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PRELIMINARY COMMUNICATIONS

ALTERATION OF THE SPECIFICITY OF BRAIN tRNA METHYLTRANSFERASES AND OF THE PATTERN OF BRAIN tRNA METHYLATION IN VIVO BY METHIONINE SULFOXIMINE

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Recently, we showed that the formation of [^{3}H]-3-methyl histamine from intraventricularly administered [^{3}H]-histamine is accelerated in the brains of mice pretreated with the convulsant L-methionine-dl-sulfoximine (MSO) (1). We also reported that tRNA methyltransferases derived from brains of MSO-treated rats methylate heterologous tRNA in vitro more extensively than tRNA methyltransferases prepared from brains of control animals (2). In the present communication we describe changes in methylation of individual bases in specific Escherichia coli tRNAs following their incubation with tRNA methyltransferases of MSO-treated rat cerebella. Preliminary results of a study of the effect of MSO on the methylation of bases in total rat brain tRNA in vivo are also presented.

Eighteen-day-old Sprague-Dawley male rats were injected with 0.94 m-mole/kg of MSO i.p. 3 hr before death, i.e. midway through the preconvulsant period. Measurements of tRNA methyltransferase activity were carried out on dialyzed 0-80% ammonium sulfate precipitates prepared from high-speed supernatant solutions (165,000 g, 150 min) of cerebella of saline and MSO-treated animals. In the in vivo experiments the rats received intracranially (25 µl) 4 µCi/kg of [Me-^{3}H]-L-methionine (5 Ci/m-mole, Amersham Corp., Arlington Heights, IL) or of physiological saline 45 min before death (3). The individual tRNAs (E. coli) were purchased from Boehringer-Mannheim (Indianapolis, IN), and S-adenosyl-[Me-^{14}C] -methionine (AdoMet) (46 mCi/m-mole) was a product of Research Products International, Elk Grove Village, IL. tRNA methyltransferases were assayed in the "extent" mode (4) in a volume of 0.6 ml which contained 0.4 to 0.6 A_{254} units tRNA, 12 nmoles (0.2 µCi) AdoMet, 1.5 m-moles spermidine, 2.5 to 3.0 mg of tissue protein, 60 µmoles Tris-HCl buffer, pH 8.3, and 0.6 µmole dithiothreitol. The reaction was stopped by the addition of 1 volume of 88% (v/v) phenol in 0.1 M sodium acetate, pH 5.1. After extraction (5), 0.1 mg of carrier tRNA and 2.5 volumes of absolute ethanol were added, and the suspension was allowed to stand at -20° overnight. The tRNA pellet was collected by centrifugation and was hydrolyzed (6). The resulting Me-^{14}C bases were separated by high pressure liquid chromatography (h.p.l.c.) (7) and their radioactivity was determined (7). For the isolation of the [Me-^{3}H]-tRNA formed in vivo, cerebral cortices were excised and frozen in liquid nitrogen. The frozen tissue was homogenized in 0.25 M sucrose containing 35 mM Tris-HCl buffer, pH 7.4, 25 mM KCl and 5 mM $MgCl_2$, and the homogenate centrifuged at 10,000 g for 15 min. The tRNA, extracted (5) from the supernatant solution, was deacylated by incubation (4 hr) at 37° in 1 mM $MgCl_2$ and 10 mM Tris-HCl buffer, pH 8.5, and was further purified by

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passage through DEAE-cellulose (8). Total hydrolysis (6) yielded the Me-^{3}H -bases which were separated by h.p.l.c. (7) and their radioactivity was determined.

