PREPARATION AND FUNCTIONAL INTERACTION OF AN AMINOACYL-tRNA SYNTHETASE COMPLEX AND A PEPTIDE INITIATION FACTOR

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Abstract

Five aminoacyl-tRNA synthetases exist in rabbit reticulocytes as an enzyme complex of about 5 x 10^5 molecular weight. The complex binds to ribosomes. In this state it appears to promote the synthesis of Met-$tRNA_f^{Met}$ bound to ribosomes with a presumptive initiation factor and ApUpG. The molar ratio of the synthetase complex and $tRNA_f^{Met}$ is near unit in reticulocytes. Disruption or removal of the complex from ribosomes may be a primary factor in the relatively low synthetic efficiency for protein synthesis of most fractionated cell-free systems.

Introduction

The primary lesion in neoplasis may involve a block in cell differentiation. This might be at either the level of transcription or translation or at both of these levels. Fractionated cell free systems of high synthetic capacity are needed to search for hypothetical inhibitor or promotor factors. However, fractionated systems for translation of mRNA from eukaryotic organisms have had the invariable characteristic of relatively low synthetic activity compared with the intact cells or unfractionated cell lysates from which they were derived. This is a limiting factor in the detection and assay of regulatory elements that have a quantitative effect in normal or neoplastic cells. A component that would cause a two-fold change in the rate of synthesis of a protein within the intact cell might well have a dramatic effect on its physiology, but probably would not be detected in most fractionated systems. This difficulty may be reflected in the *in vitro* assay of mRNA. Results from a number of laboratories have lead to the widely accepted concept that there is little or no discrimination in the translation of different mRNA's at the level of peptide initiation (1, 2, 3). However, recent work from several laboratories (4, 5) strongly supports earlier indication of factors with specificity for different species or classes of mRNA (6). Also, such systems may be particularly important in studying certain types of cellular modifications such as hypothetical suppressors of nonsense mutations in eukaryotic cells.

We have been interested in the regulatory role tRNA and the aminoacyl-tRNA synthetases may play in intact reticulocytes as well as the upper limit they may establish for the rate of *in vitro* protein synthesis. Previously we demonstrated that some but not all of the aminoacyl-tRNA synthetases are associated to a high degree with ribosomes during their preparation and appear to have been bound to the ribosomes, probably to the 60S ribosomal sununit, in the intact cell (7). Here we describe an enzyme complex that appears to contain five aminoacyl-tRNA synthetases including the enzyme for $tRNA_f^{Met}$. The complex binds to isolated 60S ribosomal subunits. The Met-tRNA synthetase of the complex appears to function in synthesis of Met-tRNA while both deacylated $tRNA_f^{Met}$ and the complex are bound to the ribosomes.

Material and Methods

A detailed description of the preparation of rabbit reticulocytes, reticulocyte ribosomes, reticulocyte peptide elongation enzymes, tRNA and assay conditions for these factors are given elsewhere (8, 9). Detail of the assay procedure used for aminoacyl-tRNA synthetases has been published (7). Generally this activity was determined in 0.5 ml reaction mixtures containing 100 mM Tris · HCl, pH 7.5, 2×10^{-6} M ^{14}C · amino acid (specific activity 100 mCi/mmole), 2 mM ATP (previously adjusted to pH 6.5 with KOH), 20 mM β-mercapto-ethanol, and 100 μg of rabbit liver tRNA. KCl and $MgCl_2$ were added to give the concentrations for each amino acid previously given (7).

The assay and isolation of the 40S ribosomal subunit Met-$tRNA_f^{Met}$ binding factor has been described (10) as has the preparation of the subunits from reticulocyte ribosomes used in these studies (11). Generally, for assay of the tRNA binding factor each assay reaction contained in a total volume of 0.25 ml: 20 mM Tris · HCl, pH 7.5, 100 mM KCl, 8 mM $MgCl_2$, 10 mM β-mercaptoethanol, 20 μg of poly U, 60 pmoles Phe-tRNA (about 100 μg of tRNA charged generally with ^{14}C · phenylalanine, 100 Ci/mole), 90 μg of 40S ribosomal subunits and binding factor to give 4 to 6 pmoles total of Phe-tRNA bound. ApUpG and Met-tRNA or other codons and aminoacyl-tRNA were substituted for poly U and Phe-tRNA were indicated.

RESULTS

Isolation and Properties of the Met-tRNA Synthetase Complex

A detailed description of the isolation and characterization of the Met-tRNA synthetase complex will be given elsewhere, however, the steps of the procedure and the yield obtained is presented in Table I. The complex has been isolated from both the high speed supernatant and the salt wash fraction removed from rabbit reticulocyte ribosomes with 0.5 M KCl. We prefer the former for isolation of the complex as indicated in Table I, in that it allows the simultaneous preparation of the peptide elongation factor, EF-I, which is separated from the synthetase complex on Sepharose 4B.

