N-Formylmethionyl-tRNA$_f$ of Wheat Chloroplasts. Its Synthesis by a Wheat Transformylase*

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ABSTRACT: In a study of the role of methionine in protein chain initiation in wheat, a transformylase was isolated and partially purified from commercial wheat germ and from wheat leaves grown under sterile conditions. This enzyme catalyzed the formylation of a methionyl-tRNA$_f$ which was present in the wheat leaf chloroplasts. The enzyme used N^{10}-formyltetrahydrofolate as formyl donor and required 5 mM Mg^{2+} and 20 mM K^+ ions for maximum activity. The molecular weight of the transformylase was estimated at 45,000 by gel filtration on Sephadex G-150. The enzyme was present in isolated chloroplasts at a higher specific activity than in the remainder of the leaf extract.

This transformylase and the methionyl-tRNA$_f$ are components of a protein chain initiating system in wheat chloroplasts similar in many ways to the system in *Escherichia coli*.

Chloroplasts isolated from several different plants have been shown to contain an independent system for the synthesis of proteins (Spencer, 1965; Sissakian *et al.*, 1965). This system has some features in common with that in *Escherichia coli* including 70S ribosomes (Lyttleton, 1962; Boardman *et al.*, 1965), *N*-formylmethionyl-tRNA$_f$ (fMet-tRNA$_f$),[1] and a transformylase for its synthesis (Schwartz; *et al.*, 1967; Burkard *et al.*, 1969; Leis and Keller, 1970a–c). The latter two are components of a protein chain initiating system in chloroplasts (for a review, see Marcker and Smith, 1969).

This paper is part of a study of the role of methionine in protein chain initiation in wheat. In the previous report (Leis and Keller, 1970c), chloroplasts were isolated from wheat leaves, and the tRNAs were extracted and fractionated on benzoylated DEAE-cellulose (BD-cellulose). A species of methionine tRNA (tRNA$_f^{Met}$) was found which, when charged with methionine, could be formylated by a transformylase present in wheat extracts. Since this species was a dominant one in the chloroplasts and a minor one in bulk leaf tRNAs, it was concluded that most if not all of this tRNA$_f^{Met}$ was localized in the chloroplasts. The present paper further characterizes the chloroplast tRNA$_f^{Met}$ and describes the partial purification and the properties of the wheat transformylase.

Materials

[^{14}C]Methionine was from New England Nuclear, and BD-cellulose (Gillam *et al.*, 1967) was a gift of Dr. Bernard Dudock.

Bulk tRNA was prepared from raw wheat germ (Shiloh Farms, Sherman, N. Y.) by the method of Dudock *et al.* (1969) and was a gift from Dr. Doju Yoshikami. Leaf tRNA$_f^{Met}$ was obtained from sterilely grown wheat plants (Leis and Keller, 1970c). *E. coli* tRNA$_f^{Met}$ was prepared from bulk *E. coli* tRNA (General Biochemicals) by chromatography on BD-cellulose. The large-scale esterification of [^{12}C]methionine or [^{14}C]-methionine (122 mCi/mmole) to different species of tRNA^{Met} was as described by Leis and Keller (1970c).

N^5,N^{10}-[^{12}C]Methenyltetrahydrofolate was chemically prepared by the method of Huennekens *et al.* (1963). N^5,N^{10}-[^{14}C]-Methenyltetrahydrofolate was synthesized from [^{14}C]formate (23.6 mCi/mmole) (Amersham Searle Corp.) and tetrahydrofolate (Sigma Chemical Co.) enzymatically by the method of Rabinowitz and Pricer (1962). The formyltetrahydrofolate synthetase (*Clostridium acidi-urici*) was a gift of Dr. Jesse C. Rabinowitz. The products were purified by chromatography on a Sephadex G-10 column equilibrated with 0.010 N HCl and 0.010 M mercaptoethanol. Column fractions were stored at −20° under argon. Before use, the N^5,N^{10}-methenyltetrahydrofolate was held at pH 8 under argon for 90 sec to form N^{10}-formyltetrahydrofolate (f-H$_4$formate).

Methods

Analytical Procedures. RNA was determined in solution by the absorbance at 260 mμ. One A_{260} unit is the amount of RNA in 1.0 ml, of a solution having an absorbance of 1.0 at 260 mμ in a cell with a path length of 1.0 cm. Protein was determined by the method of Lowry *et al.* (1951) or by the absorbance at 280 and 260 mμ (Warburg and Christian, 1942). Chlorophyll was estimated by extraction into 80% acetone and determination of the absorbance at 645 and 663 mμ (Arnon, 1949).

