminoacyl transfer from aminoacyl-*t*RNA to elongating peptide chains and binding of aminoacyl-*t*RNA to ribosomes were studied with natural *m*RNA- and poly U-associated systems. Peptide chain elongation required ammonium ion at 160 mM in addition to a partially purified protein fraction from the postribosomal supernatant and GTP for optimum amino acid incorporation. Additionally, the natural *m*RNA-associated system required 3–5 mM magnesium ion and the synthetic *m*RNA associated system required 10 mM magnesium ion for optimal incorporation. The reaction mixtures which gave optimal peptide chain elongation were also used in the study of aminoacyl-*t*RNA binding by either natural or synthetic *m*RNA-associated ribosomes. Approximately 50% of the bound aminoacyl-*t*RNA was incorporated into polypeptides by the former (polyribosomes) under the assay conditions used. The binding of aminoacyl-*t*RNA to each size of ribosomes suggests the possibility of association of 40*S* subunits with each class of ribosomes and/or conformational changes in the ribosome structure. The binding of phenylalanyl-*t*RNA to monoribosomes was poly U specific and unlike polyribosomes very little polymerization occurred. Binding of phenylalanyl-*t*RNA was also shown for poly U-associated 40*S* subunits.

INTRODUCTION

IN GENERAL the factors necessary for protein chain initiation, elongation, termination, and ribosome dissociation have been characterized in both procaryotes and eucaryotes.[1,2]

The process of peptide chain elongation is composed of three major steps: (1) binding of the aminoacyl-*t*RNA to the ribosome at the *A* site (aminoacyl-*t*RNA binding site); (2) peptide bond formation between the peptidyl-*t*RNA bound to the *P* site (peptidyl donor site on ribosome) and newly bound aminoacyl-*t*RNA to the *A* site; and (3) translocation of the peptidyl-*t*RNA from the *A* site to the *P* site. Step 1 is catalyzed by a factor designated *T* in procaryotes and T_1 in eucaryotes; step 3 by a factor designated *G* in procaryotes and T_2 in eucaryotes. Factor *T* has been further subdivided into two factors, T_s and T_u in procaryotes.[3] These factors are isolated from the post-ribosomal supernatant. Peptidyl transferase, which catalyzes step 2, is associated with the larger ribosomal subunit and thus is not present in the post-ribosomal supernatant.[4,5]

Poly U-directed synthesis of polyphenylalanine has been used as a model system for the study of polypeptide chain elongation, and it was with this system that the reactions

[1] *Cold Spring Harbor Symp. Quant. Biol.* **34** (1969).
[2] P. LENGYEL and D. SÖLL, *Bact. Rev.* **33**, 264 (1969).
[3] J. LUCAS-LENARD and A. L. HAENNI, *Proc. Natl. Acad. Sci.* **59**, 554 (1968).
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catalyzed by the various factors were originally elucidated. However, aminoacyl-*t*RNA binding to ribosomes has been reported to be independent of supernatant factors in this system. This discrepancy has been resolved by the recognition that a supernatant factor and GTP are required for aminoacyl-*t*RNA binding only at low magnesium ion concentrations.[6] In all cases the aminoacyl-*t*RNA binds only to the smaller ribosomal subunit.[7–9]

The present report describes studies on peptide chain elongation in an *in vitro* protein synthesizing system isolated from pea roots. A post ribosomal supernatant factor was found which enhanced aminoacyl-*t*RNA binding in both a poly U-monoribosome and a polyribosome system. Binding of phenylalanyl-*t*RNA to isolated 40*S* ribosomal subunits in the presence of poly U was also dependent upon added supernatant enzyme and GTP.

RESULTS

A typical sucrose density gradient profile for pea root ribosomes (Fig. 1a) showed a high percentage of large polyribosomes (> 80%) and a relatively low monoribosome level. This 'polyribosome-rich' preparation was used as a source of natural *m*RNA associated-ribosome for studies on aminoacyl-*t*RNA binding and the subsequent transfer of the aminoacyl group to the elongating polypeptide. Ribosome preparations from anaerobically treated seedlings, on the other hand, contained only monoribosomes (Fig. 1b). Anaerobiosis-induced monoribosomes were washed with NH_4Cl and used for studies on poly U-directed phenylalanyl-*t*RNA binding and polyphenylalanine synthesis.

