INITIATOR-LIKE PROPERTIES OF A METHIONYL-tRNA FROM WHEAT EMBRYOS

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Summary. The two major methionyl-tRNA species from wheat embryos and E. coli have been studied as regards their capacity to form a ternary complex with GTP and the ribosomal binding enzyme from both sources. Methionyl-tRNA$_1$ from wheat resembles met-tRNA$_F$ from E. coli in its inability to interact with the homologous enzyme. It also fails to complex with the bacterial enzyme. Methionyl-tRNA$_2$ from wheat is similar to met-tRNA$_M$ from E. coli in that forms the ternary complex with the enzyme from both organisms. Wheat met-tRNA$_1$ has a markedly higher affinity for binding "non-enzymatically" to wheat ribosomes in the presence of ApUpG than does met-tRNA$_2$.

N-formylmethionyl-tRNA acts as the initiator of protein synthesis in bacterial systems and possibly in specialized organelles of higher organisms (1-3). Recent reports (4,5) suggest that one of two major methionyl-tRNA species of eukaryote cells may be involved in cytoplasmic peptide chain initiation. One of the specific properties of the formylatable methionyl-tRNA species (met-tRNA$_F$) in bacteria is its incapacity to form a ternary complex with the polymerization factor T_u, also known as S_3, (6,7) and GTP. This property is important for its function as a polypeptide chain initiator since it prevents entrance into the ribosomal A site during the elongation process and consequently blocks its incorporation into the internal positions of the polypeptide chain. Previously (8,9) we have demonstrated the existence in wheat embryo extracts of an enzyme that interacts with GTP and aminoacyl-tRNA and have demonstrated the role of this ternary complex in the binding of aminoacyl-tRNA to wheat ribosomes. Similarly we have described (10) the separation and coding properties of the two major methionyl--tRNA species from wheat embryos.

In the present communication we report studies on the interaction of both met-tRNA species from wheat and E. coli with the enzymes responsible for binding

aminoacyl-tRNA to the ribosomes (E. coli T and wheat T_1 enzymes). The results show that one of the two major wheat species (met-$tRNA_1$) does not form a ternary complex with the homologous enzyme or with that purified from E. coli. Under similar conditions, the other wheat met-tRNA species (met-$tRNA_2$) forms complexes with both enzymes. Met-$tRNA_1$ species from wheat also differs from met-$tRNA_2$ in its higher affinity for binding "non-enzymatically" to wheat ribosomes in the presence of the triplet ApUpG.

Materials and Methods. L-methionine labelled with ^3H and ^{14}C and ^{14}C-formate were purchased from New England Nuclear Corporation. E. coli tRNA and benzoylated DEAE-cellulose were purchased from Schwarz Bioresearch.

Viable wheat embryos were prepared by the procedure of Johnston and Stern (11) from a genetically pure strain of Triticum durum obtained from INDAP, Chilean government. Wheat tRNA, ribosomes and supernatant fraction were prepared as described (10). The T_1 enzyme from wheat was present in the 40-80% ammonium sulfate fraction of the supernatant (9).

Radioactive aminoacyl-tRNA was prepared by the procedure of von Ehrenstein and Lipmann (12). E. coli T factor was isolated essentially as described previously (13) and assayed by its capacity to bind radioactive ^3H-GTP to nitrocellulose membranes (Sartorius, Germany). The ternary complex between T or T_1 enzymes, aminoacyl-tRNA and GTP was formed as follows: 50 mM sodium cacodylate buffer pH 7.0, 10 mM $MgCl_2$, 150 mM NH_4Cl, 1 mM dithiothreitol, 150 uM GTP, between 40-100 pmoles of methionyl-tRNA and enough enzyme to bind 150 pmoles of GTP was incubated 10 minutes at 0° in 1 ml volume and then passed through a Sephadex G-100 column (1.45 x 55 cm) equilibrated and eluted with 50 mM sodium cacodylate pH 7.0, 10 mM $MgCl_2$, 150 mM NH_4Cl, 1 mM 2-mercaptoethanol and 75 uM GTP, with a flow rate of 10.8 ml/hour. Aliquots of 1 ml were precipitated with cold 5% trichloroacetic acid, filtered on nitrocellulose membranes and counted in a scintillation system.