Preparation of Soluble Fraction and Ribosomes from Wheat Germ. Commercially processed raw wheat germ (100 g, Shiloh Farms, Sherman, N. Y.) was washed by brief suspension in 200 ml of 2 mM dithiothreitol followed by centrifugation. This step was carried out at room temperature. All the following operations were done at 0–4°. The white, lipid-containing surface layer and the supernatant were discarded and the germ resuspended in 100 ml of cold homogenization

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[1] Abbreviations that are not listed in *Biochemistry 5*, 1445 (1966), are: fMet-tRNA$_f$, *N*-formylmethionyl-tRNA$_f$; Met-tRNA, methionyl-tRNA; tRNA^{Met}, methionine tRNA; Met-puromycin, methionylpuromycin; fMet-puromycin, formylmethionylpuromycin; BD-cellulose, benzoylated DEAE-cellulose; f-H$_4$folate, N^{10}-formyltetrahydrofolate; SD enzyme, extract of soluble cell fraction passed through Sephadex G-25 and DEAE-cellulose columns.

buffer (50 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM KCl, and 2 mM dithiothreitol). The germ was homogenized in a Waring Blendor for 3 min at low speed. The cell debris was removed from the homogenate by centrifugation at 17,000*g* for 20 min and the supernatant spun for 2 hr at 165,000*g*. The upper two-thirds of the high-speed supernatant was collected as the wheat germ soluble fraction and the remainder discarded. The ribosome pellets were rinsed once with the homogenization buffer and then stored at −20° after 1 ml of the same buffer had been layered over each pellet.

Soluble Enzyme Preparation from Wheat Leaves. Wheat plants were grown under sterile conditions as described by Leis and Keller (1970c). All the following operations were at 0–4°. Leaves (160 g) were cut into small pieces (1–2 cm) and homogenized in a Waring Blendor with 400 ml of homogenization buffer for 1 min at low speed. The homogenate was passed through several layers of gauze and the extract centrifuged at 17,000*g* for 20 min. The supernatant was spun at 78,500*g* for 195 min and the upper two-thirds of this supernatant collected. The protein of the soluble fraction was precipitated with ammonium sulfate, dissolved, and passed through Sephadex G-25 and DEAE-cellulose columns as described in the text for wheat germ. This enzyme preparation is referred to as the wheat leaf SD enzyme.

Soluble Enzyme Preparation from Chloroplasts. Chloroplasts and a supernatant fraction were isolated from wheat leaves using a modified Honda medium (Ranalletti *et al.*, 1969) and a very mild disruption procedure (Jensen and Bassham, 1966) as previously described (Leis and Keller, 1970c). All procedures were done at 0–4°. The chloroplasts were resuspended in homogenization medium (see above) and sonicated with a Bronson sonifier for 5 periods of 15 sec each in an ice bath. The sonicated extract was centrifuged at 17,000*g* for 20 min and the supernatant spun at 165,000*g* for 2 hr. The upper two-thirds of the chloroplast-soluble fraction was collected and a SD enzyme prepared from it as above. The supernatant, in modified Honda medium, from which intact chloroplasts had been removed was also used to prepare an SD enzyme. It contained 60% of the chlorophyll of the leaves due to disruption of the chloroplasts.

Soluble Enzyme and Ribosome Preparation from E. coli. *E. coli* B (40 g, grown to late log phase in an enriched medium, Grain Processing Inc.) was sonicated in 200 ml of cold 50 mM Tris·HCl (pH 7.5), 10 mM $Mg(OAc)_2$, 50 mM KCl, and 3 mM dithiothreitol with a Bronson sonifier for 5 periods of 15 sec each in an ice bath. A soluble fraction and ribosomes were prepared by centrifugation at 165,000*g* as above. An SD enzyme was prepared from the soluble fraction also as above.