The steps shown in Table I provide a purification of about 2300 fold from the high speed supernatant. This purified material has been used in most of the experiments described below. On the basis of centrifugation data in sucrose or glycerol

gradients or in the analytical ultracentrifuge, as shown in figure 1, we estimate that about two-thirds of the protein in the preparation is associated with the synthetase complex. The activity of this preparation for the formation of aminoacyl-tRNA with various amino acids is shown in Table II. The activities for arginine, isoleucine, leucine, lysine and methionine are relatively high and in an approximately constant ratio throughout the final steps of the isolation procedure. The preparation has no detectable EF-I or EF-II, but contains relatively low amounts of synthetase activity for cysteine and glutamine. The activity for these amino acids is nearly eliminated in material isolated from the protein peak in sucrose gradients of the type shown in figure 1. These enzymes appear to sediment more slowly than the main component

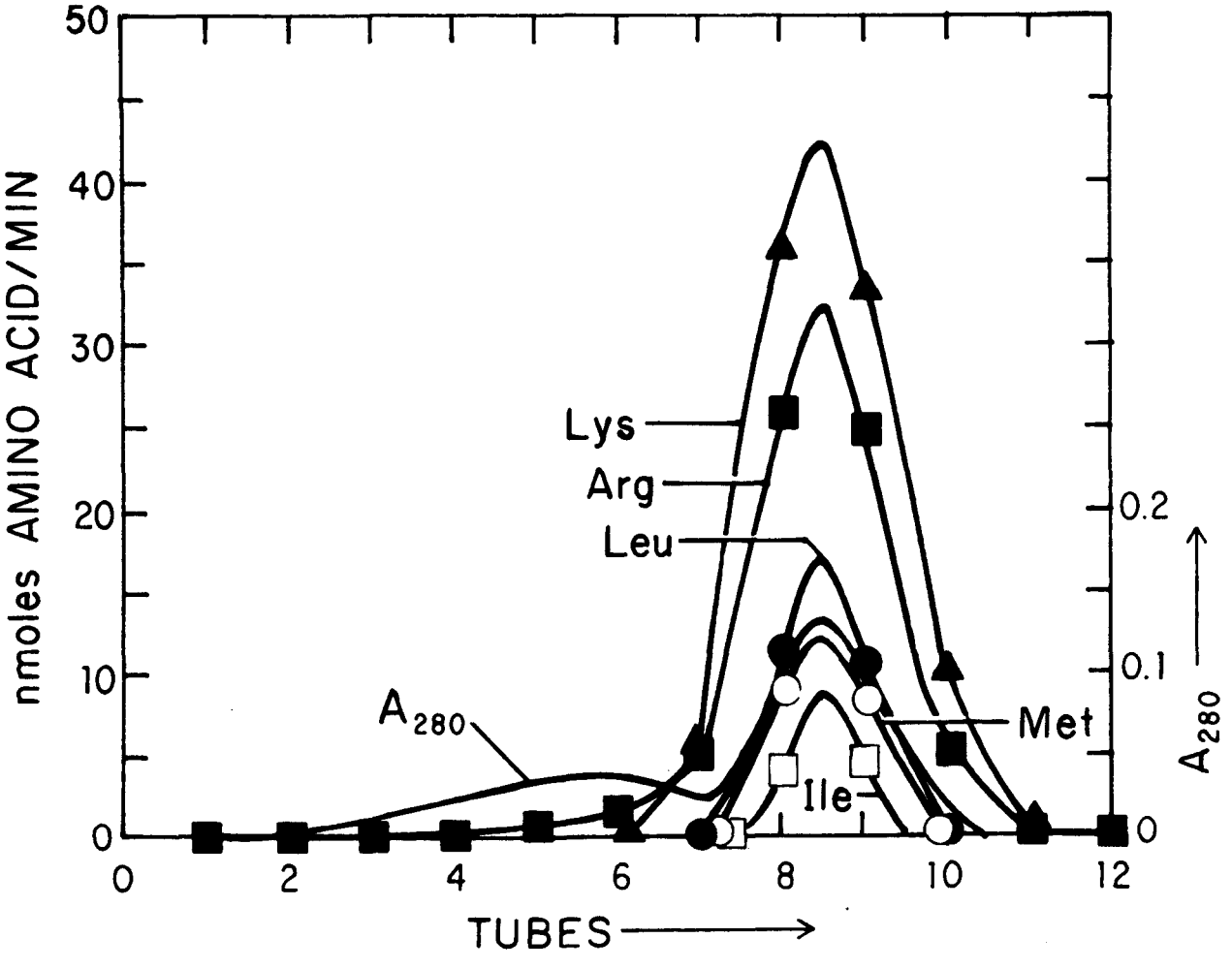

Fig. 1: Distribution on a Sucrose Gradient of Protein and Aminoacyl-tRNA Synthetase Activity of the Enzyme Complex

The Met-tRNA synthetase complex was purified through the fractionation steps shown in Table 1. Then, 0.5 mg of this material was layered on a 10 % to 30 % sucrose gradient containing 100 mM KCl, 20 mM Tris · HCl, pH 7.5, 5 mM β-mercapto-ethanol, and 1 mM dithioerythritol and centrifuged at 40,000 rpm in a SW 41 rotor for 10 hours. Aliquots of 10 µl for arginine, lysine and 50 µl for methionine, leucine and isoleucine from the 1.0 ml fractions of the gradient were used in the standard assay procedure to determine the indicate enzyme activities. The activities of each enzyme were adjusted to a common base to facilitate a direct comparison of the enzyme activities within the complex.

