THE METHYLATION OF THE PURINES OF SOLUBLE RIBONUCLEIC ACID WITH METHYL-LABELED METHIONINE*

B. B. Biswas,** Mary Edmonds, and Richard Abrams

Research Department, Montefiore Hospital
Pittsburgh 13, Pennsylvania

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Methylated purines have been shown to occur in soluble RNA of a variety of organisms (1,2). Remy (3) has observed that extracts of Escherichia coli utilize the methyl group of methionine to methylate 2,6-diaminopurine to 2-methylamino-6-aminopurine. Mandel and Borek (4) have demonstrated a decrease in the frequency of methylated adenines in soluble RNA of a methionine requiring mutant E. coli during methionine starvation. They have also reported that C^{14} from methyl labeled methionine is incorporated into the methylated adenines of E. coli (5).

We wish to present evidence for the utilization of the methyl group of methionine for the methylation of guanine found in the soluble RNA of Ehrlich ascites cells.

METHODS

15 gms. of saline washed Ehrlich ascites cells were incubated in a shaking bath with 4.6 µmoles of C^{14}-methyl labeled methionine (5 µc/µmole) in 100 ml of Robinson's medium (6) supplemented with 1 mg/ml of glucose. After 90 minutes at 37°C the re-

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**Present address, Bose Institute, Calcutta 9, India.

action mixture was chilled, and a 25 ml aliquot was removed and brought to 0.5 M with perchloric acid. After centrifugation, the acid solution was heated to 100° for 60 min. and purines isolated as described below. The remaining incubation medium was centrifuged at 600 x g. The packed cells were collected and disrupted by homogenization in cold distilled water as described by Hecht, et al (7). The homogenate was adjusted to the following final concentrations: 0.25 M sucrose, 0.025 M KCl, 0.005 M $MgCl_2$, and 0.05 M Tris buffer, pH 7.5. After centrifugation for 2 hrs. at 100,000 x g and precipitation at pH 5.0, soluble RNA was isolated from the precipitate by the phenol technique of Kirby (8). The S-RNA was reprecipitated twice prior to hydrolysis.

One half of the soluble RNA was hydrolyzed in 0.5 M HCl for 60 minutes at 100°C. Adenine and guanine fractions were recovered from the acid hydrolysate by chromatography on Dowex-50 (H^+). Unlabeled 1-methylguanine and N^2-methylguanine were mixed with the guanine fraction and an aliquot was applied to 3 MM Whatman paper. The chromatogram was developed with a butanol-ammonia mixture (6:1) for 60 hours to resolve each of the three bases (9). Each base was eluted from paper with 0.01 M HCl, and C^{14} assayed on a plated aliquot of each eluate.

The remaining soluble RNA was hydrolyzed in 0.5 M KOH for 18 hr. at 37°C. The 2',3'-AMP and 2',3'-GMP recovered from the alkaline hydrolysate by chromatography on a Dowex-1-formate column were hydrolyzed to adenine and guanine and chromatographed on Dowex-50 followed by paper chromatography as described above.

RESULTS

The results with S-RNA summarized in Table I indicate that the methylated guanines contain much more C^{14} than do adenine or guanine. Since the molar ratio of guanine to methylated guanine

in S-RNA is roughly 50, the data imply that the specific activities of methylated guanines in S-RNA were several hundred times greater than the guanine specific activity.

Table I

Incorporation of Methionine-methyl-C^{14} into Guanine Fractions

Isolated purine	Acid soluble fraction	S-RNA, acid hydrolysis	S-RNA, alkaline hydrolysis
	total counts/minute		
Guanine	4704	256	132
1-methylguanine	576	1344	1064
N^2-methylguanine	672	2520	1824
Adenine	-	212	-

The C^{14} content of the methylated guanines was very little affected by the method of isolation from RNA. Recovery from either acid or alkaline hydrolysates led to similar labeling patterns. A more rigorous demonstration of the existence of C^{14} in the methylated guanines was obtained by nitrous acid deamination of 1-methylguanine to 1-methylxanthine with very little change in specific activity (Table 2).

Table II

C^{14} in 1-Methylxanthine Derived from 1-Methylguanine

	Specific activity	Yield
	counts/min./μmole	μmoles
1-methylguanine	293	1.16
1-methylxanthine	227	0.60

The deamination product was identified by its elution characteristics on Dowex-50 and by acid and alkaline ultraviolet absorption spectra.

Although methylated adenines were not isolated, a paper chromatographic method designed to separate adenine from some of its methylated derivatives (2) removed most of the radioactivity from

adenine, suggesting that here too the C^{14} was associated with methyl derivatives.

An examination of the radioactivity in the methylated guanine carriers reisolated from the acid soluble fraction (Table I) showed considerably less total radioactivity than in those recovered from the soluble RNA. These data suggest that the pool of soluble methylated guanine compounds must be quite small. This conclusion is compatible with our failure to detect methylated guanine derivatives in the acid soluble fraction of rat liver homogenates (unpublished data). The possibility that the high radioactivity of guanine in the acid soluble fraction might have been due to a contaminant has not been excluded.

Although these data provide no evidence for the mechanism of purine methylation, they indicate that methionine can serve as the methyl donor for the methylated purines found in soluble RNA of mammalian cells.

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