Table 1 shows that the in vitro formation of [Me-^{14}C]-N^2-methyl guanine in bulk tRNA, $tRNA^{phe}$ and $tRNA^{fmet}$ was increased substantially over control values following incubation with the tRNA (guanine-N^2)-methyltransferase derived from MSO-treated animals. Conversely, the formation of [Me-^{14}C]-1-methyl adenine (9) diminished in bulk tRNA and in $tRNA^{phe}$ while increasing in $tRNA^{val}$. No evidence was found that the procedures used promoted the chemical decompositon of 1-methyl adenine. Hence, the different changes in 1-methyl adenine content in the various tRNAs are of particular interest. The relatively minor contribution of [Me-^{14}C]-5-methyl cytosine to the Me-^{14}C -base content of bulk tRNA and $tRNA^{phe}$ was decreased further by the MSO treatment. Table 1 also shows that MSO elicited a more extensive formation of [Me-^{14}C]-1-methyl adenine in $tRNA^{val}$, a poor substrate for cerebellar tRNA (adenine-1)-methyltransferase (J. Dainat and O. Z. Sellinger, unpublished observations), than in $tRNA^{fmet}$, a very effective substrate for this enzyme (J. Dainat and O. Z. Sellinger, unpublished observations). Since $tRNA^{val}$ was shown recently to accept less than 0.5 mole of methyl as 1-methyl adenine per mole of tRNA when methylated by a highly purified rat liver tRNA (adenine-1)-methyltransferase (10), it is likely that the increased methyl acceptance of $tRNA^{val}$ following MSO (Table 1) reflects improved recognition by a presumably modified cerebellar enzyme. Conversely, the failure to increase methyl acceptance of $tRNA^{fmet}$ may be linked to the fact that this tRNA normally accepts close to the maximum of 1 mole of methyl as 1-methyl adenine per mole of tRNA (10) and that, consequently, no further improvement of methyl acceptance is possible, short of involving a second adenine residue. It is possible, however, that the increased formation of [Me-^{14}C]-1-methyl adenine in $tRNA^{val}$ reflects the involvement of a second adenine residue which, like the first one, also undergoes methylation submaximally.

Table 1. Effect of MSO on the in vitro methylating capacity of cerebellar tRNA methyltransferases*

	N^2-Methyl guanine				1-Methyl adenine				5-Methyl cytosine			
tRNA	MSO+ / C	C (%)	MSO (%)	△ (%)	MSO / C	C (%)	MSO (%)	△ (%)	MSO / C	C (%)	MSO (%)	△ (%)
Bulk	1.26	25.8	34.1	+8.3	0.61	26.1	16.5	-9.6	0.70	8.6	6.3	-2.3
$tRNA^{phe}$	1.13	46.8	53.1	+6.3	1.11	32.5	36.3	+3.8	0.46	13.8	6.4	-7.4
$tRNA^{fmet}$	1.20	46.3	49.3	+3.0	1.04	15.6	14.6	-1.0	-	-	-	-
$tRNA^{val}$	0.97	55.4	55.1	-0.3	1.32	16.1	25.2	+9.1	-	-	-	-

*MSO: 0.94 m-mole/kg, i.p. The animals were 18-days old and were killed 3 hr after MSO.
+Ratio in dis./min/mg of protein of control to MSO-treated animals. The % values refer to the radioactivity after h.p.l.c. △ is the algebraic difference between C (control) and MSO values. A dash (-) indicates that the formation of [^{14}C]-5-methyl cytosine was negligible.

The pattern of in vivo labeling of the methyl bases of cerebral tRNA after a pulse which results in maximal Me-^{3}H-labeling of small molecular weight cytoplasmic RNA and in negligible labeling of

ribosomal RNA (3) is shown in Table 2. MSO affected the distribution of the Me-^{3}H-label significantly, inasmuch as it caused an apparent diminution of the total [Me-^{3}H]-methyl adenine content and an apparent doubling of the [Me-^{3}H]-7-methyl guanine content in the [Me-^{3}H]-tRNA. Other minor alterations in the distribution of the Me-^{3}H- group involved 1-methyl guanine, N_2^2-dimethyl guanine and 1-methyl hypoxanthine. No radioactivity was detected in the elution positions corresponding to the retention times of 3-methyl cytosine and N^6-N^6-dimethyladenine, the first and last methyl base to exit from the h.p.l.c. column, respectively (7). The identity of the individual brain tRNAs undergoing changes in methylation as a result of the MSO treatment is presently under investigation. Changes in 2-O-methylation also remain to be investigated (11).

Table 2. Effect of MSO on the methylation of brain tRNA in vivo*

Methyl-^{3}H-base	Control (%)	MSO (%)	Difference (%)
1-Methyl adenine	28.3	17.4	-10.9
5-Methyl cytosine	11.6	11.9	+0.3
1-Methyl guanine	8.0	10.6	+2.6
N^2-methyl guanine	16.6	17.0	+0.4
N_2^2-dimethyl guanine	9.0	11.1	+2.1
1-Methyl hypoxanthine	11.4	13.9	+2.5
5-Methyl uracil	4.9	4.8	-0.1
7-Methyl guanine	4.0	8.1	+4.1
2-Methyl adenine	1.7	2.0	+0.3
6-Methyl adenine	4.5	3.2	-1.3
Sum:	100.0	100.0	
Total radioactivity (dis./min) collected **	10,151	3,885	

*The cerebral cortex of 18-day-old rats was used. ** Represents in excess of 90% of the radioactivity applied to the h.p.l.c. column in both groups. The values are expressed as percentages of the radioactivity collected and are normalized to 100% recovery. They are the means of three determinations.

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