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METHYLATION OF *E. COLI* TRANSFER RIBONUCLEIC ACIDS BY A tRNA ADENINE-1-METHYLTRANSFERASE FROM RAT BRAIN CORTEX AND BULK-ISOLATED NEURONS

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Abstract—Brain cortices or bulk-isolated neuronal cell bodies prepared from cortices of 8-day old male rats were used as the source of a 1-methyl adenine-specific tRNA methyltransferase (tRNA-AMT). Ammonium sulfate fractionation and chromatography on spheroidal hydroxylapatite and Sephadex G-200 yielded an 80-fold purified enzyme, as determined by using *E. coli* bulk tRNA as substrate. The kinetic parameters of tRNA-AMT for the substrate *S*-adenosyl-L-methionine (SAM) ($K_m = 6\ \mu M$) and the inhibitor, *S*-adenosyl-L-homocysteine (SAH) ($K_i = 3.4\ \mu M$) were determined and several SAH analogs tested as inhibitors. *S*-Adenosyl-L-cysteine (SAC) (10^{-4}M) and *S*-adenosyl-D-homocysteine (SADH) (10^{-4}M) produced a 35 and a 21% reduction in enzyme activity, respectively. The effects of Mg^{2+}, NH_4^+ acetate and of the polyamines spermine, putrescine and spermidine on the brain tRNA-AMT mimicked the effects of these agents on hepatic tRNA-AMT (GLICK *et al.*, 1975).

Comparing the ability of cerebral tRNA-AMT to methylate *E. coli* $tRNA^{glu_2}$, $tRNA^{val}$, $tRNA^{phe}$ and bulk tRNA revealed $tRNA^{glu_2}$ as the best and $tRNA^{phe}$ as the least effective substrate.

tRNA-AMT prepared from neuronal cell bodies showed closely similar characteristics to the cortical enzyme. A comparison of the activities of tRNA-AMT in neurons and glial cells revealed higher values in the former.

tRNA adenine 1-methyltransferase [EC 2.1.1.36] (tRNA-AMT) is an enzyme or group of closely related enzymes that methylates the 1 position of adenine residues in a tRNA polynucleotide chain. Unlike the recently reported non-specific RNA adenine-1-methyltransferase extracted from the nuclear fraction of the dinoflagellate *Cripthecodinium cohnii* (WERNER *et al.*, 1976), tRNA-AMT seems to recognize specific sequences within the tRNA molecule for the insertion of the methyl group (KUCHINO & NISHIMURA, 1974).

In the past years several attempts have been made to purify this enzyme using biological material from different sources (e.g. rat liver, HeLa cells and prokaryotic cells) (BAGULEY & STAEHELIN, 1968*a*,*b*; KUCHINO & NISHIMURA, 1970; AGRIS *et al.*, 1974; KERR, 1974; GLICK *et al.*, 1975). The activity was shown to be affected by polyamines, divalent cations, ammonium acetate, SAM and SAH (YOUNG & SRINIVASAN, 1971; PEGG, 1971; HACKER, 1973; LEBOY & GLICK, 1976). In a previous study we reported a relative decrease in the methylation of *E. coli* bulk tRNA adenine residues when 2.5 mM-spermidine was present in the incubation mixture containing a crude enzyme preparation from rat brain cortex (SALAS *et al.*, 1976). At least two possibilities were suggested to explain the observed results: (a) spermidine inhibits the methylation of adenine residues in position 1; or (b) the methylation of all other bases is proportionately higher than that of adenine in position 1, and this is reflected in an apparent, relative decrease in the percentage of 1-methyladenine formed. The same study (SALAS *et al.*, 1976) also showed a relative enrichment in tRNA-AMT activity in extracts derived from bulk-isolated nerve cell bodies (SELLINGER *et al.*, 1971) as compared to similar preparations obtained from the brain cortex (neurons + glial cells). To clarify some of these issues, we decided to examine the cellular localization of tRNA-AMT and compare some of the properties of the partially purified enzyme obtained independently from the cortex and its nerve cell bodies.

