Isolation and Characterization of m^3U-Methyltransferase from Escherichia coli*

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The tRNA-modifying enzyme, S-adenosylmethionine: tRNA (uridine-5)-methyltransferase, has been purified essentially to homogeneity from an Escherichia coli strain containing an elevated level of this enzyme. A rapid, efficient method has been developed for the purification, consisting of polyethyleneimine precipitation to remove nucleic acids, followed by phosphocellulose and Blue Sepharose affinity chromatography. The enzyme is a single polypeptide chain of molecular weight 42,000. It has a pH optimum of 8.4, a Km of 12.5 \( \mu M \) for S-adenosyl-L-methionine, and a Km of 1.1 \( \mu M \) for wheat germ tRNA. The ability of the enzyme to methylate a variety of tRNA substrates including prokaryotic, eukaryotic, mitochondrial, and chloroplastic tRNAs has been characterized.

The extensive degree of nucleotide modification found n
tRNA, which varies in complexity from single methylations of the bases or sugar moieties to the formation of extremely complex "hyper-modified" nucleotides, is one of the unique features of this group of molecules (1–5). Certain "universal" modified nucleotides are found in all or almost all tRNA molecules, while other modified residues are found only in one or a few specific tRNA species (4). Ribothymine, the most common methylated nucleotide in tRNA, is found in the great majority of tRNAs, but not in all of them. It is not found in a few higher plant tRNAs (4, 6), some mammalian tRNAs (4, 7, 8), some prokaryotic tRNAs (4), and some mitochondrial tRNAs (4).

A number of tRNA methyltransferases have been highly purified from both prokaryotic and eukaryotic sources (9–13). However, significant problems often occur during the purification of these enzymes. In general, tRNA methyltransferases are quite labile, and the fractionation of individual methyltransferases from one another has often been difficult, especially in eukaryotes (14).

This paper reports the isolation and characterization of the m^3U-methyltransferase from Escherichia coli and its ability to methylate a variety of tRNAs from prokaryotes, eukaryotes, mitochondria, and chloroplasts. A rapid, efficient method has been developed for the purification of this enzyme which involves the use of polyethyleneimine to remove nucleic acid, followed by phosphocellulose chromatography and affinity elution-Blue Sepharose chromatography. This simple procedure yields essentially homogeneous enzyme. In addition, preliminary results suggest that the method may also be useful for the purification of mcm^3U-methyltransferase from E. coli and may be useful as a general method for the purification of tRNA methyltransferases.

**EXPERIMENTAL PROCEDURES**

Materials
S-adenosyl-\([\text{CH}_3]\)L-methionine (specific activity 5–15 pmol) was purchased from New England Nuclear (Boston, MA) and prepared for use as described below. Phosphocellulose was purchased from Whatman and treated prior to use as previously described (15). SE cellulose (No. 79, standard) was purchased from Millipore Bio-Spin (Bedford, MA) and prepared as previously described (16). Thioglycolate (\( \text{Na}_2\text{SO}_3 \)) was purchased from Seropharma and dimethyl sulfoxide (DMSO) was purchased from Baker Chemical Co. (Phillipsburg, NJ). A pool of E. coli containing an elevated level of this enzyme was a gift from Dr. R. Cedergren. Crude spinach chloroplast tRNA was a gift of Dr. R. Cedergren. Nucleoside diphosphates were purchased from Boehringer Mannheim GmbH. All other reagents were of the highest purity available.

Preparation of tRNA
Crude RNA from E. coli (17), bovine liver (18) and rabbit liver (18), and pure RNA (19) from wheat germ (19,20) were isolated as previously published. Mitochondrial tRNA from bovine liver was isolated as described (11). The mitochondrial tRNA was purified by TCA extraction chromatography as described by Palatini et al. (21) and then further purified by passage through an ion exchange resin (Carboxymethyl cellulose, type 100). The RNA was then digested with RNase A and RNase T1 (22).