Acceptor Assay for Methionine tRNAs. Column fractions containing tRNAs in 0.4–0.8 M NaCl with 10 mM $MgCl_2$ were assayed directly for methionine acceptor activity. The assay contained in a final volume of 1 ml the following: 0.1 ml of column fraction, 50 mM Tris·HCl (pH 7.5), 20 mM $MgCl_2$, 0.5 mM EDTA, 2 mM dithiothreitol, 2.5 mM ATP, 2.7 μM [^{14}C]-methionine (7.4 mCi/mmole by chromatographic analysis), and an amount of wheat germ SD enzyme (see text) to give complete charging. The reaction mixture was incubated for 10 min at 37°. The charged tRNA was precipitated with 2 ml of cold 8% trichloroacetic acid (pH 1.0). The precipitate was collected and rinsed on a glass fiber filter membrane (type E, Gelman Instrument Co.) and counted in a thin-window gas-flow counter (efficiency 31%). When the above procedure was used to assay tRNA that had been first precipitated from the column fractions with ethanol, 70 mM KCl was added to the incubation for good activity of the methionyl-tRNA synthetase (RajBhandary and Ghosh, 1969).

Transformylase Assay. The assay contained in a volume of 0.10 ml: 50 mM Tris·HCl (pH 7.5), 20 mM KCl, 6 mM dithiothreitol, 5 mM $MgCl_2$, [^{12}C]Met-$tRNA_f$ as indicated, 11 μM [^{14}C]f-H_4folate (23.6 mCi/mmole), and varying amounts of enzyme. Incubation was for 5 min at 37°. The [^{14}C]fMet-$tRNA_f$ was precipitated and counted as above. The incorporation was corrected for the values obtained in nonincubated controls.

Charging and Transformylation Assay for $tRNA_f^{Met}$. The two steps were carried out in a single incubation. The assay contained in a volume of 0.10 ml: 50 mM Tris·HCl (pH 7.5), 40 mM KCl, 16 mM $MgCl_2$, 2.5 mM APT, 0.5 mM EDTA, 6.8 mM ditoiothreitol, 2 μM [^{12}C]methionine, 11 μM [^{14}C]f-H_4folate (23.6 mCi/mmole), $tRNA^{Met}$ as indicated, and 0.9 mg/ml of wheat germ SD enzyme protein to give maximal charging and transformylation. After incubating for 20 min at 37°, the product was collected and counted as above.

Preparation and Analysis of f[^{14}C]Met-$tRNA_f$ and [^{14}C]Met-$tRNA_f$. Wheat germ $tRNA_f^{Met}$ (20 pmoles) was charged with [^{14}C]methionine (122 mCi/mmole) and formylated in a volume of 1.0 ml using a wheat germ SD enzyme and the components described above for the methionine acceptor assay with the addition of 55 μM [^{12}C]f-H_4folate. In a second incubation to prepare [^{14}C]Met-$tRNA_f$, the f-H_4folate was omitted. Incubation was for 40 min at 37°, and 0.1 ml of 20% KOAc (pH 5.0) was added to stop the reaction. Each tRNA was isolated by phenol treatment, Sephadex G-25 chromatography in 0.1 M KOAc (pH 5.0), and ethanol precipitation. Each was then digested with 2 μg of pancreatic ribonuclease (Worthington Biochemical Corp.) in 0.05 ml of 4 mM NH_4OAc (pH 7.0) for 5 min at room temperature (Marcker and Sanger, 1964). The digestion products were spotted on filter paper (S & S 589 Orange) saturated with pyridine–acetic acid–water (3:30:967) buffer (pH 3.5) and subjected to electrophoresis for 80 min at 54 V/cm. The electrophoretogram was dried and cut into 1-cm strips which were counted in 10 ml of Bray's scintillation fluid (Bray, 1960) in a Packard scintillation counter (efficiency 75%).

Reaction of Wheat fMet-$tRNA_f$ with Puromycin on E. coli Ribosomes. The charging, formylation, ribosome binding, and reaction with puromycin were carried out in a single incubation. The incubation mixture contained the following in a volume of 0.1 ml: 50 mM Tris·HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 2 mM dithiothreitol, 2.5 mM ATP, 0.25 mM GTP, 5 mM PEP, 4.3 μM [^{14}C]methionine (230 mCi/mmole), 0.2 mM each of 7 [^{12}C]amino acids (Glu, Gln, Asp, Asn, Ser, Gly, and Ala), 55 μM [^{12}C]f-H_4folate, 0.15 A_{260} unit of AUG (Miles Laboratories), 1 mM puromycin (Nutritional Biochemicals Corp.), 47 pmoles of wheat germ $tRNA_f^{Met}$, 0.6 mg of *E. coli* SD enzyme protein, and 3 A_{260} units of *E. coli* ribosomes. Incubation was for 30 min at 37°. The reaction was stopped with the addition of 1 ml of 0.1 M Na_2HPO_4 (pH 8.1) and 1.5 ml of ethyl acetate (saturated with Na_2HPO_4 buffer). The phases were mixed on a Vortex mixer for 10 periods of 5 sec each, then separated by centrifugation. A 1-ml aliquot of the ethyl acetate was removed for counting of ^{14}C with 10 ml of Bray's scintillation fluid. This procedure, with an extraction at pH 8.1, detects both Met-puromycin and fMet-puromycin (Leder and Bursztyn, 1966).