The trinucleotide ApUpG was synthesized by the method of Thach et al (14). Methionyl-tRNA binding to ribosomes in the presence of the triplet ApUpG was

done as described by Leder and Nirenberg (18). $N^{5,10}$-methenyl-^{14}C-tetrahydrofolate preparation and the assay for the formylation of methionyl-tRNA was performed by the method of Dickerman and Weissbach (15).

Results. Figure 1A shows that chromatography on benzoylated DEAE-cellulose of wheat methionyl-tRNA results in a clear separation of two major species which we call met-$tRNA_1$ and met-$tRNA_2$. The radioactivity appearing at the column front is free ^3H-methionine. If the wheat tRNA is acylated with methionine with an E. coli extract, only methionyl-$tRNA_1$ is esterified indicating that the bacterial methio-

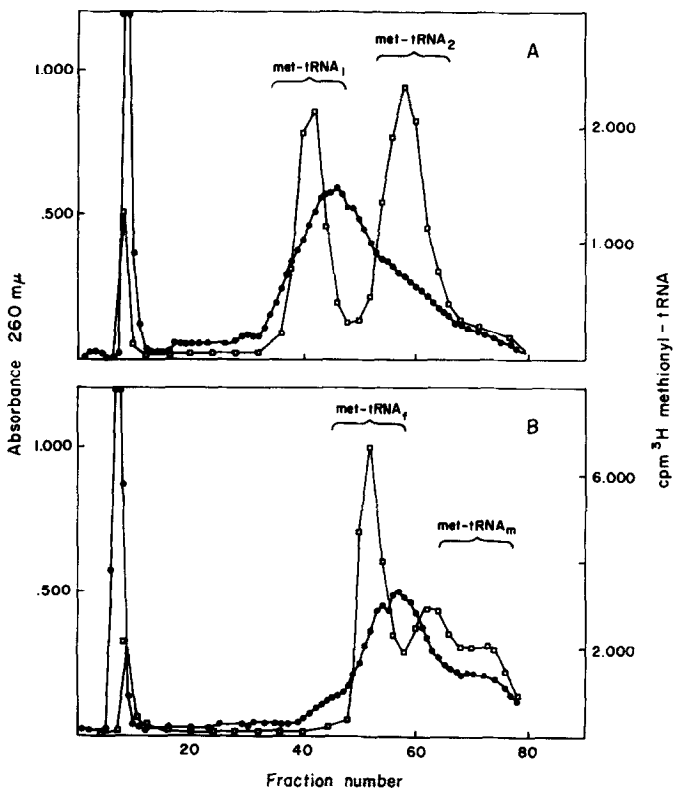

FIGURE 1. Fractionation of ^3H methionyl-tRNA from wheat and E. coli on a benzoylated DEAE-cellulose column. A benzoylated DEAE-cellulose column (19) (1.5 x 35cm) was equilibrated with a buffer containing 10 mM sodium acetate pH 5.2, 10 mM $MgCl_2$, 0.5 mM 2-mercaptoethanol and 0.4 M NaCl and eluted with a linear gradient of salt from 0.4 M - 1.0 M NaCl in the same buffer (400 ml in each chamber). The flow rate was of 80 ml/hour and the fractions contained 6 ml. The column was run at 10°.
Part A, represents the fractionotion of 1 nmole of wheat ^3H met-tRNA; aliquots of 200 ul were directly counted in the scintillation mixture described by Bray (20). Part B, shows the pattern obtained with 800 pmoles of ^3H met-tRNA from E. coli; 200 ul aliquots were counted.

nyl-tRNA synthetase does not recognize the wheat species 2. Fractionation of wheat met-tRNA on a reverse phase chromatography column described by Kelmers et al (16) results in a reverse order of appearence of the two species (10). Figure 1B shows the fractionation of met-tRNA from E. coli in a similar benzoylated DEAE-cellulose column. Table I shows that the two fractions of the E. coli met--tRNA differ in their capacity to be formylated and thus correspond to met-$tRNA_F$ and met-$tRNA_M$ (1). Attempts to formylate either of the wheat met-tRNA species with wheat or E. coli extracts under similar conditions were not succesful. Since a brief report (17) states that one of the two major wheat met-tRNA species can be formylated by the E. coli transformylase, we are presently purifying the bacterial enzyme (15) to check this point again. Incubation of wheat met-tRNA with a crude E. coli extract at 37° results in a large amount of hydrolysis of the met-tRNA.