**Assay of Methyltransferase**
M^3U-methyltransferase was assayed in a total volume of 20 \( \mu l \) containing 0.5 M Tris-\( \text{HCl} \), pH 7.7, 0.1 M NaCl, 100 \( \mu \)M adenosine-\( \text{[14C]} \)methionine, 10 \( \mu \)M spermidine, 10 mM magnesium acetate, 10 \( \mu \)M spermidine, and 1 to 2 \( \mu l \) of enzyme extract. After incubation at 37° for the desired time, usually 20 minutes during purification procedures, the reaction was stopped by the addition of 0.5 to 1 ml of ice cold 10% trichloroacetic acid. The precipitated reaction mixture was kept at 0–4° for at least 30 minutes to ensure complete precipitation of the RNA. Precipitated samples were filtered through glass fiber filters (Advantec, Japan) which were then washed twice with two ml of cold 10% trichloroacetic acid. As previously noted (11), the filters must be carefully washed with 20% trichloroacetic acid to ensure reproducibility. With the reaction on, the top of the filtration apparatus was removed and each filter was given a final wash with 20% trichloroacetic acid. The bands removed for these assays were obtained by using a non-methylated RNA substrate, i.e., crude, with wild-type E. coli strain, in one

unit, the quantity of material contained in 1 ml of solution which has an absorbance of 1 at 260 nm when measured in a 1-cm lightpath cell; PEl, polyethyleneimine; PC, phosphocellulose.

Details of "Experimental Procedures" are presented in miniprint form. Miniprint is easily read with the aid of a standard magnifying glass.
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The nucleic acid (usually 0.3 to 0.45%) can readily be determined. For example, the extract is brought to 0.2% PEI as described. After the PEI-treated extract is stirred for 15 min a small aliquot is removed and centrifuged in a Sorvall SS-34 rotor at 10,000 rpm (12,000 x g) for 15 min. A clear, golden supernatant indicates that the nucleic acid precipitation is complete. A cloudy supernatant indicates that more Polymin P is required, and should be added as described above. A260/A280 ratios at this point in the purification are not useful, since the Polymin P interferes strongly with these readings. Following nucleic acid removal by Polymin P precipitation and centrifugation, solid (NH4)2SO4 was added to the clear golden supernatant to bring it to 85% of saturation. Following nucleic acid removal by Polymin P precipitation and centrifugation, solid (NH4)2SO4 was added to the clear golden supernatant to bring it to 85% of saturation. After the (NH4)2SO4 dissolved, the precipitated extract was stored at -20°C for at least 2 h and then centrifuged at 12,000 rpm for 30 min in a Sorvall GSA rotor. The resulting pellets were stored at -20°C until further use.

Phosphocellulose Chromatography—The (NH4)2SO4 pellets of the extract following PEI precipitation were each dissolved in 20 to 40 ml of dialysis buffer containing 10 mM Tris-HCl, pH 7.6, 0.1 mM Na2EDTA, 1.0% glycerol, and 0.5 mM dithiothreitol. The sample was dialyzed, usually for a total of about 24 h, against several changes of this buffer in a ratio of 10 volumes of dialysis buffer to 1 volume of sample. The dialysis was continued until the addition of a few drops of 1 M BaCl2 to a 1-ml aliquot of the buffer failed to produce a white precipitate of BaSO4.

As the concentration of (NH4)2SO4 in the extract decreases during dialysis, a white precipitate usually forms in the sample. This is readily removed, with little or no loss of enzymic activity, by centrifugation at 12,000 rpm for 15 min in a Sorvall GSA rotor.

The dialysate, having an A260 of 20, an A280 of 13, and a volume of 443 ml, was applied to a 450-ml phosphocellulose column (2.6 X 85 cm) previously equilibrated with 0.1 M potassium phosphate, pH 6.5, in PC buffer, containing 10% glycerol, 0.5 mM Na2EDTA, and 0.5 mM dithiothreitol. Following sample application, the column was washed with 50 ml of 0.15 M potassium phosphate, pH 6.5, in PC buffer, and then with 400 ml of 0.35 M potassium phosphate, pH 7.2, in PC buffer. Fractions (14 ml) were collected at a flow rate of 120 ml/h. 