Results

Isolation of $tRNA_f^{Met}$ from Wheat Germ. Bulk tRNA isolated

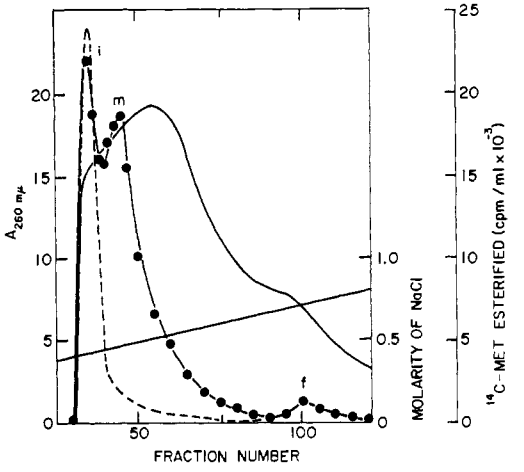

FIGURE 1: Fractionation of wheat germ tRNAs on BD-cellulose in the presence of $MgCl_2$. Bulk tRNA (420 mg) isolated from commercially processed wheat germ was adsorbed to BD-cellulose in a column (1.3 × 90 cm) equilibrated with 0.3 M NaCl and 10 mM $MgCl_2$. The column was developed with a 1400-ml linear gradient of NaCl from 0.3 to 1.2 M in 10 mM $MgCl_2$. Fractions of 6.5 ml were collected at a flow rate of 0.5 ml/min. Aliquots (0.1 ml) of column fractions were assayed directly for methionine acceptor activity using wheat germ (—●—) or *E. coli* (-----) SD enzyme; (—) A_{260}.

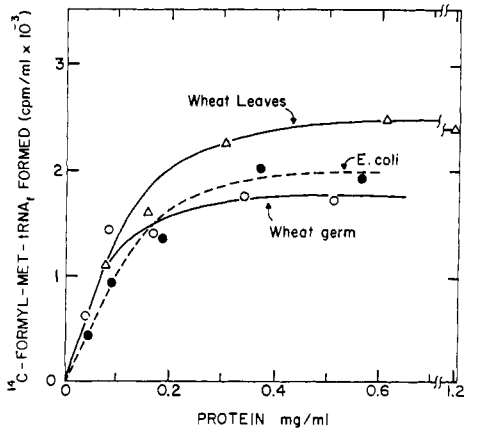

FIGURE 2: Comparison of transformylase activity of wheat germ, wheat leaf, and *E. coli* extracts. Wheat germ [^{12}C]Met-tRNA$_f$ (11–15 pmoles) was incubated under the conditions for the transformylase assay with varying concentrations of wheat germ SD enzyme (—○—), wheat leaf SD enzyme (—△—), or *E. coli* SD enzyme (—●—).

from commercially processed raw wheat germ was chromatographed on BD-cellulose in the presence of 10 mM $MgCl_2$ with a NaCl gradient (Gillam *et al.*, 1967). Three peaks of tRNA$_f^{Met}$s, two major and one minor species, were detected (Figure 1). The two major species eluted together in the early part of the NaCl gradient. They correspond to the two cytoplasmic species, tRNA$_i^{Met}$ and tRNA$_m^{Met}$, previously described (Leis and Keller, 1970a–c). The minor speces eluted at a higher NaCl concentration (0.72 M) and was completely resolved from tRNA$_i^{Met}$ and tRNA$_m^{Met}$ on this column. When this species was first detected it was analyzed by gel filtration on Sephadex G-100 and was found to be a monomeric tRNA. It represented 4% of the total tRNA^{Met} present in wheat germ.