Table 1. Purification of an Aminoacyl-tRNA synthetase complex

Fractionation Step	Total Recovery Protein mg	Enzyme units*	Specific Activity units/mg ptn	Purification
High Speed Supernatant	1.37×10^5	2320	0.017	–
40%–70% $AmSO_4$	5000	1340	0.264	15.7
Sepharose 4B	138	590	4.25	248.0
Hydroxylapatite	10.6	150	14.2	829.0
Phosphocellulose	3.07	124	40.4	2360

*nmoles of Arg-tRNA/minute formed in the standard assay procedure.

Table 2. Aminoacyl-tRNA Synthetase activity in the Met-$tRNA_f$ Complex

Amino Acid	Purified Complex activity*/mg ptn	Purified Complex Total Activity Recovered	Initial Total Activity	% Recovered in Complex	Present in Complex
Arg	32.2	124	2320	5.3	Yes
Cys	1.3	5.0	469	1.1	? (No)
Glu	1.1	4.2	443	0.9	? (No)
Ile	4.5	17.3	1175	6.5	Yes
Leu	12.9	49.7	1344	3.7	Yes
Lys	42.6	164	2547	6.4	Yes
Met	10.5	40.4	603	6.6	Yes
All others	0.5	–	–	0.5	No

*nmoles of aminoacyl-tRNA formed/min in standard assay.
Activity for all other amino acids was less than 0.5 nmoles of aminoacyl-tRNA formed per mg protein in complex and recovery was less than 0.1×10^{-3}.

Table 3. Properties of the Aminoacyl-tRNA Synthetase Complex

1. Molecular weight	about 500,000
2. $S_{w,20}$	14.7
3. SDS Gel Subunits	about 14
4. Met charging for both $tRNA_M^{Met}$ and $tRNA_f^{Met}$	
5. Binds to and charges on the 60S ribosomal subunit	

in sucrose gradients. The significance of their presence in the preparation at this stage of purification is not clear. They may belong to another, slightly smaller complex, however, we conclude that they are not an integral part of what we propose to call the Met-tRNA synthetase complex. The level of aminoacyl-tRNA synthetase activity for all of the other common amino acids is below the limits of the assay system used. Physical characterization of the Met-tRNA synthetase complex has not been completed, however, as summarized in Table 3, it has a sedimentation coefficient of 14.7 S and a molecular weight of about 500,000. As anticipated it gives multiple bonds on SDS gel electrophoresis. We believe we can detect 14 distinct bands, which might be subunits of the various enzymes of the complex, however, this is an approximate value. Nucleic acid including tRNA is below detectable levels at this stage of purification and does not appear to be an integral part of the complex.

A most interesting feature of the Met-tRNA synthetase complex is its propensity toward binding to ribosomes, particularly to the 60S ribosomal subunit, and its apparent ability to carry out its synthetic functions while so bound. The distribution on sucrose gradients of Arg-tRNA and Lys-tRNA synthetase activity in the presence of isolated 40S or 60S ribosomal subunits is shown in figure 2. The complex from the salt wash fraction was mixed with the indicated type of ribosomal subunits then layered on sucrose gradients which were then centrifuged for different times to bring the ribosomal subunits to the indicated position in the gradient. The position of the synthetase complex was determined by assaying appropriate aliquots of fractions taken from the gradient. Similar results have been obtained with binding of purified complex. The five amino-acyl-tRNA synthetases present in the complex give similar patterns, indicating that the complex was maintained as a unit. Nearly all of the synthetase activity was bound to the 60S ribosomal subunits, but very little binding was observed with 40S subunits. Phe-tRNA synthetase present in the salt wash also binds to the 60S subunit but is not present in the purified complex. This reflects tight binding of the complex to the 60S subunit for it to remain associated with the subunit during sucrose gradient centrifugation. Also, it should be noted that the activity of the synthetases were determined by taking aliquots of gradient fractions into reaction mixtures that differed from the gradient only in sucrose concentration and having tRNA, the appropriate amino acid and ATP. In other similar studies we have observed that synthetase activity is maintained when ribosomes are added to reaction mixtures under conditions in which the synthetases will be bound to the ribosomes. These observations appear to indicate that synthetases maintain their synthetic activity when bound to the ribosomes.