EXPERIMENTAL PROCEDURES

Materials

S-Adenosyl-L-[methyl-^{14}C]methionine (specific activity 45 mCi/mmol) was purchased from Research Products International (Elk Grove Village, IL); *E. coli* bulk tRNA from Schwartz-Mann (Orangeburg, NY); *E. coli* $tRNA^{phe}$ and *E. coli* $tRNA^{val}$ from Miles Laboratories (Elkhart, IN); *E. coli* $tRNA^{glu_2}$ and *S*-adenosyl-L-methionine from Boehringer Mannheim (Indianapolis, IN). The methylated

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Abbreviations used: tRNA-AMT, tRNA adenine-1-methyltransferase; SAM, *S*-adenosyl-L-methionine; SAH, *S*-adenosyl-L-homocysteine; SAC, *S*-adenosyl-L-cysteine; SADH, *S*-adenosyl-D-homocysteine;

bases used as standards were from Sigma Chemical Company (St. Louis, MO); μBondapak C_{18} columns (30 × 0.4 I.D. cm) from Waters Associates, Inc. (Milford, MA); trifluoroacetic acid from Matheson, Coleman and Bell (East Rutherford, NJ); ammonium phosphate monobasic from J. J. Baker (Phillipsburg, NJ); spheroidal hydroxylapatite from Gallard-Schlesinger (Carle Place, NY); *S*-adenosyl-L-homocysteine and its analogs from Sefochem Fine Chemicals (Jordan Valley, Israel). Spermidine phosphate was from Sigma Chemical Co. (St. Louis, MO), putrescine dihydrochloride and spermine tetrahydrochloride were from Mann Research Laboratories (New York, NY).

Apparatus

A solvent delivery system equipped with a dual piston pump, model 6000A, a universal liquid chromatography injector model U6K and an absorbance detector model 440 were from Waters Associates, Inc. (Milford, MA). The chromatographic run was recorded on an Omniscribe recorder, Texas Instruments (Austin, TX).

Methods

Hydrolysis of [methyl-^{14}C] tRNA. Trifluoroacetic acid (1 ml) was added to [methyl-^{14}C]tRNA in a combustion tube (25 × 2.0 I.D. cm) (A. H. Thomas Co., Philadelphia, PA) sealed under vacuum and incubated at 170°C for 30 min. After hydrolysis the trifluoroacetic acid was evaporated under a stream of N_2 at room temperature and the dry residue stored at 4°C.

Chromatographic analysis. The tRNA hydrolysate (SALAS *et al.*, 1976) containing between 2000 and 10,000 dpm was dissolved in water (0.2 ml) and applied to the column via the injector septum at room temperature using a 100 μl syringe (Precision Sampling Corp., Baton Rouge, LA). After sample injection the flow rate was maintained at 2 ml/min. For optimal separation (SALAS & SELLINGER, 1977) of the [methyl-^{14}C] bases in the hydrolysate, buffer solutions were changed 350 s after injection. Fractions (0.02–0.1 ml) were collected in plastic mini-vials (Rochester Scientific Co., Inc., Rochester, NY) to which 2 ml of tissue solubilizer-scintillation mixture was added (Amersham, Searle, Arlington Heights, IL). The radioactivity of the samples was determined in a Beckman 250 liquid scintillation spectrometer. All buffers and samples were filtered through Millipore filters (0.45 μm pore size) and were degassed *in vacuo* prior to use.

Methylation of E. coli *tRNA* in vitro. Both the 'rate' and 'extent' methylation assay mixtures (PEGG, 1971) as applied to brain tissue have been described elsewhere (SALAS *et al.*, 1976). For the study of the kinetic parameters and of the effects of SAH analogs on enzyme activity the assays were performed in the 'rate' mode.