This minor species could be formylated after being charged with methionine by a wheat germ enzyme extract. A formylation assay was set up using [^{14}C]f-H$_4$folate. This assay established that the minor species was a tRNA$_f^{Met}$ similar to *E. coli* tRNA$_f^{Met}$ and that wheat germ extract contained an active transformylase like that in *E. coli*.

This same species of tRNA$_f^{Met}$ was found as a major species of tRNA^{Met} in chloroplasts isolated from wheat leaves grown under sterile conditions (Leis and Keller, 1970c). This proved that tRNA$_f^{Met}$ is a chloroplast tRNA and suggested that the tRNA$_f^{Met}$ found in wheat germ was derived from plastids. It also eliminated the possibility that the tRNA$_f^{Met}$ was derived from contaminating bacteria or molds.

Partial Purification of Wheat Germ Transformylase. The protein in the wheat germ soluble fraction (see Methods) was precipitated with ammonium sulfate (47 g/100 ml) to concentrate the enzymes. The precipitate was dissolved in a minimum volume of 20 mM Tris·HCl (pH 7.5) and 2 mM dithiothreitol, and low molecular weight material was removed by passage through a Sephadex G-25 column equilibrated with the same buffer. Endogenous RNA was removed by adsorption of the Sephadex-treated enzyme to DEAE-cellulose in a small column and elution of the protein with 0.3 M KCl, 20 mM Tris·HCl (pH 7.5), and 2 mM dithiothreitol. The enzyme preparation which had been passed through the Sephadex and DEAE-cellulose columns will be referred to as the wheat germ SD enzyme. The SD enzyme was stored frozen in small portions at −20° under argon. It could be kept for longer than 4 months without appreciable loss of activity. The transformylase activity of the SD enzyme was tested (see Methods) by incubating it with wheat germ [^{12}C]Met-tRNA$_f$ and [^{14}C]-f-H$_4$folate and measuring the [^{14}C]fMet-tRNA$_f$ formed (Figure 2).

A threefold purification of the transformylase was obtained by passing the SD enzyme through an ascending-flow Sephadex G-150 column equilibrated with 0.1 M KCl, 20 mM Tris·HCl (pH 7.5), and 2 mM dithiothreitol (Figure 3). The transformylase eluted after most of the high molecular weight protein had emerged from the column. The molecular weight of the transformylase appeared to be about 45,000 since ovalbumin (molecular weight 45,000) gave the same elution volume when tested on the same column (Figure 3). The enzyme which had been passed through the Sephadex G-150 column had a specific activity of 132 pmoles of fMet-tRNA$_f$ formed/mg of protein under the conditions of the transformylase assay (see Methods).

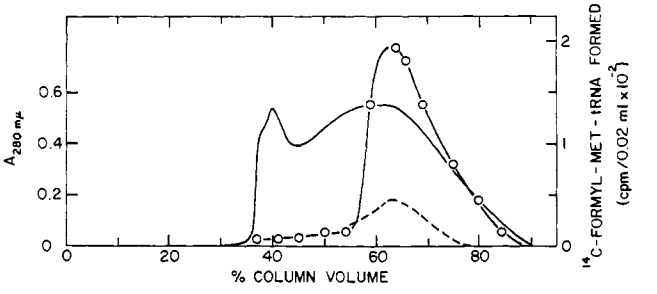

FIGURE 3: Gel filtration of the wheat transformylase on Sephadex G-150. A wheat germ SD enzyme 18 mg (1.5 ml, of protein/ml) was applied to a Sephadex G-150 column (1 × 122 cm) equilibrated with 0.1 M KCl, 20 mM Tris·HCl (pH 7.5), and 2 mM dithiothreitol. The column was run at 4° with a flow rate of 0.4 ml/min by ascending flow. Fractions (1.0 ml) were collected and 0.02-ml aliquots were assayed for transformylase activity (see Methods): (—) A_{280}; (—○—) transformylase activity; (-----) A_{280} pattern for ovalbumin (molecular weight 45,000) chromatographed on the same column.