Purification and Properties of the tRNA Binding Factor

The relations outlined above carry the implications that the enzymes of Met-tRNA synthetase complex may function in a physiologically significant manner while they are associated with ribosomes within intact reticulocytes. A protein component capable of promoting codon-directed binding of $tRNA_f^{Met}$ to 40S ribosomal subunits, presumably a peptide initiation factor, was used to further test this hypothesis. This protein factor appears to be similar or identical to the factor that has been reported by several laboratories (12, 13, 14, 15) and extensively

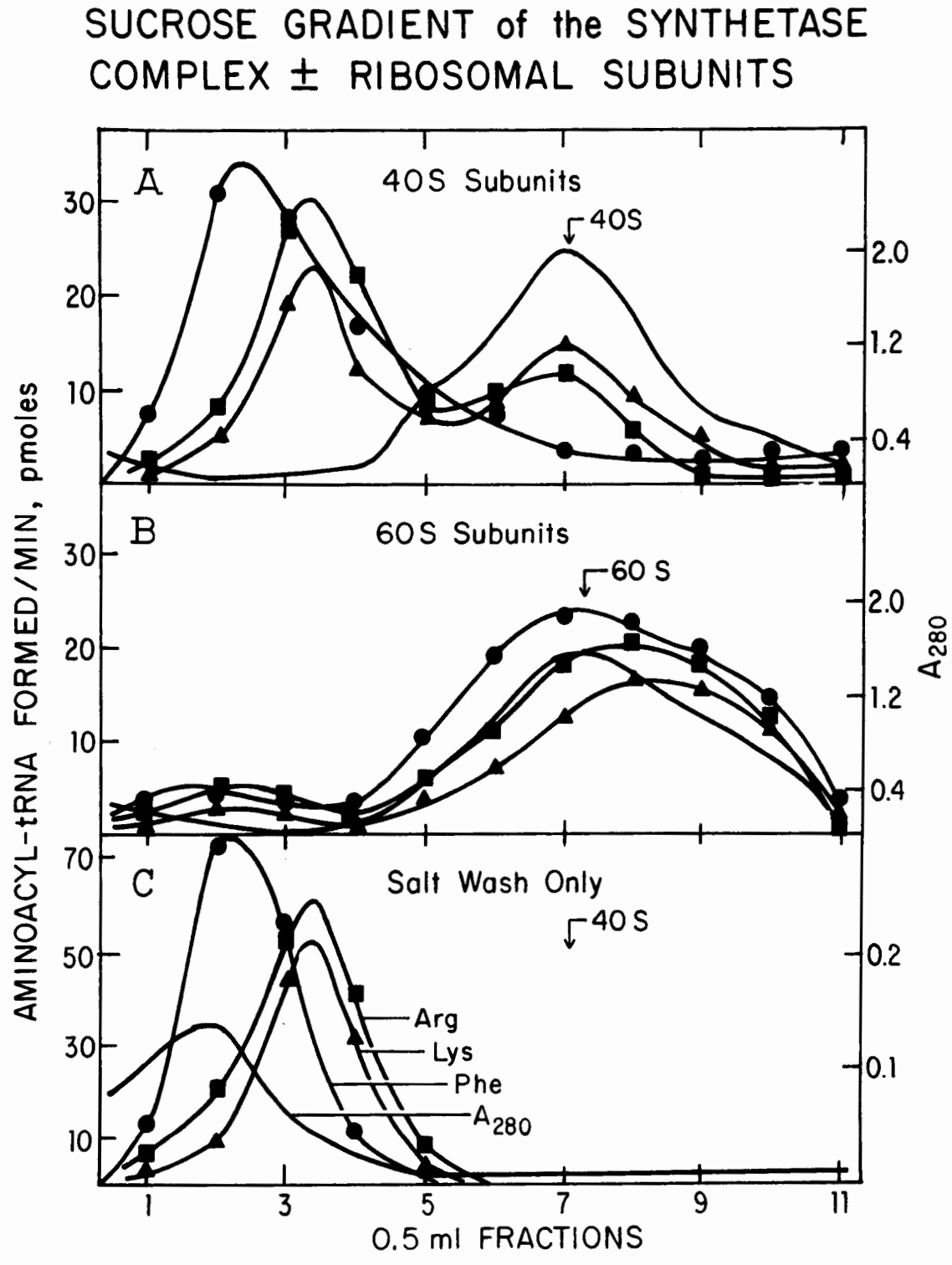

Fig. 2: Distribution of the Met-tRNA Synthetase Complex with Ribosomal Subunits in a Sucrose Gradient

Ribosomal subunits, 0.5 mg, were mixed with 250 μg of salt wash protein in 0.25 ml samples containing 20 mM Tris ·HCl, pH 7.5, 25 mM KCl, 5.0 mM MgCl, and 1.0 mM β-mercaptoethanol. The samples were layered on 5 ml, 10—30 % linear sucrose gradients containing the same salt concentrations as the samples. The gradients were centrifuged 1.5 hr at 60,000 rpm in a SW 65 rotor for gradients containing salt wash alone or 40S subunits. Centrifugation time was reduced to 1.0 hr for the gradient containing the 60S subunit. The distribution of the Met-tRNA synthetase complex is indicated by the activities arginine and lysine. (●) Phe-tRNA; (▲) Lys-tRNA; (■) Arg-tRNA.

purified from brine shrimp by Zasloff and Ochoa (16). The steps and yield of the procedure that we have used to purify the factor from reticulocytes is outlined in Table 4. The procedure provides about a 2500-fold purification of the factor from the supernatant of the high speed centrifugation used to collect ribosomes from the reticulocyte lysate. The product from the procedure appears as primarily a single component on SDS gel electrophoresis and a single component of 3.7 $S_{w,20}$ in the analytical ultracentrifuge. It has a molecular weight of about 86,000 as determined by gel filtration chromatography.