Preparation of tRNA-AMT. Male, Sprague–Dawley rats (8 days-old) were decapitated and the brain cortices (approx 15 g) quickly removed and homogenized in a glass–Teflon Potter–Elvehjem homogenizer to a final volume of 3.5 times the tissue weight. The buffer solution contained 10 mM-Tris–HCl pH 7.6, 1 mM-EDTA and 1 mM-dithiothreitol (DTT). The homogenate was centrifuged at 165,000 *g* for 150 min and the clear supernatant fractionated with a saturated solution of ammonium sulfate at pH 7.0 (PEGG, 1971). The final pellet was recovered by centrifugation, resuspended in 10 mM-Tris–HCl buffer pH 8.0, containing 1 mM-EDTA and 1 mM-DTT (buffer A) and was dialyzed overnight against 3 l of the same buffer. The dialysis residue (ca. 10 ml) was then made 1 mM in Na phosphate and applied to a column (25 × 1.5 cm) of spheroidal hydroxylapatite, previously equilibrated with the same buffer. The column was then washed with a volume of buffer, equivalent to that of the sample, following which a linear gradient of Na phosphate (1–400 mM; 300 ml) in buffer A was used to elute the enzyme. The total tRNA methyltransferase activity in the eluates was determined using *E. coli* bulk tRNA as methyl acceptor. Peak I (Fig. 1A) was pooled and concentrated by precipitation with solid ammonium sulfate to 80% saturation. The precipitate was collected by centrifugation and dissolved in 2.0 ml of 50 mM-Tris, pH 8.0 containing 0.1 mM-EDTA and 1 mM-DTT and was dialyzed overnight against 2 l of the same buffer. The non-diffusible material was applied to a column (130 × 0.9 cm) of Sephadex G-200 previously equilibrated with the 50 mM-Tris buffer. Enzyme activity was eluted using the same Tris buffer and fractions were pooled and concentrated by solid ammonium sulfate precipitation (80%). The precipitate obtained after centrifugation was dissolved in a small volume of buffer A. Measured aliquots of the enzyme were stored at −70°C.

tRNA-AMT from neuronal cell bodies. Neuronal cell bodies, prepared from cerebral cortices of 8 day-old male rats as previously described (SELLINGER *et al.*, 1971; SELLINGER & AZCURRA, 1974) were suspended and homogenized in the same buffer used for whole cortices (3.5 ml/g

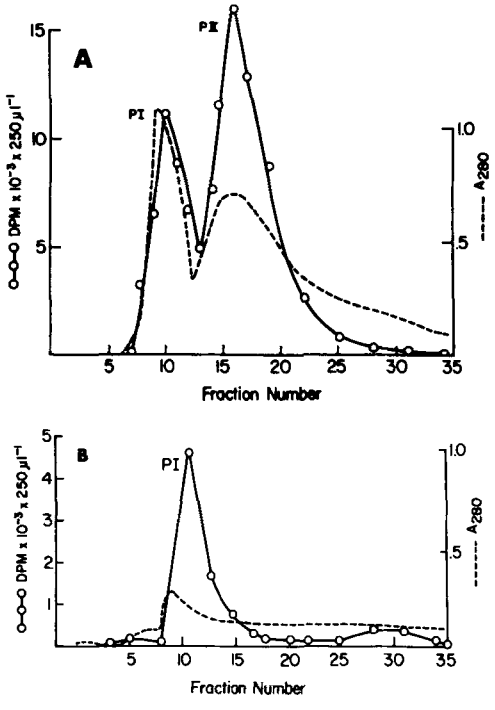

FIG. 1. Spheroidal hydroxylapatite chromatography of rat brain tRNA methyltransferases. The column was eluted with a linear 1–400 mM-Na phosphate gradient. Aliquots (250 μl) were analyzed for tRNA methyltransferase activity in the 'rate' mode (PEGG, 1971). (A) whole brain cortex tRNA methyltransferases. (B) neuronal tRNA methyltransferases. For details see Methods.