TABLE I

Formylation of methionyl-tRNA's by E. coli transformylase

Experiment	tRNA added	cpm ^{14}C-formyl incorporated
1	E. coli met-$tRNA_F$ (8 pmoles)	320
	" met-$tRNA_M$ (9.2 pmoles)	59
	" tRNA (commercial unacylated 2 A_{260})	5
2	E. coli met-tRNA unfractionated (20 pmoles)	304
	Wheat met-tRNA " (23 pmoles)	25
	" met-$tRNA_1$ (24 pmoles)	32
	" met-$tRNA_2$ (22 pmoles)	31
	None	42

The formylation reaction was carried out exactly as described by Dickerman and Weissbach (15). The enzyme used was the fraction of the E. coli supernatant that precipitates between 40 and 60% ammonium sulfate and which was treated with protamine sulfate to eliminate contaminating nucleic acids.
The specific activity of the $N^{5,10}$-methenyl-^{14}C-tetrahydrofolate was 28 uC/umole.

Figures 2 and 3 show the elution pattern of different met-tRNA species on Sephadex G-100 columns in the presence of the wheat T_1 enzyme preparation. In 2A, the calibration of the column demonstrates that the enzyme, measured by its ca-

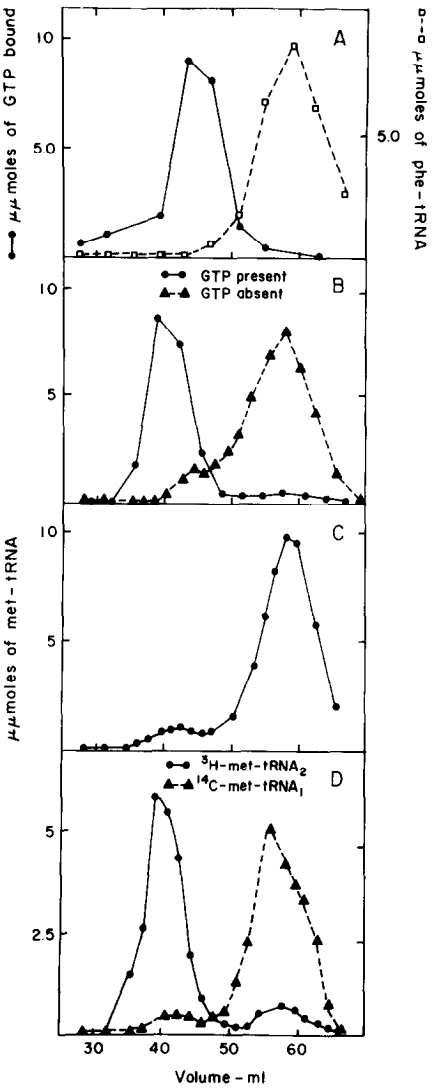

FIGURE 2. Isolation of the ternary complex GTP:methionyl-tRNA:wheat T_1 enzyme by gel-filtration. The general method employed is given in the text. The same amount of wheat T_1 enzyme preparation was used in all experiments (250 ug).
Part A, shows the elution volumes of the wheat T_1 enzyme measured by the capacity of aliquots (100 ul) to retain ^{3}H-GTP on nitrocellulose filters (8) and of ^{14}C--phe-tRNA run separately on the same column.
Part B, shows the elution of wheat ^{14}C-met-tRNA$_2$ (100 pmoles) incubated with wheat T_1 enzyme and GTP. Also shown in this figure is a control experiment in which GTP was left out in the incubation and of the elution buffer.
Part C, shows the elution of wheat ^{14}C-met-tRNA$_1$ (90 pmoles) incubated and eluted with the complete system.
Part D demonstrates the elution of an incubation to form ternary complex in which 50 pmoles of ^{14}C-met-tRNA$_1$ and 50 pmoles of ^{3}H-met-tRNA$_2$ were added together. Aliquots were precipitated, filtered and conted in a scintillation system that discriminates the ^{3}H and ^{14}C radioactivity.