The factor has the capacity to promote binding of $tRNA_f^{Met}$ or, to a less efficient degree, $tRNA^{Phe}$ to 40S ribosomal subunits. This binding is prevented by addition of 60S ribosomal subunits to the reaction mixture. The requirements for binding of Phe-tRNA are presented in Table 5. The binding reaction is strictly codon dependent and inhibited by 60S ribosomal subunits. The factor does not promote ApUpG directed binding of $Met\text{-}tRNA_M$, as shown in Table 6, even though ApUpG directs binding of $Met\text{-}tRNA_M$ at higher Mg^{++} ion concentrations. Met-tRNA bound to 40S ribosomal subunits with the factor has relatively low reactivity for reaction with puromycin even though aminoacylated forms of $Met\text{-}tRNA_f$ react readily under similar conditions. The reaction of $Met\text{-}tRNA_f$ and N-acetylMet-$tRNA_f$ with puromycin are presented in Table 7. For these experiments either Met-tRNA or N-acetylMet-tRNA were bound under standard conditions to 40S ribosomal subunits and then the 60S ribosomal subunits and puromycin were added to the reaction mixtures. $Met\text{-}tRNA_f$ was not appreciably reactive with puromycin and could not be activated with either of the peptide elongation enzymes or ribosomal salt wash fractions that promotes peptide initiation with globin mRNA. In contrast N-acetylMet-tRNA is bound to the ribosomes in relatively large amounts, most of which is reactive with puromycin without the addition of other factors to the reaction mixture. It should be noted that Met-tRNA rather than N-formylMet-tRNA, is thought to function in peptide initiation in eukaryotic organisms.

Table 4. Purification of the Initiator tRNA Binding Factor

Fraction	Total Volume (ml)	Total Protein (mg)	Total Units* ($x10^{-3}$)	Specific Activity (units/mg)	% Yield
High Speed Supernatant	2,000	69,000	278	4	100
40–70 Ammonium Sulfate	120	6,000	120	20	43.2
DEAE Cellulose	80	520	83	160	29,9
Cellulose Phosphate	5.4	6.25	31.2	5,000	11.2
Pulverized glass	3.1	2.06	22.4	10,800	8.1
Hydroxylapatite	2.0	1.54	17.0	11,000	6.1

*pmoles Phe-tRNA bound as determined in the standard assay system.

A detailed description of the fractionation procedure and assay of the factor is presented elsewhere (10).

The tRNA binding factor promotes binding of deacylated $tRNA_f^{Met}$ or $tRNA^{Phe}$ with relatively high efficiency as compared with Met-$tRNA_f$ or Phe-tRNA. Results for Met-tRNA and deacylated $tRNA_f^{Met}$ are shown in figure 3. For these experiments, *E. coli* tRNA estimated to be more than 75 % $tRNA_f^{Met}$ was either charged with ^{35}S • methionine with *E. coli* synthetase or labeled with tritium by exchange procedure as described previously (18, 19). Binding of the uncharged ^{3}H • $tRNA_f^{Met}$ was determined in the standard assay procedure used for Met-$tRNA_f$.

The significance for peptide initiation of this binding of deacylated $tRNA_f^{Met}$ to 40S ribosomal subunits is not clear, however, this property has been used in the experiments described below with the Met-tRNA synthetase complex.

Table 5. Characteristics of the standard assay system

Additions	tRNA Binding pmoles		
	Factor —	Addition +	Factor Activity
Complete*	1.20	4.85	3.65
– poly (U)	0,08	0.04	0
– 40S subunits	0.02	0.01	0
+ 60S subunits	0.55	1.45	0.90
4 mM Mg^{++}	0.20	0.55	0.35
+ GTP	1.20	4.80	3.60
N-acetylPHe-tRNA†	130	4.56	3.26

* The complete system is the standard assay system with 0.36 μg of protein from the hydroxylapatite fraction prepared as described.

† In place of Phe-tRNA.

Table 6. Specificity of $tRNA_f^{Met}$ Binding Factor with ApUpG Codon

tRNA Species	Binding Factor	pmoles bound
Met-$tRNA_f$	—	0.10
	+	2.75
Met-$tRNA_M$	—	0.11
	+	0.24

Binding of Met-tRNA was carried out in the standard assy with system with Met-$tRNA_M$ substituted for Met-$tRNA_f$ where indicated.