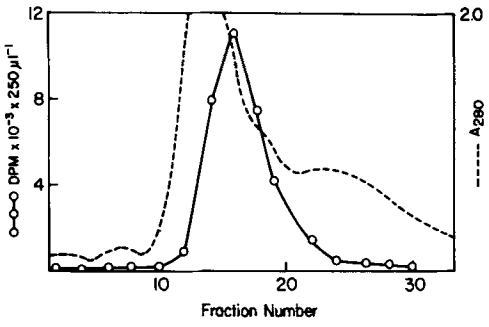

FIG. 2. Chromatography of tRNA-AMT from whole rat brain cortex on Sephadex G-200. Aliquots (250 µl) were tested for tRNA-AMT activity in the 'rate' mode. For details, see Methods.

of neuronal pellet). Subsequent treatment of this preparation was identical to that described for the cortical enzyme.

Protein determination. Protein was determined according to LOWRY *et al.* (1951), using crystalline bovine serum albumin as standard.

RESULTS

Spheroidal hydroxylapatite chromatography resolved two peaks of methyltransferase activity, as shown in Fig. 1A. The first peak contained most of the tRNA-AMT, while the second peak contained some tRNA-AMT + significant tRNA-guanine N^2-methyltransferase and tRNA-guanine-N^2_2-dimethyltransferase activity. In contrast, equivalent preparations derived from cortical neuronal cell bodies contained virtually only tRNA-AMT enzyme activity, as shown in Fig. 1B. It should be noted that the elution volumes for the first peak in Figs. 1A and 1B were the same regardless of the source of the enzyme. Also, the ammonium sulfate precipitation and dialysis steps prior to chromatography were found to be essential for the successful separation of tRNA-AMT from the other tRNA-methylating activities.

Peak 1 (Fig. 1A) was further purified by passage through a column of Sephadex G-200 (Fig. 2). The effluent fractions containing tRNA-AMT activity were treated as described in Methods and were stored at −70°C for up to 6 months without detectable loss of activity.

To determine the methyl base specificity of tRNA-AMT unequivocally, bulk *E. coli* tRNA was methylated using enzyme preparations partially purified through the hydroxylapatite and/or Sephadex G-200 steps. The resulting [methyl-^{14}C]tRNA was hyrolyzed with trifluoroacetic acid at 170°C for 30 min and following the evaporation of the acid, the hydrolyzates were subjected to high performance liquid chromatography (HPLC) as described in Methods (SALAS & SELLINGER, 1977). The radiometric analysis of the HPLC eluates revealed that virtually all of the radioactivity was confined to the elution position corresponding to 1-methyladenine (Fig. 3). Separate methylation assays using cortical enzyme preparations revealed, in addition to [methyl-^{14}C]1-methyladenine, residual amounts of [methyl-^{14}C]1-methylguanine and N^2-methylguanine, both representing no more than 10% of the total [methyl-^{14}C]-tRNA formed. Table 1 summarizes the purification procedure of tRNA-AMT from whole brain cortex.