**Fig. 1. Phosphocellulose chromatography.** The 0 to 85% (NH4)2SO4 fraction of the PEI supernatant was applied to a 450-ml phosphocellulose column as described in the text. Arrows indicate the start of each buffer as follows: (a) 0.1 M potassium phosphate, pH 6.5, in PC buffer; (b) 0.15 M potassium phosphate, pH 6.5, in PC buffer, (c) linear gradient of 0.15 M potassium phosphate, pH 6.5, to 0.35 M potassium phosphate, pH 7.2, in PC buffer. Fractions (14 ml) were collected at a flow rate of 120 ml/h. ---, A260; ----, m5U-methyltransferase activity. The fractions containing enzyme activity were pooled as indicated.
buffer. The enzyme was eluted from the column with a 3.2-liter linear gradient of 0.15 M potassium phosphate, pH 6.5, to 0.35 M potassium phosphate, pH 7.2, both in PC buffer. The elution profile of this column is shown in Fig. 1. Most of the protein was not retained on the phosphocellulose column. The enzyme, however, was retained on the column, and was eluted subsequently with approximately 0.2 M potassium phosphate. Fractions containing the m’U-methyltransferase activity were pooled, and the sample brought to 85% of (NH₄)₂SO₄ saturation and centrifuged as described above. This purification step was very effective, achieving a more than 40-fold purification.

It should be noted that the potassium phosphate concentration required to elute the enzyme may vary with different lots of phosphocellulose. New lots should therefore be tested prior to use on a large scale. Furthermore, the enzyme activity has been observed occasionally to split into two peaks upon phosphocellulose chromatography. The reason for this is not known. Splitting was found to vary with different lots of phosphocellulose and the occurrence of splitting did not appear to affect the properties of the m’U-methyltransferase. Splitting is believed to be a column artifact and not to represent two forms of the m’U-methyltransferase enzyme. Since, in most cases when splitting was observed, the enzyme mcmo’U-methyltransferase was also found to split. Moreover, both enzymes split in the same proportions. In addition, there is no evidence for two forms of m’U-methyltransferase as judged by a single sharp peak observed upon gel filtration chromatography on Ultrogel AcA 54, and one major protein band on SDS-polyacrylamide gels.

**Blue Sepharose Affinity Chromatography**—One of three (NH₄)₂SO₄ pellets from the m’U-methyltransferase activity peak of the phosphocellulose column was dialyzed into dialysis buffer as described above for phosphocellulose chromatography. When all of the (NH₄)₂SO₄ was removed, the sample was then dialyzed into 10 mM potassium phosphate, pH 7.0, 2 mM Na₂EDTA, 20% glycerol, and 0.5 mM dithiothreitol, resulting in 4.5 ml of sample having an A₅₅₀ of 5.6 and an A₂₆₀ of 3.6. An aliquot of this sample (PC enzyme) was removed for further purification and the remainder was frozen at -70°C in aliquots for future use. PC enzyme (1.5 ml) was applied to a 3-ml Blue Sepharose column (0.6 x 10.5 cm), previously equilibrated with 2 mM Na₂EDTA, 20% glycerol, and 0.5 mM dithiothreitol (BSC buffer) containing 10 mM potassium phosphate, pH 7.0. The column was washed successively with 3 ml of 10 mM potassium phosphate, pH 7.0, in BSC buffer, 9 ml of 40 mM potassium phosphate, pH 7.0, in BSC buffer, and 12 ml of 10 mM potassium phosphate, pH 7.0, in BSC buffer. The enzyme was then affinity eluted with 12 ml of BSC buffer containing 10 mM potassium phosphate and wheat germ tRNA at a concentration of 1 A₅₅₀ unit/ml. The column was then washed with 6 ml of 10 mM potassium phosphate, pH 7.0, in BSC buffer. The affinity elution step does not remove all of the enzyme from the column. The remaining m’U-methyltransferase (approximately 40% of the total) was eluted from the column with a 12-ml wash of 200 mM potassium phosphate, pH 7.0, in BSC buffer. This enzyme is considerably less pure than the affinity-eluted enzyme. One-milliliter fractions were collected and assayed as described above. The affinity-eluted m’U-methyltransferase activity pool was pooled as shown in Fig. 2a. The enzyme was then freed of tRNA and concentrated by the use of a small (1.5 ml) DEAE-cellulose column built in a 5-ml pipette. The affinity-eluted enzyme (16 ml) was applied to the small DEAE-cellulose column which was previously equilibrated with 10 mM Tris-HCl, pH 7.6, 1 mM Na₂EDTA, 10% glycerol, and 0.5 mM dithiothreitol (DEAE-buffer) conditions.