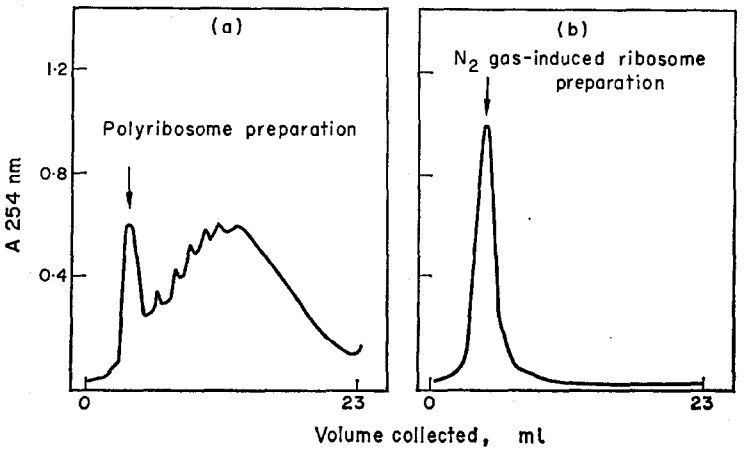

FIG. 1. SUCROSE GRADIENT PROFILE OF RIBOSOMES FROM PEA ROOT TIPS.
(a) Polyribosome preparation. (b) N_2 gas-induced monomeric ribosome preparation. The ribosome preparations were layered onto linear 10–34% sucrose gradients and centrifuged at 23 000 rpm in the SW 25 rotor for 2 hr.

The enzyme fraction used in the aminoacyl transfer and binding assays was prepared by salt fractionation of the post-ribosomal supernatant. The fraction obtained from a 45 to 65% ammonium sulfate cut was most active relative to both transfer and binding (unreported

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data) and hence was used in all assays. This enzyme fraction was heat sensitive with all activity being lost by heating the preparation to 50° for 4 min (Tables 2 and 3).

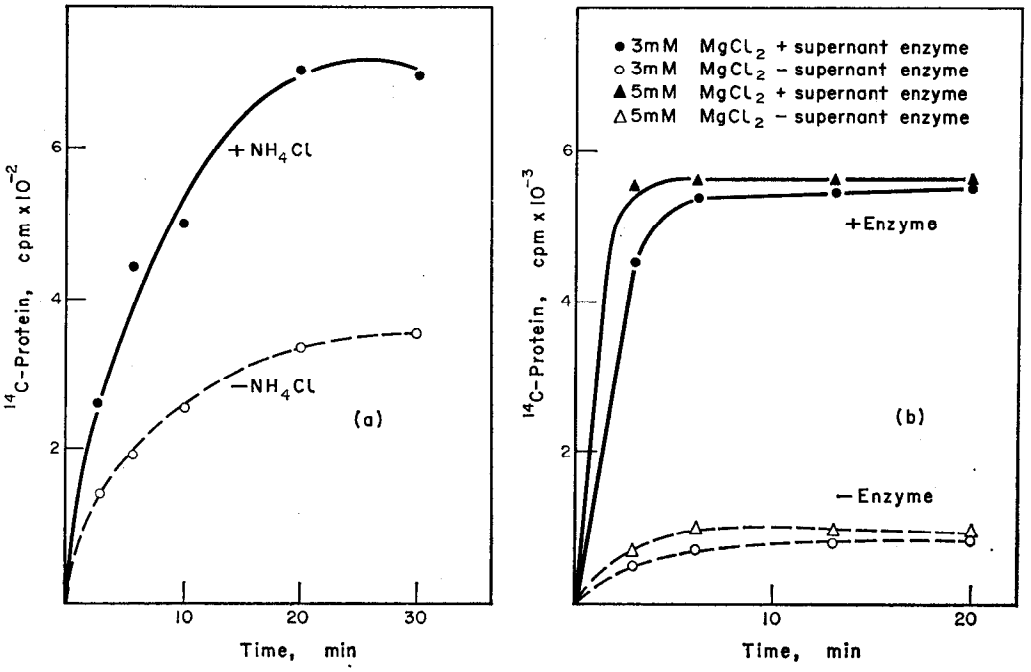

FIG. 2. KINETICS OF INCORPORATION OF ^{14}C-AMINO ACID FROM ^{14}C-AMINOACYL-*t*RNA BY NON-WASHED POLYRIBOSOMES.

(a) Incorporation of ^{14}C-leucine in the presence and absence of NH_4Cl. In addition to the standard incubation medium described in methods, 26·4 A260 units of ribosomes, 16 680 cpm of ^{14}C-leucyl-*t*RNA (6720 cpm/1·0 A260 unit *t*RNA) and 1·2 mg of supernatant protein were used in a final vol. of 3 ml. Aliquots of 0·5 ml were taken for measurement of incorporation at the indicated times.

(b) Incorporation of ^{14}C-amino acid at variable $MgCl_2$ concentrations. In addition to the standard incubation medium described in methods, 11·8 A260 units of ribosomes, 56 150 cpm of ^{14}C-aminoacyl-*t*RNA (97 605 cpm/1·0 A260 unit *t*RNA), 250 μg of supernatant protein and a varied concentration of $MgCl_2$ were used in a final reaction volume of 2·5 ml. Aliquots of 0·5 ml were taken for measurement of incorporation at the indicated times.