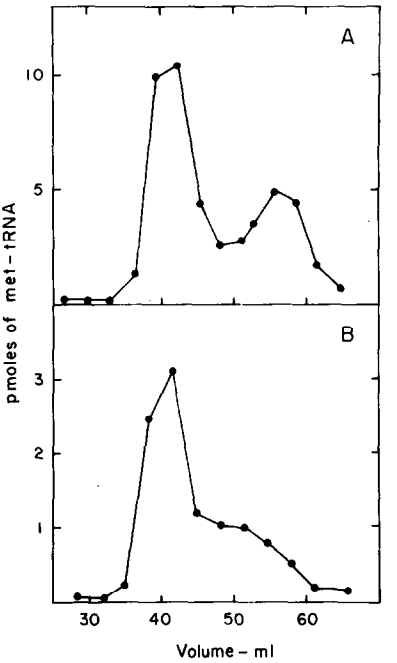

FIGURE 3. Formation of ternary complex with wheat T_1 enzyme and E. coli met-tRNA species. The general procedure is described in the text. The same amount of wheat T_1 enzyme is used as in figure 2. In part A, 120 pmoles of E. coli ^{14}Cmet--tRNA$_M$ was used to form the ternary complex; in part B, 40 pmoles of met-tRNA$_F$ were used.

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pacity to retain GTP on nitrocellulose membranes, elutes considerably ahead of free aminoacyl-tRNA. Wheat met-tRNA$_2$ when run in the presence of T_1 but in the absence of GTP elutes as free aminoacyl-tRNA (2B). In the presence of GTP and the enzyme, however, all met-tRNA$_2$ added complexes and elutes a little ahead of the free enzyme. Using this column the molecular weight of T_1 has been estimated to be approximately 50.000 and of the ternary complex $\backsim$ 70.000.

On the other hand, wheat met-tRNA$_1$ under similar conditions fails to form a complex (2C). The difference between the two wheat met-tRNA species is more strikingly demonstrated in 2D where both species, labelled with different isotopes, were incubated together with the enzyme and GTP and passed through the column. It is clear from this experiment that met-tRNA$_1$ is unable to form the ternary complex and that this incapacity is not a result of some contaminant that may hinder complex formation.

Figure 3 in parts A and B shows that the wheat enzyme can form a ternary complex with both E. coli met-tRNA$_F$ and met-tRNA$_M$, indicating that the heterologous protein cannot distinguish the structural peculiarities of met-tRNA$_F$.

Figure 4 shows the interaction of the different met-tRNA species with the purified T enzyme from E. coli. As reported by Ono et al (6), the bacterial enzyme can form a ternary complex with the homologous met-tRNA$_M$ but not with met-tRNA$_F$ (4A and 4B). In this case, however, the E. coli enzyme can also recognize the difference in the wheat met-tRNA's and complexes met-tRNA$_2$ but not met-tRNA$_1$ (4C and 4D).