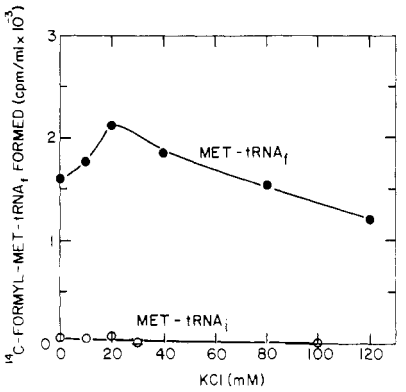

FIGURE 4: The effect of KCl concentration on wheat germ transformylase activity. Transformylase activity of 0.2 mg/ml of wheat germ SD enzyme was assayed as described in Methods except that the KCl concentration was varied: (—●—) [^{12}C]Met-tRNA$_f$; (—○—) [^{12}C]Met-tRNA$_i$.

Isolation of Transformylase from Sterilely Grown Wheat Leaves. To establish that this transformylase was really a wheat enzyme and not due to bacterial or fungal contamination, wheat plants were grown under sterile conditions (Leis and Keller, 1970c) and extracts of these wheat leaves were tested for transformylase activity. The bacterial contamination of leaves grown under these conditions was found to be extremely low, 2×10^{-7} g of bacteria/g of leaf assuming 10^{-12} g/bacterium (Leis and Keller, 1970c). Only 1–2 mold colonies were seen in each crop of wheat seedlings germinated (20 ft^2) and these contaminated plants were not harvested. The transformylase activity of the SD enzyme prepared from these sterilely grown wheat leaves was equal to that from wheat germ (Figure 2). It is also shown in Figure 2 that the transformylase in an *E. coli* extract was active with the wheat germ Met-tRNA$_f$.

Properties of the Wheat Germ Transformylase. The requirements for the transformylase reaction are shown in Table I. The enzyme required Mg^{2+} for activity; the optimum Mg^{2+} concentration was previously determined to be 5 mM (Leis and Keller, 1970b). Transformylation was completely dependent upon the addition of both wheat germ SD enzyme and Met-tRNA$_f$. The presence of K^+ stimulated the transformylation reaction; 20 mM KCl gave optimum activity (Figure 4). Na^+ or NH_4^+ could replace K^+ without loss in enzyme activity. In a separate experiment, the pH optimum was determined to be about 7.5, with good activity between 6.5 and 8.0.

TABLE I: Requirements for Transformylation of Wheat Germ Methionyl-tRNA$_f$ with a Wheat Germ SD Enzyme Preparation.[a]

Incubation	[^{14}C]fMet-tRNA$_f$ Formed (pmoles)
Complete system	14.0
− KCl	10.9
− $MgCl_2$	2.3
− Met-tRNA$_f$	0
− Wheat germ SD enzyme	0.8

[a] The conditions of the transformylase assay were as described in Methods with 0.2 mg/ml of wheat germ SD enzyme. The complete system contained 14.2 pmoles of wheat germ [^{12}C]Met-tRNA$_f$.

TABLE II: Charging and Transformylation Assays of tRNA^{Met}s with a Wheat Germ SD Enzyme Preparation.[a]

tRNA	[^{14}C]Met- or f[^{14}C]Met-tRNA Formed[b] (pmoles)	[^{14}C]fMet-tRNA Formed[c] (pmoles)
tRNA$_i^{Met}$ (wheat germ)	66.3	0
tRNA$_m^{Met}$ (wheat germ)	58.6	0
tRNA$_f^{Met}$ (wheat germ)	23.4	22.6
tRNA$_f^{Met}$ (wheat leaf)	19.3	19.7
tRNA$_f^{Met}$ (*E. coli*)	112.0	101.0

[a] The two assays of a particular tRNA^{Met} were done at the same time under identical conditions. In the first assay ^{14}C was in methionine, and in the second it was in f-H$_4$folate. Since the amount of tRNA in the two assays was the same, the data from the pair of assays can be compared to see what fraction of the tRNA^{Met} present was formylated after it was charged with methionine. [b] The assay was the charging and transformylation assay as described in Methods except that [^{14}C]methionine (7.4 mCi/mmole) and [^{12}C]f-H$_4$folate were used. [c] The assay was the charging and transformylation assay as described in Methods with [^{12}C]methionine and [^{14}C]f-H$_4$folate.