Specific Requirements for Aminoacyl Transfer from Aminoacyl-tRNA

The data reported here show that NH_4Cl, magnesium ion and GTP were required for transfer of the aminoacyl group from aminoacyl-*t*RNA to elongating peptides by polyribosomes or poly U-associated monoribosomes, respectively (Table 2). Maximum transfer activity with both systems occurred at 160 mM NH_4Cl or 80 mM NH_4Cl + 80 mM KCl while activity was quite low in the absence of NH_4Cl (Fig. 2a and Fig. 3a). Addition of higher concentrations of KCl (up to 80 mM) only partially alleviated the requirement for NH_4Cl. The optimal magnesium concentration for polyphenylalanine synthesis by poly U-directed monoribosomes was 10 mM (Fig. 3b), whereas NH_4Cl washed or non-washed polyribosomes required only 3–5 mM magnesium for maximum incorporation (Fig. 2b and Table 1). At higher concentrations (e.g. 10 mM) transfer activity by polyribosomes was

considerably reduced. Maximal transfer activity with both systems was enhanced by GTP and supernatant enzyme (Table 2). In addition polyphenylalanine synthesis directed by salt-washed monoribosomes showed an absolute requirement for poly U (Table 2).

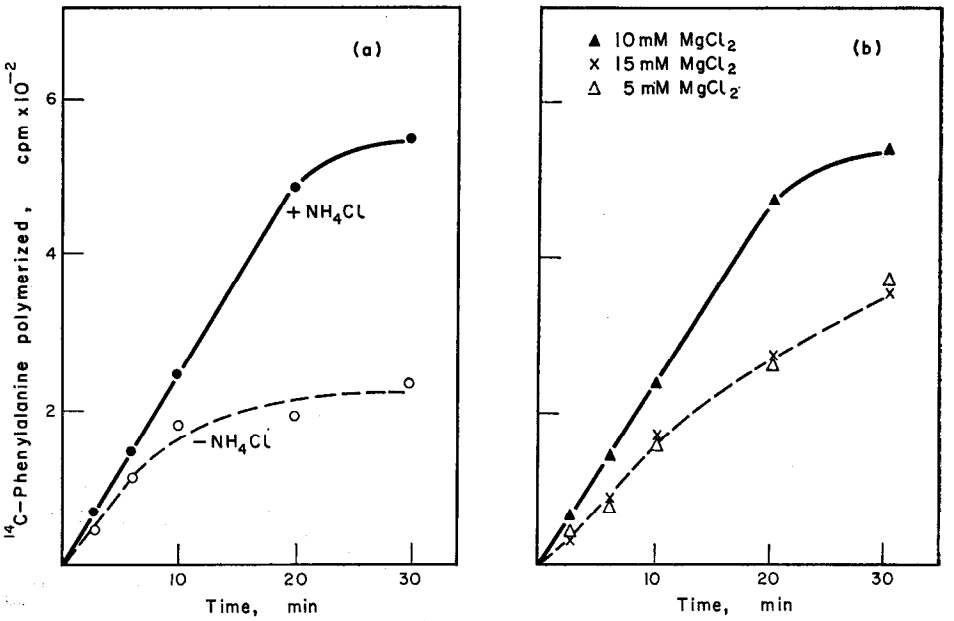

FIG. 3. KINETICS OF INCORPORATION OF ^{14}C-PHENYLALANINE FROM ^{14}C-PHENYLALANYL-*t*RNA BY POLY U-DIRECTED MONORIBOSOMES.

(a) $\pm NH_4Cl$. In addition to the standard incubation medium, 11·2 A260 units of monoribosomes, 250 μg of poly U, 200 μg of supernatant enzyme and 7900 cpm of ^{14}C-phenylalanyl-*t*RNA were used in a final vol. of 2·5 ml. The assay was carried out as shown in Fig. 2a. (b) At variable $MgCl_2$ concentrations. The assay medium was the same as given in Fig. 3a and the assay was carried out as shown in Fig. 2a.

Binding of Aminoacyl-tRNA to Ribosomes

By Polyribosomes. The kinetics of binding of aminoacyl-*t*RNA to polyribosomes are shown in Fig. 4. Binding activity increased linearly for 10 min before reaching a plateau and was GTP- and enzyme-dependent (Table 3). A major difficulty that arises in assessing the

TABLE 1. AMINOACYL TRANSFER FROM AMINOACYL-*t*RNA BY SALT WASHED POLYRIBOSOMES

Polyribosomes	^{14}C-Amino acids polymerized from ^{14}C-aminoacyl-*t*RNA (cpm) −Enzyme	+Enzyme	Polyribosomes	^{14}C-Amino acids polymerized from ^{14}C-aminoacyl-*t*RNA (cpm) −Enzyme	+Enzyme
At 5 mM Mg^{2+}			At 10 mM Mg^{2+}		
Non-washed	109	2571	Non-washed	142	924
Washed	79	2560	Washed	101	1075

Incubation was at 37° for 20 min. 5580 cpm of ^{14}C-aminoacyl-*t*RNA was used per assay.