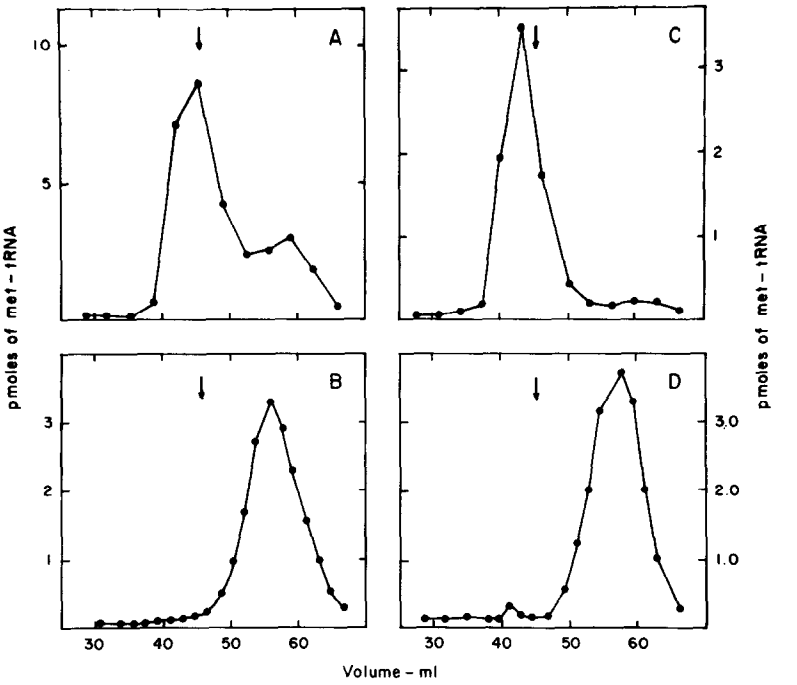

FIGURE 4. Formation of ternary complex with E. coli T enzyme and different met-tRNA species. In all these experiments 30 ug of purified E. coli T enzyme were used. In A, the complex formation was attempted with 100 pmoles of E. coli ^{14}C-met-tRNA$_M$ and in B, with 50 pmoles of ^{3}H-met-tRNA$_F$. In C, the incubation contained 50 pmoles of wheat met-tRNA$_2$ and in D, 50 pmoles of met-tRNA$_1$. The arrow shows the elution volume of the free E. coli T enzyme.

Another difference between the two met-tRNA species of wheat resides in their different ability to bind non-enzymatically to wheat ribosomes in the presence of the trinucleotide ApUpG. Table II shows the effect of different Mg^{++} ion concen-

TABLE II

Binding of wheat methionyl-tRNA species to wheat ribosomes in the presence of ApUpG

Mg^{++} concentration mM	Δ pmoles bound with ApUpG met-$tRNA_1$	met-$tRNA_2$
5	0.06	0
7	0.12	0
10	0.36	0
15	0.67	0
20	0.83	0.11
30	1.16	0.26

The binding reaction contained in a 50 ul volume: washed wheat ribosomes 2 A_{260}, ApUpG 0.28 A_{260}, 50 mM Tris-HCl pH 7.4, 50 mM KCl and 1.2 pmoles of ^3H methionyl-$tRNA_1$ and 1.8 pmoles of ^3H methionyl-$tRNA_2$ (spec. act. of ^3H methionine was 3470 uC/umole; 1 pmole ~ 610 cpm), and was incubated at 25° for 15 minutes. All the values have been corrected by subtracting the binding obtained in the absence of ApUpG (at 20 mM Mg^{++} these blanks were 0.1 pmoles for met-$tRNA_1$ and 0.02 pmoles for met-$tRNA_2$).

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trations on the binding of the two met-tRNA species to wheat ribosomes. It is obvious that met-$tRNA_1$ is much more efficient in its interaction with ribosomes than met-$tRNA_2$. This difference is especially significant at the lower Mg^{++} ion concentrations.

Discussion. The results presented above demonstrate that the two major species of wheat embryo methionyl-tRNA differ markedly by several criteria: 1) capacity to form a ternary complex with GTP and the T enzymes from wheat and E. coli; 2) affinity for binding to wheat ribosomes in the presence of the triplet ApUpG and 3) capacity to be esterified by the E. coli methionyl-tRNA synthetase. The properties of met-$tRNA_1$ are thus compatible with an initiator function in protein synthesis. The failure to form a ternary complex with T_1 enzyme and GTP would prevent insertion of that particular methionyl residue in the internal positions of the peptide chain as in the case with E. coli met-$tRNA_F$. Also the high ribosomal binding efficiency of met-$tRNA_1$ especially at the lower Mg^{++} concentration would present an obvious advantage in an initiator aminoacyl-tRNA. In this respect, it is conceivable that an initiation factor similar to F_2 which binds

Fmet-tRNA to bacterial ribosomes may be still present in our washed wheat particles.

There are many obvious experiments that should answer the question of whether met-$tRNA_1$ or a modified form of this compound is the true initiator of protein synthesis in the cytoplasm of plant cells. We will attempt to perform such experiments in the near future.

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NOTE:- Richter and Lipmann [Nature 227, 1212 (1970)] have just published a note reporting different results with yeast, while Leis and Keller [BBRC 40, 416 (1970)] have independently reached our conclusion with respect to wheat embryos.

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