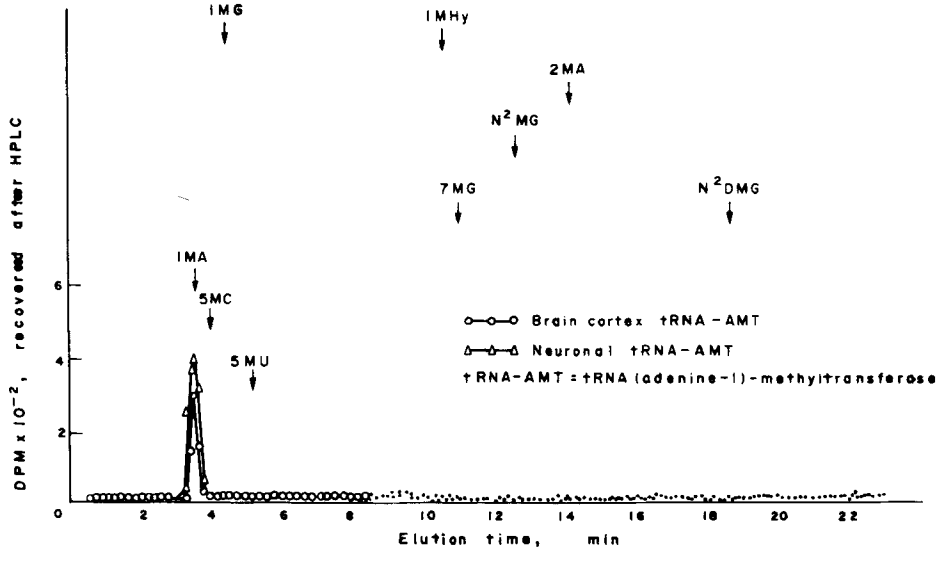

FIG. 3. High performance liquid chromatography (HPLC) of *E. coli* [methyl-^{14}]tRNA hydrolyzates on µBondapak C_{18}. The enzyme activity was measured in the 'extent' mode (PEGG, 1971). For details, see Methods.

TABLE 1. PURIFICATION OF tRNA-AMT FROM RAT BRAIN CORTEX*

Fraction	Vol. (ml)	Units†/ml	Total units	Protein (mg/ml)	Units/mg protein	Yield (%)	x-fold
Homogenate	52	61,534	3.2×10^6	19.8	528	100	0
High speed supernatant	48	41,665	2.0×10^6	6.7	1057	62.5	2.0
Ammonium sulfate ppt.	13	99,995	1.3×10^6	15.2	1118	40.6	2.1
Hydroxylapatite	3	137,852	4.1×10^5	11.3	12,200	12.9	23.1
Sephadex G-200	3	11,360	5.7×10^4	0.27	42,074	1.8	79.7

* 14.4 g of tissue
† D.p.m. [methyl-^{14}C] tRNA/30 min.

Comparing the effects of NH_4^+ and Mg^{2+} revealed a range of concentrations over which the activity of both the cortical and neuronal tRNA-AMT failed to change much. The range was 3–7 mM for Mg^{2+} and 0.2–0.8 M for NH_4^+. Using 100 mM-Tris buffer, maximal tRNA-AMT activity was found between pH 8.2–8.4.

The findings shown below are from experiments in which we used tRNA-AMT from whole brain cortex (unless otherwise stated); the enzyme derived from nerve cell bodies yielded analogous results. Figure 4 is a comparative study of the effect of different concentrations of spermidine, spermine and putrescine on whole cortex tRNA-AMT. Spermidine produced a maximal stimulatory effect at 2–3 mM, as previously demonstrated for the unfractionated brain cortex tRNA methyltransferases (SALAS *et al.*, 1976). Alternatively, 1-mM-spermine and 40–60 mM-putrescine were the most suitable concentrations to produce maximal tRNA-AMT activity. In another experiment the kinetic parameters for tRNA-AMT were examined and an apparent K_m of 6 μM determined for SAM. The methylation of tRNA was competitively inhibited by SAH with a K_i of 3.4 μM.

Table 2 lists the effect of a number of SAH analogs on the activity of the neuronal tRNA-AMT. Of all compounds tested, the strongest inhibitory effect was produced by (10^{-4}M) *S*-adenosyl-L-cysteine (SAC) (35%), while a weaker effect was observed with (10^{-4}M) *S*-adenosyl-D-homocysteine (SADH) (21%).

A further characterization of tRNA-AMT was undertaken using individual *E. coli* tRNA species as substrates. As shown in Table 3, the extent methylation of the various *E. coli* tRNAs varied markedly, that of $tRNA^{glu_2}$ being the most and that of $tRNA^{phe}$ being the least effective. The extent methylations of $tRNA^{phe}$ and $tRNA^{val}$ were equivalent to 12% and 20% respectively, of that noted with $tRNA^{glu_2}$, in good agreement with similar values obtained using rat liver tRNA-AMT (KUCHINO & NISHIMURA, 1974; GLICK *et al.*, 1975).