level of binding in this system is that part of the bound aminoacyl-*t*RNA is transferred to peptidyl-*t*RNA as has been shown for other systems.[10] Thus the total counts incorporated reflect both binding and transfer activity. In this study approximately 50% of the aminoacyl-*t*RNA was incorporated into hot acid insoluble polypeptides, the remainder being ribosome-bound aminoacyl-*t*RNA (Table 3). To show the binding of aminoacyl-*t*RNA to ribosomes in a more specific manner, assay mixtures were centrifuged on sucrose gradients. The results indicate that each size class of ribosomes consistently bound aminoacyl-*t*RNA (Fig. 5). The radioactivity peaks denoting bound aminoacyl-*t*RNA shown in Fig. 5a did not exactly coincide with their respective absorbancy peaks with the various classes of polyribosomes. On the other hand the radioactivity peaks denoting ^{14}C-amino acid polymerized (i.e. hot TCA insoluble radioactivity) shown in Fig. 5b coincided with their respective absorbancy peaks.

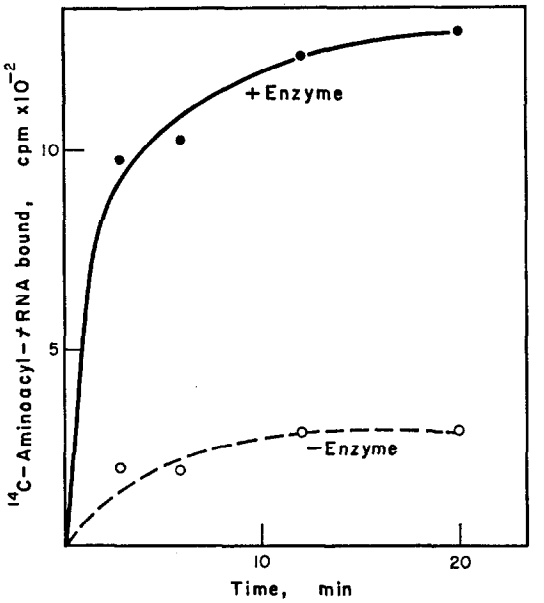

FIG. 4. KINETICS OF BINDING OF ^{14}C-AMINOACYL-*t*RNA TO POLYRIBOSOMES.
In addition to the standard incubation medium, 11·8 A260 units of ribosomes, 56 150 cpm of ^{14}C-aminoacyl-*t*RNA and 250 μg of supernatant enzyme were used in a final vol. of 2·5 ml. Incubation was at 0°. Aliquots of 0·5 ml were taken at the indicated times and assayed.

By poly U-associated monoribosomes. The kinetics for binding of phenylalanyl-*t*RNA by the poly U-monoribosome system are shown in Fig. 6. The results are similar to those obtained with polyribosomes with the exception that the binding required 20 min for saturation with poly U-monosomes and that more binding occurred in this system than in the polyribosome system in the absence of added enzyme. Figure 7 shows that binding activity increased with increasing magnesium concentration up to 20 mM either in the presence or absence of added enzyme. However, when the values obtained for enzymatic binding at various magnesium concentrations were corrected by subtracting 'non-enzymatic' binding, the optimal magnesium concentration was again 10 mM.

[10] D. C. N. EARL and S. T. HINDLEY, *Biochem. J.* **122**, 267 (1971).

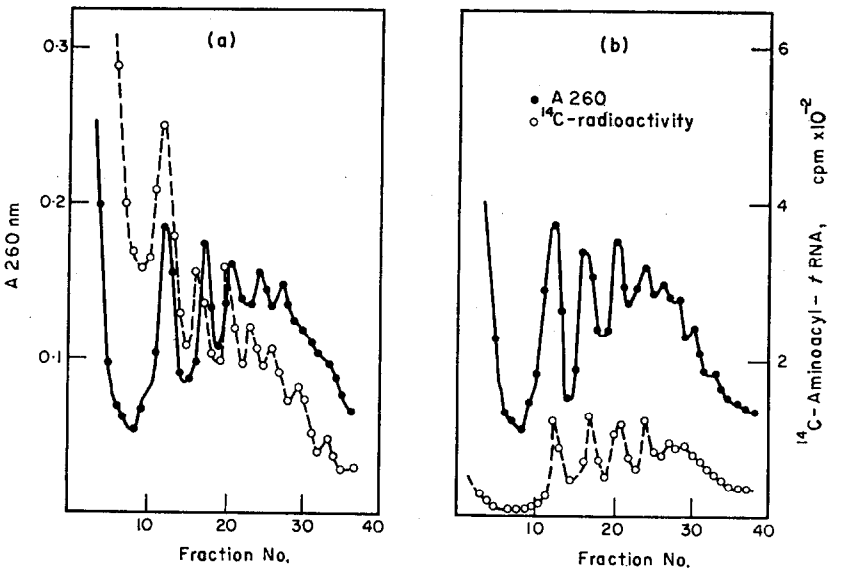

FIG. 5. EVIDENCE FOR THE BINDING OF ^{14}C-AMINOACYL-*t*RNA TO POLYRIBOSOMES.