Table 7. Puromycin Reactivity of Bound Met-tRNA$_f$ and Ac-Met-tRNA$_f$

Species Bound	Additions	Met-Puromycin pmoles	Formed percent
Met-tRNA$_f$	None	0.23	14
1.65 pmoles	EF-I + GTP	0.20	12
	EF-II + GTP	0.25	15
	EF-I + EF-II + GTP	0.28	17
	Salt Wash + GTP	0.43	26
AcMet-tRNA$_f$	None	2.62	90
2.90 pmoles	Salt Wash + GTP	2.51	87

Binding of Met-tRNA or N-acetyl Met-tRNA was carried out in the standard assay system then 60S ribosomal subunits were added to the reaction mixture to give a 1 : 1 molar ratio with the 40S subunits. Then the other components indicated in the table were added. Quantities added were: salt wash, 80 μg; EF-II, 2 μg; GTP, 0.2 mM; puromycin, 0.5 mM. Then the reaction mixture was incubated 20 minutes at 37° and reactivity with puromycin determined by extraction of the product formed with ethylacetate saturated with methionine by a procedure similar to that described by Leder and Bruztyn (17).

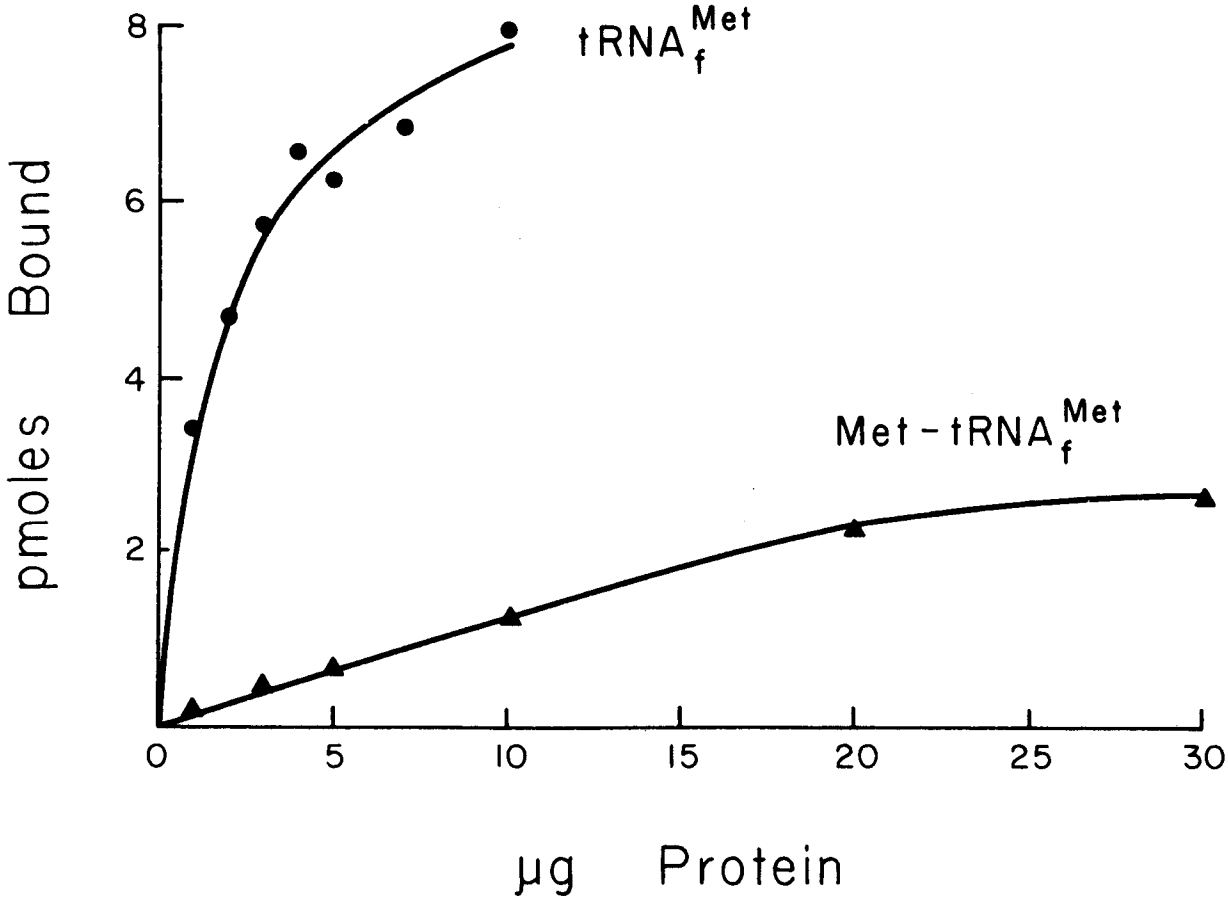

Fig. 3: Binding of tRNA$_f^{Met}$ or Met-tRNA$_f$ with the Binding Factor
Deacylated tRNA$_f^{Met}$ or Met-tRNA$_f$ was bound to 40S ribosomal subunits with ApUpG as described in the text.