To determine the cellular distribution of tRNA-AMT, fractions of equivalent purity were prepared from whole cerebral cortex, bulk-isolated neuronal cell bodies and glial cells (SELLINGER *et al.*, 1971) and cultured astrocytes derived by growing 3-day old whole rat brain cortex in primary culture for 12–14 days (CUMMINS & GLOVER, in press). Table 4 shows that [methyl-^{14}C]1-methyladenine represented 41.5% of the total radioactivity recovered after HPLC in the neurons as against 30.2% and 28.1% in the two types of glial cells, with an intermediate 32.1% in the whole cortex. Conversely N^2-methylguanine formation (SALAS *et al.*, 1977) was significantly higher in both

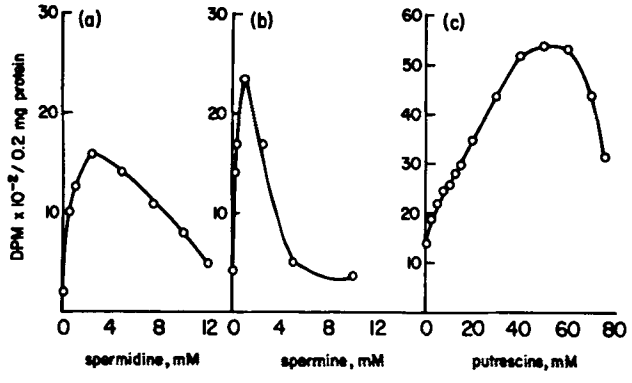

FIG. 4. Effect of polyamines on tRNA-AMT activity using bulk *E. coli* tRNA. The enzyme activity was measured in the 'rate' mode using cortical tRNA-AMT.

TABLE 2. THE INHIBITION OF BRAIN tRNA-AMT

Compound	% Inhibition 10^{-4}M	10^{-5}M	10^{-6}M
S-Adenosyl-L-homocysteine	84	34	2.2
S-Adenosyl-D-homocysteine	21	12	2.2
S-Adenosyl-L-cysteine	35	0	0
S-5'-Butyl-5'-deoxyadenosine	0	0	0
S-Adenosyl-5'-deoxy-5'-thioethanol	0	0	0
S-5'-Isobutyl-5'-deoxyadenosine	0	0	0
2-*O*-Methyladenosine	0	0	0
9-β-D-Arabinofuranosyladenosine	0	0	0
9-β-D-Arabinofuranosylhypoxanthine	0	0	0
1-β-D-Arabinofuranosyluracil	0	0	0
Adenosine	11	0	0

Neuronal cells from 8-day old rat cerebral cortex were used as the source of the enzyme. tRNA-AMT was incubated in the presence of *E. coli* tRNA, 2.5 mM-spermidine and *S*-adenosyl-L-[methyl-^{14}C]methionine for 30 min at 37°C, followed by 10% cold TCA precipitation. Radioactivity was recovered on Millipore filters (see Methods).

bulk-isolated (42.2%) and cultured (34.1%) glial cells than in the neuronal cell bodies (18.2%).

DISCUSSION

A procedure is described which yields an 80-fold purified tRNA-AMT, essentially free of contaminating guanine-specific tRNA methyltransferases, from the 8-day old rat cerebral cortex and its neurons. The procedure requires relatively small amounts of tissue (ca. 15 g). It is of interest that a much higher degree of purification and hence a much more laborious procedure was found necessary to free tRNA-AMT of rat liver (GLICK *et al.*, 1975) and of HeLa cells (AGRIS *et al.*, 1974) of contaminating tRNA methyltransferases. No differences were detected in the present study between the tRNA-AMT purified from the whole cortex and that purified from its neuronal cell bodies (Figs. 1–3).