The incubation conditions were the same as those in Fig. 4 except that 16·0 A260 units of ribosomes, 67 380 cpm of ^{14}C-aminoacyl-*t*RNA and 100 μg of supernatant protein were used in a final reaction vol. of 1 ml at 0° for 20 min in duplicate. 0·8 ml of the reaction mixture from each tube was placed on a sucrose gradient (10–34%) containing 50 mM Tris buffer pH 7·5, 5 mM $MgCl_2$, 80 mM KCl and 80 mM NH_4Cl for fractionation (SW 27, 1 × 3·5 in tube; 25 000 rpm × 105 min) at the end of incubation time. Fractions of 20 drops each were collected for absorption measurement at 260 nm and radioactivity determination. (a) Radioactivity collected on the glass fiber filter after cold TCA precipitation. (b) Radioactivity collected on the glass fiber filter after heating with TCA at 90° for 15 min.

Practically all of the ^{14}C-phenylalanyl-*t*RNA bound in this assay was due strictly to binding of the phenylalanyl-*t*RNA to the monoribosomes based on the hot acid solubility of the membrane associated ^{14}C-radioactivity (Table 3). In contrast to the polyribosome system very little polymerization occurred in the binding assay with poly U-linked monoribosomes. Binding activity was poly U-specific and like the polyribosome system was

TABLE 2. REQUIREMENTS FOR AMINOACYL TRANSFER FROM AMINOACYL-*t*RNA TO PROTEIN BY PEA RIBOSOMES

Reaction mixture	^{14}C-Phenylalanine polymerized by monoribosomes* (%)	^{14}C-Amino acids polymerized by polyribosomes† (%)	Reaction mixture	^{14}C-Phenylalanine polymerized by monoribosomes* (%)	^{14}C-Amino acids polymerized by polyribosomes† (%)
Complete	100 (479 cpm)	100 (4539 cpm)	Enzyme heated at 50° for 4 min	<1	40–50
−GTP	<2	<2	−Poly U	<3	—
−Enzyme	<5	<20			

Incubation was at 37° for 20 min.
* 1580 cpm of ^{14}C-phenylalanyl-*t*RNA per assay.
† 11 230 cpm of ^{14}C-aminoacyl-*t*RNA per assay. Polyribosomes were not salt-washed.

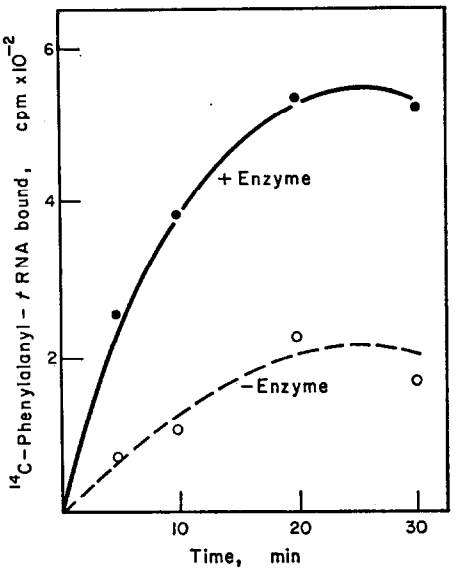

FIG. 6. KINETICS OF BINDING OF ^{14}C-PHENYLALANYL-*t*RNA TO POLY U-DIRECTED MONORIBOSOMES. The reaction medium was the same as shown in Fig. 3a except that 12 630 cpm of ^{14}C-phenylalanyl-*t*RNA was used. The assay was carried out as shown in Fig. 4.

enhanced by GTP and supernatant enzyme (Table 3). The enzyme-dependent binding activity of aminoacyl-*t*RNA was lost by heating the enzyme fraction to 50° for 4 min. The 'non-enzymatic' binding activity (i.e. minus enzyme or plus heated enzyme) remained when the ribosomes were heated to 50° for 4 min (Table 3).

TABLE 3. REQUIREMENTS FOR THE ENZYMATIC BINDING OF AMINOACYL-*t*RNA TO PEA RIBOSOMES

Reaction mixture	^{14}C-Phenylalanyl-*t*RNA and monoribosomes* (%)		^{14}C-Aminoacyl-*t*RNA and polyribosomes† (%)	
	Bound	Polymerized	Bound	Polymerized
Complete	100 (510 cpm)	5	100 (1055 cpm)	50
—Ribosomes	2	—	2	—
—Mg^{2+}	8	—	—	—
—GTP	59	<5	10	—
—Enzyme	40	<5	20	10
—Enzyme and GTP	40	<5	—	—
Enzyme heated at 50° for 4 min	43	—	—	—
—Poly U	5	—	—	—
Ribosome and enzyme both heated at 50° for 4 min	40	—	—	—

Incubation was at 0° for 20 min.
* 2415 cpm of ^{14}C-phenylalanyl-*t*RNA per assay.
† 11 230 cpm of ^{14}C-aminoacyl-*t*RNA per assay. Polyribosomes were not salt-washed.