**Table I**

| Enzyme fraction | Total protein* | Enzyme units† | Specific activity |
|-----------------|---------------|--------------|-----------------|
| S-20            | 63,228        | 1.45 x 10⁴   | 22              |
| PEI (NH₄)₂SO₄   | 12,523        | 1.12 x 10⁴   | 99              |
| PC              | 95            | 3.64 x 10⁴   | 3,852           |
| Blue Sepharose DEAE | For 1.5 ml of 12.5 ml | 0.54 | 5.6 x 10⁴ | 10,370 |
|arteological      | Theoretical for 13.5 ml | 4.86 | 5.0 x 10⁴ |

*The protein concentration of all enzyme fractions was determined by the method of Bradford (31). The protein concentration of the highly purified Blue Sepharose enzyme was also determined by the method of Warburg and Christian (32) with the table by Layne (33) and the results were essentially identical (±0.02 mg/ml).

†An enzyme unit is the amount of enzyme that catalyzes 1 pmol of methylation/min at 30°C.

This was the yield when 1.5 ml of 13.5 ml of PC enzyme were applied to the Blue Sepharose column.
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The m^5U-methyltransferase was also chromatographed together with molecular weight standards on Ultrogel AcA 54 in the absence of SDS. Under these conditions, the enzyme was found to have a $M_r$ of 39,000 (Fig. 3b).

**Fig. 3. Molecular weight determination.** The following abbreviations were used for the molecular weight standards: BSA, bovine serum albumin (68,000); Oval, ovalbumin (45,000); CA, carbonic anhydrase (32,000); Lac, lactoglobulin (18,000); Lys, Lysozyme (14,500).

**a,** estimation of molecular weight (denaturing conditions). The Blue Sepharose affinity eluted m^5U-methyltransferase was subjected to SDS-polyacrylamide gel electrophoresis alongside molecular weight standards. The arrow indicates the location of the single prominent band in the lane in which the enzyme was applied.

**b,** estimation of molecular weight (nondenaturing conditions). One hundred microliters of PC enzyme were applied to an Ultrogel AcA 54 column (0.65 x 140 cm) along with molecular weight standards. One-milliliter fractions were collected at a flow rate of 8 ml/h and assayed for m^5U-methyltransferase activity as described above. The arrow indicates the fraction of peak enzyme activity.

The enzyme was then eluted with 0.22 M KCl in DEAE-buffer. The fractions were assayed, and the activity peak pooled (Fig. 2b). The enzyme was pipetted into prefrozen Eppendorf tubes and stored at -70°C until needed. Since the enzyme at this stage is quite sensitive to freeze-thawing, it is usually stored in 50-μl aliquots which are thawed once for use. The enzyme is stable at -70°C for at least 6 months. To recover the tRNA for reuse, the small DEAE-cellulose column was washed with 1 M KCl in DEAE-buffer, and the tRNA precipitated with 2 volumes of ethanol.

The complete purification procedure is summarized in Table 1.

**Determination of the Molecular Weight and Purity**

SDS-polyacrylamide gel electrophoresis of the affinity-eluted Blue Sepharose enzyme resulted in only a single major band of $M_r = 42,000$ (Figs. 3a and 4a). The densitometer tracing of this gel (Fig. 4) shows that the enzyme is essentially homogeneous, with only traces of impurities present. This enzyme preparation was also chromatographed on Ultrogel AcA 54. A single major protein peak was observed which coincided with the m^5U-methyltransferase activity peak, indicating that the major band on the gel was the m^5U-methyltransferase (data not shown).
Optimization of pH and Ionic Strength

Using the highly purified enzyme, the effect of pH on m3U-methyltransferase activity has been determined over a range of pH 7.6 to pH 8.8 with both Hepes and Tricine buffers. Both buffers resulted in the same activity, and with either buffer, an optimum of pH 8.4 is observed (data not shown). A 25% inhibition of enzyme activity occurs at pH 7.8 and pH 8.8.

The effect of ammonium acetate and spermidine on m3U-methyltransferase has also been investigated. Ammonium acetate has a broad optimal concentration range around 50 mM, at which concentration the methylation rate is enhanced 30% relative to the rate in the absence of ammonium acetate. The optimal concentration of spermidine in the presence of 50 mM ammonium acetate has been determined to be 20 mM, at which concentration a slight stimulation (1.0- to 1.1-fold) is observed (data not shown).