No other wheat Met-tRNA was formylated by the transformylase under these conditions (Table II). The most extensive tests were done with the cytoplasmic initiating tRNA$_i^{Met}$ to see if it could be formylated under any conditions. There was no formylation of Met-tRNA$_i$ even when the Mg^{2+} concentration was varied from 1 to 50 mM (Leis and Keller, 1970b) or when the K^+ concentration was varied from 0 to 100 mM (Figure 4). To see if this lack of formylation was due to the presence in the preparations of tRNA$_i^{Met}$ or tRNA$_m^{Met}$ of a substance which could inhibit transformylation, these preparations were added to tRNA$_f^{Met}$ which was then assayed for formylation. There was no inihibition of formylation of Met-tRNA$_f$ by the presence of either of these cytoplasmic tRNA^{Met}s. We conclude that the wheat germ transformylase will recognize, of the tRNA^{Met}s so far isolated from wheat, only Met-tRNA$_f$. The wheat transformylase will, however, formylate, *E. coli* Met-tRNA$_f$ (Table II). Also shown in Table II is the fact that formylation of the Met-tRNA$_f$ preparation from the column (Figure 1) was quantitative; all of the tRNA which could be charged with methionine could be formylated.

Identification of fMet-Adenosine from fMet-tRNA$_f$. In order to prove that transformylation had occurred, tRNA$_f^{Met}$ was charged with [^{14}C]methionine in the presence of [^{12}C]f-H$_4$folate using the wheat germ SD enzyme. The RNA was isolated and digested with pancreatic ribonuclease. The digestion products were separated by paper electrophoresis at pH 3.5 (Figure 5). The major radioactive product was identified as f[^{14}C]Met-adenosine. When the formyl donor was omitted from the charging incubation, the only radioactive digestion

TABLE III: Formation of fMet-Puromycin from Wheat fMet-tRNA$_f$ in the Presence of *E. coli* Ribosomes.[a]

Incubation	f[^{14}C]Met-Puromycin[b] Formed (pmoles)
Complete system	55.7
− tRNA$_f^{Met}$	5.4
− f-H$_4$folate	4.6

[a] The puromycin reaction was carried out at 5.5 mM $MgCl_2$ in a single incubation as described in Methods with the label in [^{14}C]methionine. The system included wheat germ tRNA$_f^{Met}$, *E. coli* ribosomes and SD enzyme, ATP, GTP, AUG, f-H$_4$-folate, and puromycin. The figures in the table have been corrected for a control without puromycin. In a separate experiment the reaction was shown to be completely dependent on the addition of ribosomes and soluble fraction. [b] Some [^{14}C]Met-puromycin could also be present since the ethyl acetate extraction was done at pH 8.1.

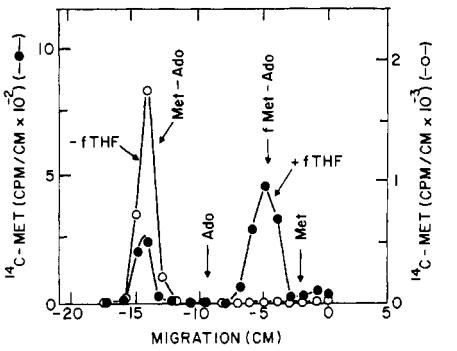

FIGURE 5: Electrophoresis of products from pancreatic ribonuclease digestion of fMet- and Met-tRNA$_f$. The preparation of the tRNAs, digestion, and electrophoresis of the products was as described in Methods. When *E. coli* fMet-tRNA$_f$ was treated under identical conditions, the radioactive product had the same mobility as the fMet-adenosine peak which is labeled fMet-Ado. Methionine and adenosine (Ado), added as internal markers, were detected by ninhydrin spray and ultraviolet absorbance: (—●—) the product from charging in the presence of f-H$_4$folate; (—○—) the product from charging in the absence of f-H$_4$folate.

product was [^{14}C]Met-adenosine (Figure 5). In another experiment (not shown) the radioactive label was present in the formyl group of f-H$_4$folate. The only labeled digestion product found had the mobility of fMet-adenosine. These experiments show convincingly that Met-tRNA$_f$ was formylated by a transformylase in wheat germ.