By poly U-linked 40S *ribosomal subparticles.* The 40*S* ribosomal subunits were prepared as previously described.[11] Binding of phenylalanyl-*t*RNA to the 40*S* subunits was enzyme-, GTP-, and poly U-dependent (Table 4). Again very little of the activity measured in this assay was due to incorporation of phenylalanyl group into polypeptide form.

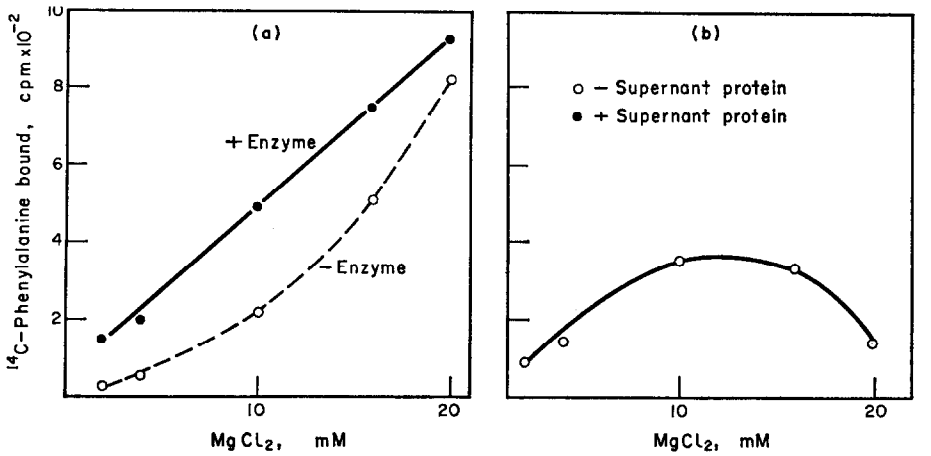

FIG. 7. THE EFFECT OF $MgCl_2$ CONCENTRATION ON THE BINDING OF ^{14}C-PHENYLALANYL-*t*RNA TO MONORIBOSOMES.

The reaction mixture described in material and methods was modified to contain $MgCl_2$ in the amounts indicated. 2400 cpm of ^{14}C-phenylalanyl-*t*RNA was used in each assay. (a) supernatant protein. (b) The values were obtained by subtracting the amount of phenylalanyl-*t*RNA bound to ribosomes in the absence of supernatant protein from the amount bound in the presence of supernatant protein.

DISCUSSION

The optimal $MgCl_2$ concentration for amino acid incorporation by polyribosomes or by viral RNA-directed washed ribosomes (with initiation factors) is normally 5 mM or less.[12–15] When salt-washed ribosomes are used for poly U-directed polyphenylalanine

TABLE 4. AMINOACYL-*t*RNA BINDING ACTIVITY OF RIBOSOMAL SUBUNITS FROM PEA

Ribosomal subunits in reaction mixture†	^{14}C-Phenylalanyl-*t*RNA bound (cpm)		^{14}C-Polyphenylalanine (cpm)	
	−Enzyme	+Enzyme	−Enzyme	+Enzyme
40*S* + poly U	7975	11 441	181	85
60*S* + poly U	142	388	92	320
40*S* − poly U	—	88	—	—

* Incubation was at 0° for 20 min. 17 136 cpm of ^{14}C-phenylalanyl-*t*RNA was used per assay.
† 2·0 A260 units of 40*S* or 2·2 A260 units of 60*S* subunits were used per assay.

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synthesis the optimal $MgCl_2$ concentration is in most cases reported to be 10–20 mM. This high $MgCl_2$ requirement can be reduced to 5 mM by addition of the optimum amount of ribosomal protein.[16] Our results for $MgCl_2$ requirements in both polyribosome and poly U-directed systems are similar with the other systems reported. We are now investigating the possibility of lowering $MgCl_2$ requirements by adding ribosomal protein to a poly U-directed washed monoribosome system.

The stimulation of amino acid incorporation in an *in vitro* protein synthesizing system by NH_4Cl was reported for bacterial systems.[17–19] Although many bacterial aminoacyl-*t*RNA transfer assays include NH_4Cl and KCl (80 mM each), the reason for their inclusion has never been explained. It seems unlikely that the NH_4Cl requirement would be restricted to those assay systems which use NH_4Cl-washed ribosomes or to those in which aminoacyl-*t*RNA is used instead of free amino acids. In ribosome systems from wheat germ[20] and rice embryo[21] KCl alone appears to fulfill the monovalent ion requirement. This does not eliminate the possibility that the enzyme fraction prepared by $(NH_4)_2SO_4$ still contains sufficient NH_4^+ ion to obscure this requirement as demonstrated here for pea ribosomes. Ammonium ion is also present in commercially available phosphoenol pyruvate kinase. Thus when phosphoenol pyruvate kinase with phosphoenol pyruvate is used as an energy regenerating system the requirement of NH_4Cl would also be obscured.