Specificity of Methylation

The specificity with which m3U-methyltransferase either from E. coli SN01 (pTN015) or E. coli MRE 600 forms m3U has been confirmed by 2-dimensional thin layer chromatography. When the methylated tRNA, either wheat germ tRNA\textsuperscript{\textit{A}}Y, bovine liver tRNA\textsuperscript{\textit{A}}\textit{x}, or Dictyostelium discoideum vegetative stage mitochondrial tRNA, was digested to mono-nucleotides and chromatographed as described above, the only methylated product was m3U (data not shown). Moreover, it has previously been shown that all of the m3U produced by the methylation of wheat germ tRNA\textsuperscript{\textit{A}}Y with m3U-methyltransferase of E. coli MRE 600 is present in the normal position that m3U occupies in tRNA, i.e. the 23rd nucleotide from the 3'-end of the molecule in the sequence GT\textsuperscript{\textit{C}} (6, 20).

Methylation of Various tRNA Substrates

A variety of tRNA substrates have been methylated by the highly purified m3U-methyltransferase. The extent of methylation of these tRNAs has been determined (Table II), along with that of wheat germ tRNA\textsuperscript{\textit{A}}Y and bovine liver tRNA\textsuperscript{\textit{A}}x, both of which are known to completely lack m3U (4). Unfractioned E. coli tRNA, presumably because all of the molecules already possess m3U, is not a substrate for the enzyme.

The initial rates of methylation have also been determined for these tRNAs (Fig. 5). The data points represent the per cent of complete methylation which has occurred in the times indicated for each substrate.

**TABLE II**

| tRNA                    | Extent of methylation of various tRNAs |
|-------------------------|----------------------------------------|
|                         | CH\textsuperscript{3}U-H\textsuperscript{3}H incorporated into tRNA | pmoles CH\textsuperscript{3}U-H\textsuperscript{3}H | pmoles tRNA |
|                         | pmoles %                                 |
| Bovine liver tRNA\textsuperscript{\textit{A}}x | 1.75 | 87.5 |
| Wheat germ tRNA\textsuperscript{\textit{A}}Y | 1.97 | 98.5 |
| Rabbit liver            | 0.13 | 6.5  |
| Spinach chloroplast     | 0.02 | 100  |
| *D. discoideum* (vegetative stage) | 0.07 | 35.0 |
| *D. discoideum* - mitochondrial (vegetative stage) | 0.24 | 12.0 |
| A. nidulans             | 0.10 | 5.0  |
| B. subtilis             | 0.01 | 0.5  |
| *E. coli               | 0  | 0    |

*The concentration of tRNA was calculated, using an average molecular weight of 25,000, to be 2000 pmoles/A\textsubscript{260} unit.

FIG. 5. Initial rates of methylation. The initial rates of methylation were determined for several tRNA substrates. Each point indicates the percent of methylation which has occurred relative to complete methylation of the individual species. Values represent the average of at least two experiments. Numbers adjacent to the curves indicate the tRNAs methylated as follows: 1) spinach chloroplast tRNA; 2) B. subtilis tRNA; 3) A. nidulans tRNA; 4) rabbit liver tRNA; 5) D. discoideum tRNA (vegetative state); 6) bovine liver tRNA\textsuperscript{\textit{A}}x; 7) wheat germ tRNA\textsuperscript{\textit{A}}Y; 8) D. discoideum mitochondrial tRNA.

Kinetic Studies

The initial velocities were determined for the methylation of wheat germ tRNA\textsuperscript{\textit{A}}Y over a tRNA concentration range of 60 to 2000 nm and an Ado-Met concentration of 2.5 to 30 \muM. Duplicate assays were incubated for 2 min which is within the linear range of the reaction, and lines were fitted to the data points by unweighted least squares analysis. From the data, the \textit{K}_{m} values have been determined to be 12.5 \muM for Ado-Met and 1.1 \muM for wheat germ tRNA\textsuperscript{\textit{A}}Y.

DISCUSSION

Blue Sepharose and Affi-Gel Blue (Bio-Rad) have been used by a number of investigators (37-44) for the purification of enzymes which interact with nucleic acids and related molecules. The enzymes have an affinity for the dye, Cibacron Blue F3G-A, which is bound to the agarose matrices in these resins. Since only 60% of the m3U-methyltransferase was removed from the Blue Sepharose column by the affinity elution process described above, attempts were made to improve the recovery. Affinity elution of the enzyme with a wheat germ tRNA\textsuperscript{\textit{A}}Y gradient (0 to 1 A\textsubscript{260} in 10 mm potassium phosphate, pH 7.0, in BSC buffer) improved neither the purity nor the yield of the enzyme (data not shown), while attempts to affinity elute more of the enzyme by raising the tRNA concentration in the buffer to 2.5 A\textsubscript{260} units/ml resulted in enzyme of lower purity (Fig. 4, Scan b).