Localization of the Transformylase in the Wheat Leaf. Since wheat leaf tRNA$_f^{Met}$ has been shown to be a chloroplast tRNA, we were interested to see if the transformylase was a chloroplast enzyme. Chloroplasts were therefore isolated from wheat leaves by a modification of the method of Jensen and Bassham (1966) as previously described (Leis and Keller, 1970c). The mild isolation procedure used yielded some intact chloroplasts, but 60% of the chloroplasts were disrupted releasing their contents into the soluble fraction. Assay of the pellet of intact chloroplasts showed that it did contain transformylase; the specific activity was 48 pmoles of fMet-tRNA$_f$ formed/mg of protein in the standard assay. The activity in the chloroplast pellet could not be due to contamination of supernatant fraction, since the specific activity of the latter was lower, 30 pmoles of fMet-tRNA$_f$ formed/mg of protein. Some, but not all, of the transformylase activity of the supernatant fraction can be accounted for by enzyme released from broken chloroplasts. A further analysis of the cellular localization of the transformylase would require the isolation of intact chloroplasts by the nonaqueous procedure of Hall and Whatley (1967) and the isolation and analysis of leaf mitochondria.

Reaction of fMet-tRNA$_f$ with Puromycin on E. coli Ribosomes. A Met-tRNA which is formylated in the cell cannot function in chain elongation and therefore is presumed to be a chain-initiating tRNA. Since in the wheat cell, tRNA$_f^{Met}$ is localized in chloroplasts or plastids where it functions on 70S ribosomes (Lyttleton, 1962; Boardman *et al.*, 1965), it was decided to test for reaction with puromycin using 70S ribosomes. *E. coli* ribosomes were used because of difficulty in isolating sufficient ribosomes from wheat chloroplasts. The puromycin reaction was carried out in a single incubation with [^{14}C]methionine, ATP, wheat germ tRNA$_f^{Met}$, *E. coli* ribosomes, and SD enzyme preparation, and puromycin, with and without f-H$_4$folate (Table III). The reaction was stopped with the addition of pH 8.1 buffer, and the solution was extracted with ethyl acetate as previously described (Leis and Keller, 1970b). At this pH both fMet- and Met-puromycin were extracted into the ethyl acetate (Leder and Bursztyn, 1966). The wheat fMet-tRNA$_f$ exhibited a strong reaction with puromycin on the *E. coli* 70S ribosomes at 5 mM Mg^{2+}. The reaction was weak in the absence of formylation, as in the case of *E. coli* Met-tRNA$_f$ (Bretscher and Marcker, 1966).

Discussion

In the chloroplasts of wheat, a transformylase utilizes f-H$_4$-folate to formylate the α-amino group of methionine esterified to a tRNA$_f^{Met}$. The resultant fMet-tRNA$_f$ can function in protein chain initiation on the 70S ribosomes of chloroplasts. In contrast, in the cytoplasm of the wheat cell, unblocked Met-tRNA$_i$ can function in protein chain initiation on 80S ribosomes (Leis and Keller, 1970b,c; Marcus *et al.*, 1970). This appears to be a characteristic initiating system in the cytoplasm of many eukaryotes. The chloroplast system resembles that in *E. coli*; both use an fMet-tRNA$_f$ on 70S ribosomes. It was therefore interesting to see if there were any differences between these two systems. The wheat transformylase was partially purified, and its molecular weight was estimated by gel filtration on Sephadex G-150 as 45,000. The value is approximately twice the molecular weight reported for the purified *E. coli* transformylase described by Dickerman *et al.* (1967). In addition, the wheat transformylase required 20 mM K^+ for maximal activity, whereas the *E. coli* enzyme did not. Also, the wheat germ enzyme required only 5 mM Mg^{2+}, whereas the optimum for the *E. coli* enzyme was 30 mM. Though the two enzymes show differences, they both recognize the same tRNA substrates; they both formylate wheat and *E. coli* Met-tRNA$_f$s and they are both inactive with wheat Met-tRNA$_i$. The chloroplast Met-tRNA$_f$ could bind to the initiating site on *E. coli* 70S ribosomes and react with puromycin. This reaction showed a strong dependence on formylation as did the reaction of *E. coli* Met-tRNA$_f$ (Bretscher and Marcker, 1966). It will be of interest to see if initiation on chloroplast 70S ribosomes is also dependent on formylation of Met-tRNA$_f$.

One would predict a similar initiation system in the other

organelle of plants, the mitochondrion. A $tRNA_f^{Met}$ and a transformylase have been identified in the mitochondria of yeast and rat liver (Smith and Marcker, 1968) and HeLa cells (Galper and Darnell, 1969). We are presently attempting to isolate wheat mitochondria to see if they do contain a $tRNA_f^{Met}$ and transformylase.

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