In this study we demonstrated the enzymatic binding of aminoacyl-*t*RNA to pea ribosomes in reaction mixtures which gave optimal conditions for peptide bond formation. The binding assay was performed at 0° according to the method of Krisko *et al.* used in mammalian system.[22] The binding of phenylalanyl-*t*RNA to monomeric ribosomes was poly U-specific and was, as shown by others,[6,17,20,23–28] dependent on GTP and supernatant enzyme. Washed ribosomes retained a significant capacity for binding of aminoacyl-*t*RNA without added enzyme. Since this binding activity was not lost by heating the ribosomes to a temperature which normally inactivate soluble binding enzymes, it seems unlikely that the 'non-enzymatic' binding activity of ribosomes is due to residual enzyme activity remaining on the ribosomes after salt washing. Of course, this possibility cannot be ruled out since the binding enzyme might be protected from inactivation by heat by being associated with the ribosomes.

Binding of phenylalanyl-*t*RNA was demonstrated for poly U-associated 40*S* subunits as reported in bacterial[3,7] and mammalian systems.[8,9] Although binding activity was enhanced by supernatant enzymes there was considerable 'non-enzymatic' activity associated with 40*S* subunits. Kan *et al.*[29] purified a factor from the 1 M NH_4Cl wash of *E. coli* ribosomes, (F_{AB}; aminoacyl-*t*RNA binding factor), which stimulated binding of aminoacyl-

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*t*RNA to 30*S* ribosomal subunits. They also reported that this factor was different from the initiation factors (F_1 and F_2) and the *T* factor of *E. coli.*

It would be interesting to see if the 'non-enzymatic' binding activity associated with 40*S* subunits or with 80*S* monoribosomes (contributed by 40*S* subunits) represents a similar activity.

We were also able to demonstrate aminoacyl-*t*RNA binding to polyribosomes in the presence of enzyme and GTP. About 50% of the aminoacyl-*t*RNA bound to the polyribosomes was incorporated into polypeptides under the assay conditions used. This would be expected since part of the isolated polyribosomes would have peptidyl-*t*RNA in the ribosomal *P* site. Thus some peptide bond formation would be expected to occur spontaneously with aminoacyl-*t*RNA newly bound at the ribosomal *A* site. Earl and Hindley[10] recently showed in a population of *in vivo* labeled polyribosomes that 50% of the bound aminoacyl-*t*RNA was present as peptidyl-*t*RNA in the *P* site (i.e. puromycin releasable).

The binding of aminoacyl-*t*RNAs to various classes of polyribosomes is shown in Fig. 5. Monomeric ribosomes bound more aminoacyl-*t*RNA than any of the size classes of polyribosomes. With the various classes of polyribosomes, the radioactivity peaks denoting bound aminoacyl-*t*RNA did not exactly coincide with their respective absorbancy peaks. Thus polyribosomes binding aminoacyl-*t*RNA apparently have a slightly different conformation or composition as reflected by their slightly changed sedimentation characteristics. Horez and McCarty[30] have shown in the reticulocyte system that 40*S* subptaricles are associated with each class of polyribosomes. Schreier and Noll[31] and Chuang and Simpson[32] independently demonstrated that in poly U-linked protein synthesizing systems changes in ribosomal conformation are associated with translocation.

These observations would be consistent with the possibility of the association of 40*S* subunits with the ribosomes and/or with conformational changes in the ribosome structure. A model with three *t*-RNA binding sites proposed by Wettstein and Noll[33] would account for the binding of aminoacyl-*t*RNA to polyribosomes at an entry site.[34] However, it is difficult to explain our observed non-coincidence of ^{14}C-radioactivity and absorbancy peaks with this model. This evidence seems to support the notion that during protein synthesis ribosomal subunits are cycled, and only two binding sites are present on the ribosomes.[35,36] We are currently using this natural *m*RNA-associated ribosomal system for further investigation of mechanisms of protein synthesis.

EXPERIMENTAL

Pea seeds were germinated in rolls of moist paper.[37]

Preparation of ribosomes. (A) *Polyribosomes.* The apical 5 mm sections of 2- or 3-day-old pea seedlings were cut onto dry ice. The frozen apical sections were homogenized in a 0·25 M sucrose solution containing 50 mM Tris buffer pH 7·5–20 mM KCl, and 20 mM $MgCl_2$ (TKM buffer *A*) with a Willem's polytron for 4 sec at speed 8. The homogenate was filtered through Mira cloth, and the filtrate was centrifuged at 17 000 *g* for 15 min. Ribosomes were prepared from the supernatant solution by layering the sample over 5 ml of 1·5 M sucrose in TKM buffer B (50 mM Tris pH 7·5, 20 mM KCl, 5 mM $MgCl_2$) and centrifuging at