In view of the ease and simplicity of the purification scheme described here, an attempt was made to extend the procedure to other tRNA methyltransferases. Preliminary results from studies on the purification of mcmo5U-methyltransferase from E. coli SN01 (pTN015) indicate that this enzyme can also be purified by chromatography on Blue Sepharose resin. The Blue Sepharose procedure reported here, and variations thereof, may indeed serve as a general method for the purification of tRNA methyltransferases.

The molecular weight of m3U-methyltransferase was determined by SDS-polyacrylamide gel electrophoresis to be 42,000. A M\textsubscript{r} of 38,000 was determined for this enzyme upon gel filtration on Ultrogel AcA 54 in the absence of SDS. This
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indicates that the native form of the enzyme is probably a monomer. These values are in excellent agreement with a Mₐ of 42,000, which has recently been observed for m^3U-methyltransferase synthesized in a minicell system directed by a 2.9-kilobase E. coli chromosomal DNA fragment containing the structural gene for m^3U-methyltransferase (27).

E. coli m^3U-methyltransferase catalyzes the synthesis of m^3U in E. coli tRNAs at a specific uridine residue 23 nucleotides from the 3'-end of the molecule. The enzyme has a broad substrate range and is capable of methylating a relatively large number of tRNAs from a wide range of different organisms as indicated in Table II. Indeed there is no known tRNA which has a uridine at the 23rd position from the 3'-end which is not a substrate for this enzyme. This enzyme has even been shown to form m^3U in viral tRNA-like moieties at a site corresponding to that occupied by m^3U in tRNA (45, 46). Apparently, the m^3U-methyltransferases of higher eukaryotes, such as wheat germ and mammals, have a narrower substrate range, in each case, there is a subset of tRNAs which lack m^3U and have an unmodified uridine instead (4, 6, 8).

Kinetic studies with highly purified m^3U-methyltransferase gave a Km value of 12.5 µM for Ado-Met which is 30-fold greater than that reported for another highly purified methyltransferase, the 1-methyladenosine methyltransferase of rat liver (11). Higher Km values have been previously observed for Ado-Met with E. coli as compared to mammalian tRNA methyltransferases (1). The isoelectric point (pl, 4.8) and the sensitivity of the m^3U-methyltransferase to Ado-Hcy (50% inhibition of the methylation rate at an Ado-Hcy concentration of 2 µM) have previously been determined for m^3U-methyltransferase isolated from E. coli MRE 600 (47).

Table II shows the extent of m^3U formation with highly purified m^3U-methyltransferase and a variety of tRNAs. As expected, Bacillus subtilis and E. coli tRNAs are either very poor substrates or are not methylated at all by this enzyme. The other prokaryotic tRNA studied, from Anacystis nidulans, is clearly a suitable substrate and methylates to an extent about 10-fold greater than that of B. subtilis. Among the eukaryotic tRNAs studied, D. discoides total tRNA and rat liver tRNA are both substrates, with rat liver tRNA being methylated to about twice the extent of D. discoides tRNA. It is interesting that, of the two organism tRNAs studied, the spinach chloroplast tRNA is methylated to only a very limited extent, comparable to that of prokaryotic tRNA, while D. discoides mitochondrial tRNA is quite a good substrate, being methylated to an extent more than 10-fold higher than spinach chloroplast tRNA. The methylation of mitochondrial tRNA was not totally unexpected, since two mitochondrial tRNAs recently sequenced, Neurospora crassa tRNA^{NM} (48) and yeast tRNA^{NM} (49), have a uridine at the 23rd position from the 3'-end of the molecule.

Studies of the kinetics of methylation (Fig. 5) show that the prokaryotic tRNAs studied are methylated with more rapid kinetics than the eukaryotic species tested. An exception to this is the chloroplast tRNA, which methylates with similar kinetics to B. subtilis and A. nidulans tRNAs. This may imply structural similarities between certain chloroplast tRNAs and those of prokaryotes. On the other hand, D. discoides mitochondrial tRNA is methylated with kinetics quite dissimilar to the rapid kinetics observed with spinach chloroplast and prokaryotic tRNAs.

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