TABLE 3. METHYLATION OF INDIVIDUAL *E. coli* tRNA SPECIES

tRNA	Relative activity
Bulk	1.00
$tRNA^{val}$	0.71
$tRNA^{phe}$	0.43
$tRNA^{glu_2}$	3.57

Enzymes were from 8-day old rat cerebral cortex. 'Extent' assay was performed in the presence of 2.5 mM-spermidine, 1 mg of brain protein and tRNA between 1.5 and 20 nmol. Incubation: 2 h.

*Activity with bulk tRNA: 6.3×10^4 d.p.m./mg/120 min.

The study of tRNA-AMT inhibitors (Table 2) demonstrated that 10^{-4}M-SAH inhibited tRNA-AMT almost totally while 10^{-6}M-SAH failed to inhibit the enzyme. Inhibition by 10^{-4}M-SADH and SAC was

TABLE 4. A COMPARISON OF tRNA METHYLTRANSFERASE METHYLATING CAPACITY IN CEREBRAL CORTEX, BULK-ISOLATED CORTICAL NEURONS AND GLIAL CELLS AND CULTURED ASTROCYTES*

[Methyl-^{14}C] Base	Whole cortex	Bulk-isolated† Neuronal perikarya	Glial cells	Cultured astrocytes‡
1-Methyladenine	32.1	41.5	30.2	28.1
5-Methylcytosine	16.8	13.7	9.6	16.7
1-Methylguanine	2.4	9.0	N.D.	10.0
1-Methylhypoxanthine	7.7	5.7	8.2	7.4
N^2-Methylguanine	28.4	18.2	42.2	34.1
N^2_2-Dimethylguanine	9.5	9.9	9.8	trace
Minor bases	3.1	2.0	N.D.	3.7
Total radioactivity ± (d.p.m.)	11,143	12,713	1476	9848

* Values are expressed as % of total recovered radioactivity after HPLC set to 100%. The actual recovery of d.p.m. among the methylated bases as % of the d.p.m. injected into the HPLC machine was above 90% in most cases and approached 95% in some. tRNA methyltransferases: ammonium sulfate precipitate (0–80%) of the 165,000 *g*/150 min supernatant obtained from the homogenate of the indicated tissue or cell source. N.D. Not detected.

† By the procedure of SELLINGER *et al.* (1971); ‡ By the procedure of CUMMINS & GLOVER (1978). 'Extent' assay, 2 h. Spermidine: 2.5 mM; Substrate: *E. coli* tRNA (bulk), 10 μg.

35% and 21% respectively, indicating a lower effectiveness of these analogs, when compared to SAH (HILDESHEIM *et al.*, 1973*a*, *b*; TREWYN & KERR, 1976). Comparing the inhibitions by SAC and SADH revealed that the length of the amino acid chain is not a critical factor determining their efficacy as tRNA-AMT inhibitors and that 10^{-5}M-SADH is slightly more effective than 10^{-5}M-SAC.

The concentration of NH_4^+ necessary for maximal stimulation of brain tRNA-AMT was higher than that (0.1–0.25 M) previously reported for both non-neural (PEGG, 1971; YOUNG & SRINIVASAN, 1971; HANCOCK & ELEFTHERIOU, 1971) and neural (HANCOCK & ELEFTHERIOU, 1971) tRNA methyltransferases and, more recently, by LEBOY & GLICK (1976) for the highly purified rat liver tRNA-AMT. The rat brain tRNA-AMT also appeared to differ from the purified rat liver enzyme (LEBOY & GLICK, 1976) in terms of its Mg^{2+} requirements, since appreciable activity was determined in the presence of 2–10 mM-Mg^{2+} with no polyamines or NH_4^+ co-present, whereas LEBOY & GLICK (1976) reported negligible activity in the presence of 0.5–10 mM-Mg^{2+}, when compared to the activity measurable in the presence of optimal NH_4^+ concentrations. Alternatively, comparing the total d.p.m. incorporated into tRNA (Figs. 4A-C), reveals that putrescine (Fig. 4C) produced the maximal stimulatory effect.