Charging of $tRNA_f^{Met}$ Bound to Ribosomes

Earlier studies indicated that $tRNA_f^{Met}$ is accumulated on ribosomes when intact reticulocytes are incubated with NaF (20). NaF blocks initiation of peptides on reticulocyte ribosomes in intact reticulocytes with little or no effect on peptide elongation, thus allowing the breakdown of polysomes by the completion and release of nascent peptides. The block in peptide initiation caused by NaF appears to be beyond the step at which $tRNA_f^{Met}$ presumably as the charged species, is bound to the ribosomes. It is not clear why most of the $tRNA_f^{Met}$ is in the deacylated form. It may be due to the action of a Met-$tRNA_f^{Met}$ hydrolase that has the ability to deacylate this species of tRNA while it is bound to the 40S ribosomal subunit (21). These NaF ribosomes that bear primarily deacylated $tRNA_f^{Met}$ can be easily prepared by procedures similar to those used for regular ribosomes. Like regular ribosomes they have high proportion of the total Met-tRNA synthetase complex present in the cell associated with them at isolation. We have used these ribosomes to test the hypothesis that the $tRNA_f^{Met}$ could be charged while it was bound to the ribosomes.

For these experiments NaF ribosomes that bear both deacylated $tRNA_f^{Met}$ and Met-tRNA synthetase were added to reaction mixtures that contain the concentrations of salts, ATP and ^{35}S • methionine used in the standard assay system. No additional tRNA or enzyme fraction was added to the reaction. After incubation the reaction mixture was diluted about 20-fold by the addition of a solution containing the same salts at the concentrations of those in the reaction mixture, and then the ribosomes were pelleted by centrifugation. The RNA was extracted from the ribosomal pellet with sodium dodecylsulfate and phenol then the tRNA extracted with 1.0 M NaCl as previously described (8). Unfractionated tRNA also containing $tRNA_M^{Met}$ was charged with ^{3}H • methionine and an appropriate aliquots mixed with the ^{35}S • Met-tRNA extracted from the NaF ribosomes. The mixture of ^{35}S and ^{3}H • Met-tRNA's were chromatographed on BD cellulose essentially as described by Kerwar, Spears and Weissbach (22) and the distribution of the two labels followed. The results are presented in figure 4. Nearly all of the ^{35}S • methionine from the NaF ribosomes is in peak I which is Met-$tRNA_f$. Nearly all of the tRNA present on the NaF ribosomes was charged, as indicated by a comparison of the amount of ^{35}S • Met-tRNA formed by first extracting the tRNA and then charging it in a subsequent step.

The experiment described above with NaF ribosomes has served as a model for an apparently comparable experiment carried out with the tRNA binding factor and the Met-tRNA synthetase complex. For these experiments 40S ribosomal subunits were incubated with unfractionated tRNA, tRNA binding factor and ApUpG under conditions of the standard assay. After incubation, the reaction mixture was diluted with a solution containing the same concentration of salts used in the standard reaction mixture, and then the subunits bearing the uncharged tRNA were collected by centrifugation and resuspended in a solution containing 4 mM MgCl, 25 mM KCl, and 10 mM Tris-HCl, pH 7.5. To aliquots of this solution were added 60S ribosomal subunits to give a molar ratio of 60S to 40S subunits of about unity. These ribosomes were added in the amount of 2.0 mg/ml to the standard