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229 400 *g* for 85 min.[38] When washed polyribosomes were prepared 0·5 M NH_4Cl was included in 5 ml of 1·5 M sucrose containing TKM buffer B. (B) *Monomeric ribosomes.* 3-day-old seedlings were submerged in distilled H_2O and bubbled with N_2 gas for 1 hr.[39] Ribosomes were isolated from the homogenate of apical root tips (5 mm) by the above procedure for polyribosome preparation. The ribosomal pellets were washed twice with TKM buffer *B* containing 0·5 M NH_4Cl. (C) *Preparation of ribosomal subunits.* Monoribosomes prepared from N_2 gas-treated tissue were suspended in TKM buffer B containing 0·5 M NH_4Cl. Ribosomal subunits were separated on 15–30% linear sucrose gradients in TKM buffer B containing 1 mM dithiothreitol (DTT). Centrifugation was at 25 000 rpm for 15 hr (Spinco SW 27, 1 × 3·5 in tube). Fractions containing the respective 40*S* and 60*S* subunits were pooled, and pelleted.[11] The ribosomal pellets were suspended in TKM buffer B. All steps were carried out at 0–4°.

Preparation of postribosomal supernatant enzymes. The epicotyls (*ca.* 3 mm long) from 3-day-old pea seedlings were homogenized in a 0·45 M sucrose solution containing 50 mM Tris buffer pH 7·5, 0·5 mM $MgCl_2$,[40] and 1 mM DTT with a Willem's polytron. The homogenate was filtered through Mira cloth, and the filtrate was centrifuged at 17 000 *g* for 15 min. The supernatant was centrifuged again at 229 400 *g* for 85 min to pellet ribosomes. The postribosomal supernatant was fractionated with $(NH_4)_2SO_4$ (saturated solution adjusted to pH 7·5 with NH_4OH) between 45% and 65% saturation. The precipitate from the 65% saturated $(NH_4)_2SO_4$ was collected by centrifugation and dissolved in 25 mM Hepes buffer pH 7·5 containing 1 mM DTT. The solution (15–20 mg protein/2 ml) was desalted by passing through a column of Sephadex G25 equilibrated with 25 mM Hepes buffer pH 7·5 containing 1 mM DTT.

Preparation of aminoacyl-tRNA. Pea *t*RNA (50–80 A 260 units) was prepared from 3-day-old seedlings by the method of Vanderhoef *et al.*[41] The purified *t*RNA was acylated either with a mixture of 10 μCi of ^{14}C-leucine (sp. act. 50 μCi/μmol) and the other 19 ^{12}C-amino acids (0·01 mM each) or with 20 μCi or reconstituted ^{14}C-amino acid mixture (Schwarz) in a reaction mixture containing 50 mM Hepes buffer pH 8·0, 8 mM KCl, 5 mM $MgCl_2$, 1 mM ATP, 6·4 mM phosphoenol pyruvate, 0·1 mg of pyruvice kinase, and 0·5 mg of supernatant enzyme fraction in a final vol. of 1 ml. After incubation at 37° for 20 min the reaction mixture was chilled and diluted with 2 ml of 10 mM sodium acetate buffer (pH 4·5) containing 0·3 M NaCl and 10 mM $MgCl_2$. Aminoacyl-*t*RNA was then recovered by the method of Vanderhoef *et al.*[41] ^{14}C-Phenylalanyl-*t*RNA was purchased from New England Nuclear Corporation (0·191 μCi/5·2 mg *t*RNA).

Aminoacyl-tRNA transfer and binding assays. The transfer assay was carried out in a 0·5 ml reaction mixture containing 50 mM Hepes (pH 7·5), 80 mM KCl, 80 mM NH_4Cl, 5 mM $MgCl_2$, 0·3 mM GTP, 40–50 μg of the supernatant enzyme fraction, 2·0 A 260 units of ribosomes and ^{14}C-aminoacyl-*t*RNA as indicated in each legend. When monomeric ribosomes were used 50 μg of poly U and ^{14}C-phenylalanyl-*t*RNA (indicated in each legend) were included in the reaction mixture and the $MgCl_2$ concentration was raised to 10 mM. Incubation was at 37° for 20 min. The reaction was terminated by adding trichloroacetic acid (TCA) to a final concentration of 5%. The acidified reaction mixture was then heated at 90° for 15 min. Hot trichloroacetic acid-insoluble radioactivity retained on Whatman GFA glass fibre discs, determined in a liquid scintillation spectrometer, was taken as a measure of incorporation of amino acid into polypeptide. For the aminoacyl-*t*RNA binding assay the reaction mixture was incubated for 10 min at 0°, after which time the reaction was terminated by dilution followed by filtration on a millipore membrane (Schleicher and Schull HA 0·45 μ) according to the method of Nirenberg and Leder.[42] The membrane was then dried and counted in a liquid scintillation spectrometer.

Acknowledgement—This research was supported by AEC contract AT(38-1)-643 to J.L.K.

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