The *in vitro* stimulation of tRNA methyltransferases by spermine, spermidine and putrescine (Fig. 5) is a well documented phenomenon (PEGG, 1971; YOUNG & SRINIVASAN, 1971; HACKER, 1973; LEBOY & PIESTER, 1973; CUMMINS *et al.*, 1975; SALAS *et al.*, 1976, 1977; DAINAT *et al.*, 1977). It is of interest to note, however, that while spermine affected brain tRNA-AMT and non-neural tRNA methyltransferases optimally at approx 1–1.5 mM (PEGG, 1971; YOUNG & SRINIVASAN, 1971; HACKER *et al.*, 1973), spermidine and putrescine affected brain tRNA-AMT and non-neural tRNA methyltransferases differently. For example, PEGG (1971) found maximal stimulation of rat liver tRNA methyltransferases by 15–25 mM-putrescine, while brain tRNA-AMT (Fig. 4) required 40–60 mM-putrescine for optimal activity, a value which compared favorably with the values of 60 mM and 40–50 mM found optimal for the tRNA methyltransferases of leukemic cells (HACKER, 1973) and purified rat liver tRNA-AMT (LEBOY & GLICK, 1976). Our results also show (Fig. 4) that, like the rat liver tRNA methyltransferases (PEGG, 1971; YOUNG & SRINIVASAN, 1971), brain tRNA-AMT required 2–3 mM-spermidine, whereas the purified liver tRNA-AMT (LEBOY & GLICK, 1976) was maximally stimulated by 12–14 mM-spermidine. It is of further interest that HACKER (1973) noted two spermidine concentration optima for the tRNA methyltransferases of the leukemic cells, one at 3 and the other at approx 10 mM. Although the ranking of the polyamines' effectiveness as stimulators of cerebral tRNA-AMT agrees with the accepted notion that putrescine is the most effective polyamine (HACKER, 1973), MOLLER *et al.* (1977) recently showed 10 mM-spermine to be most effective in stimulating tRNA methylation by adult rat brain extracts and 20 mM-spermidine to be most effective with the fetal and the newborn rat brain enzymes.

When tRNA-AMT was tested with different individual *E. coli* tRNA species (Table 3), $tRNA^{glu_2}$ and $tRNA^{phe}$ proved to be the best and the least effective substrates, respectively. These results are in complete accord with the findings of KUCHINO & NISHIMURA (1974) and of GLICK *et al.* (1975) who used adult rat liver tRNA-AMT. Examining the methylation of $tRNA^{f^{met}}$ by tRNA methyltransferases of the immature (6 days) and weanling (21 days) rat cerebellum (DAINAT *et al.*, 1977, and unpublished observations) we recently noted that in the 6-day old animal 1-methyl-adenine formation failed to exceed 10% of the total methylated bases formed, while at 21 days it rose to approx 38% in the absence of spermidine, being virtually nil in its presence. These findings suggest a finely tuned and developmentally controlled sensitivity of this reaction toward polyamines.

The results shown in Table 4 demonstrate that neurons contain appreciable levels of tRNA-AMT activity, in confirmation of our previous circumstantial findings (SALAS *et al.*, 1976). To find out whether tRNA-AMT is *selectively* enriched in neurons, we prepared tRNA-AMT-containing fractions from bulk-isolated and cultured glial cells and compared them to equivalent cortical and neuronal fractions. The results indicate that, although both glial preparations contained tRNA-AMT activity, the per cent contribution of [methyl-^{14}C]1-methyladenine to the total methylated bases was significantly lower in the glial cells than in the whole cortex or the neurons. On the premise that the content in tRNA-AMT of each isolated cell type reflects the *in vivo* situation, neurons are therefore a relatively richer source of tRNA-AMT than are glial cells. Whether the neuronal and the glial tRNA-AMT activities have different site specificities, as do the enzymes of *Phaseolus vulgaris* (DUBOIS *et al.*, 1974), and whether the enrichment of tRNA-AMT in nerve cells has a functional correlate, i.e. the control of the synthesis of neuron-specific proteins remains to be established.

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