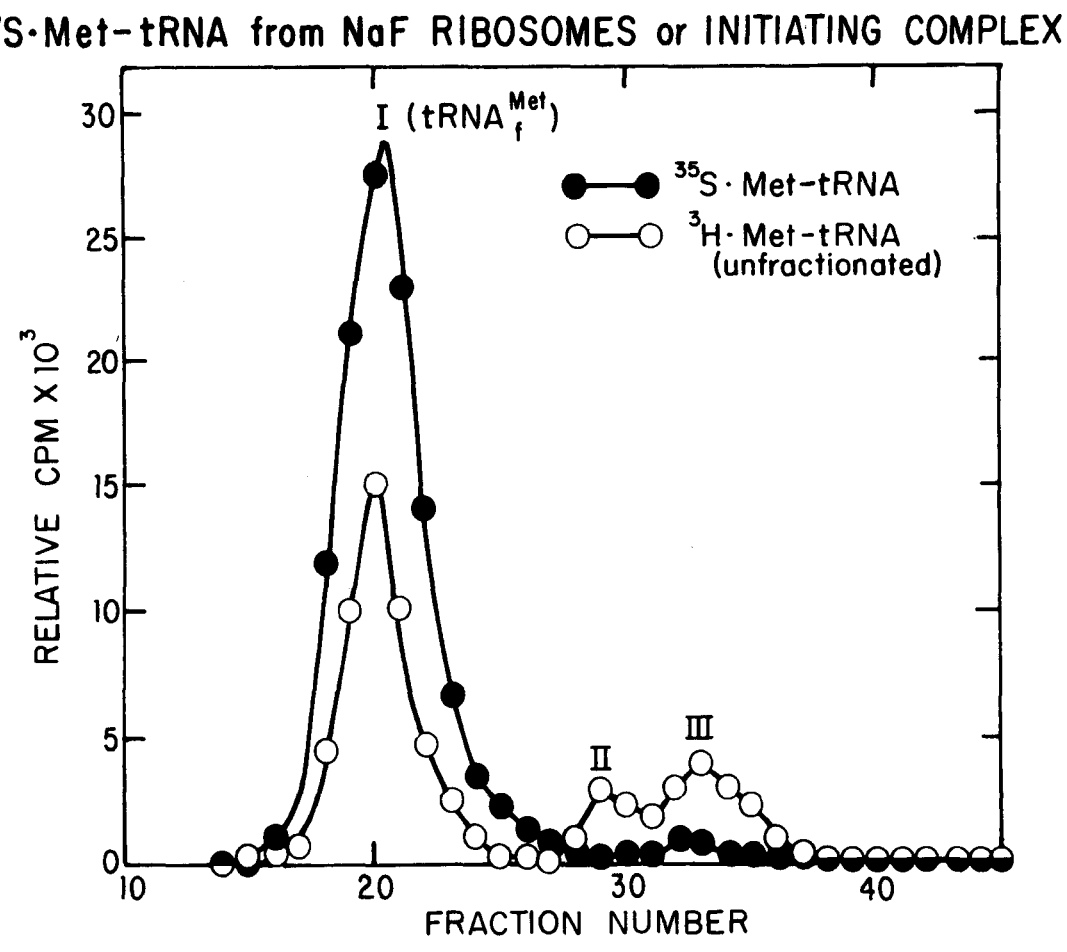

Fig. 4: Distribution of Met-tRNA from NaF Ribosomes or from tRNA Originally Bound to 40S Ribosomal Subunits with the Binding Factor.

Deacylated tRNA bound to NaF ribosomes or to ribosomal subunits with the tRNA binding factor was charged with ^{35}S - methionine, reisolated and chromatographed on BD cellulose with unfractionated ^{3}H - Met-tRNA as described in the text.

reaction mixture modified to contain 2.5 mM KCl. It also contained purified Met-tRNA synthetase complex, ATP and ^{35}S - methionine. After incubation, the tRNA was extracted from the reaction mixture then chromatographed on BD cellulose with unfractionated ^{3}H - Met-tRNA as described above. The distribution of ^{35}S and ^{3}H - methionine were nearly identical to those shown in figure 4. Nearly all of the tRNA bound to the 40S ribosomal subunits was charged during the incubation.

These results with NaF ribosomes and with the tRNA binding factor plus the Met-tRNA synthetase complex appear to indicate that Met-tRNA$_f^{Met}$ can be charged while it is bound with ApUpG and the binding factor to the ribosomes. In addition, the results appear to indicate that with ApUpG the binding factor is able to promote the selective binding of tRNA$_f^{Met}$ with high discrimination against tRNA$_M^{Met}$.

Discussion

We consider the development of a highly active, fractionated cell-free system of primary importance for study of the factors at the molecular level that control and regulate the synthesis of specific proteins. To date this objective has not been accomplished. We believe that the phenomena described here may give some insight into an aspect of the problem that generally is not considered. Classically, it has been assumed that aminoacyl-tRNA is formed free in solution by the respective synthetase enzymes and would be carried to the ribosomes by free diffusion. This may not be the primary mechanism in intact cells.

The molar ratio of tRNA to ribosomes in most cells does not appear to favor a diffusion limited reaction. Maaløe and Kjedlgaard (23) estimated 7–15 molecules of unfractionated tRNA per ribosome in *E. coli*. We find a similar ratio, estimated to be about 10, in rabbit reticulocytes. This is the total tRNA. Minor species may account for 1 % or less of the total. It appears that most tRNA species are present in reticulocytes at less than a 1 : 1 molar ratio. We estimate this ratio to be about 0.3 for Met-$tRNA_f^{Met}$.

The molar ratio of the Met-tRNA synthetase complex to $tRNA^{Met}$ and ribosomes is also an interesting figure. We have attempted to estimate the molar amount of Met-tRNA synthetase complex in intact reticulocytes from the specific activity of the Arg-tRNA synthetase in the purified complex, a molecular weight of 5×10^5 for the complex, and Arg-tRNA synthetase activity in cell lysates in which free arginine and other small molecules had been removed by gel filtration and protein synthesis was blocked with cycloheximide. We estimate 0.2 moles of Met-tRNA synthetase complex per mole of ribosome. Thus, $tRNA_f^{Met}$ and the Met-tRNA synthetase complex are present in similar molar amounts. It should be stressed that value is subject to some error, but we doubt that it is off by more than a factor of 2. Loftfield (24) has pointed out that all measurements of K_D for the dissociation of the enzyme tRNA complex and all K_m's for this reaction fall in a range of 10^{-7} M or less. This appears to indicate that there will be a relatively low proportion of these components that exist as free enzyme and tRNA within the cell. Jacobson has reached a similar conclusion (25). It is conceptually difficult from the physical standpoint to see how the charging reaction could take place on a ribosome in concert with the other reactions of peptide synthesis that occur on the ribosome but this may be indicated.

The data and considerations presented here carry the potential implication that charging of tRNA as well as the reactions of peptide elongation may be carried out on the ribosomes. Disruption of this organized system or changing the concentration or ratio of components in the cell-free system from those in intact cells might account for the characteristic reduction in synthetic capacity.

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