THE METHYLATION OF TRANSFER RNA BY METHYL COBAMIDE

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It has been reported that small amounts of methylated bases occur in transfer ribonucleic acid (Littlefield and Dunn, 1958) and the methylation of these bases has been shown to occur at the polynucleotide level after the formation of transfer RNA. S-adenosylmethionine has been found to serve as a methyl donor in the methylation of various t-RNAs using enzyme preparations from microbial systems, (Fleissner and Borek, 1963; Gold and Hurwitz, 1963), while Hurwitz et al., (1964) have reported that methyl cobamide and methyl tetrahydrofollate will not serve as methyl donors for the methylation of t-RNA.

In this note, we would like to report data showing that methyl cobamide will serve as a methyl donor for the methylation of t-RNA using enzyme preparations from rat liver and from Propionibacterium shermanii and t-RNA preparations from rat liver, Escherichia coli and P. shermanii.

MATERIALS AND METHODS

Methyl cobamide was synthesized from C^{14}-methyl iodide and vitamin B_{12} in the presence of ammonium chloride and zinc and magnesium powders by the method of Zagalak (1963).

Enzyme Preparation. The enzyme preparation was made from the supernatants from 105,000 x g centrifugates of rat liver homogenates,

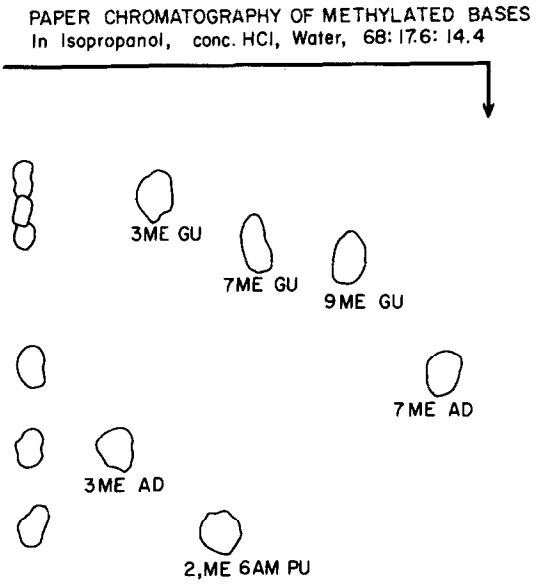
precipitating the t-RNA by streptomycin sulfate and bringing the resultant supernatant to 30% saturation with ammonium sulfate. The precipitate so obtained was discarded. The supernatant was brought to 50% saturation with ammonium sulfate and the precipitate obtained by centrifugation was further purified by dissolving in 1 x 10^{-3} M tris buffer pH 8.2 and dialyzing overnight against the same buffer to remove ammonium sulfate. The dialyzed solution was used as enzyme preparation.

Assay System. The incubation mixture consisted of 50 µmoles tris buffer pH 8.2, 10 µmoles reduced glutathione, 10 µmoles $MgCl_2$, 10 µmoles ATP, 180 µg of enzyme protein preparation, 4 mg of t-RNA, 30.3 mµmoles of methyl cobamide, the total brought to a volume of 2.5 ml. The system was incubated at 37° for 1 hour and the reaction stopped by the addition of 0.5 ml of 6 N HCl and 1 ml of 20% trichloroacetic acid. The mixture was then centrifuged, the supernate discarded, the precipitate washed twice with 5 ml of 5% cold trichloroacetic acid and twice with 5 ml of alcohol-ether mixture (3:1). The precipitate was dissolved in 2.5 ml, 0.1 M KOH and incubated for 18 hours at 37° C. 0.5 ml of this incubation mixture plus 15 ml of scintillation fluid were used to prepare solutions for counting in the Packard Tricarb Liquid Scintillation Counter.

RNA Sources. The t-RNA from rat liver and from P. shermanii was isolated by phenol extraction of the 105,000 x g supernatant according to the method of Kirby (1956). E. coli Strain B s-RNA was purchased from Calbiochem.

Separation of Methylated Bases. The nucleotides obtained by alkaline hydrolysis were separated by paper electrophoresis and the radioactive areas were eluted and hydrolyzed with hydrochloric acid to give the free bases. These were separated by paper chromatography, using isopropanol, hydrochloric acid and water in a ratio of 68:17.6:14.4. A typical diagram of separation of known methylated bases in this system is given in Figure I.

FIGURE I



The methylated bases that were isolated from the various radioactive spots on the paper chromatograms were examined with regard to their absorption spectra in the ultraviolet region at pH 1 and at pH 8 and the spectra were compared with and found to be identical to the spectra of the pure compounds, in those cases in which an identification of the unknown was made, (see Table III).

RESULTS

In Table I are given the data of a representative experiment on the methylation of t-RNA from rat liver using labeled methyl cobamide as methyl source and examining the effect of the addition of enzyme, of enzyme plus homocysteine, enzyme plus methionine and enzyme plus S-adenosylmethionine on the methylation.

TABLE I

Methylation of s-RNA by Methyl B_{12}

		cpm
MeB_{12}	No Enzyme	1,264
MeB_{12}	+ Enz.	2,002
MeB_{12}	+ Enz. + Homocyst.	2,336
MeB_{12}	+ Enz. + Methionine	2,259
MeB_{12}	+ Enz. + S-adenosyl-Methionine	2,034

In Table II are given data on the methylation of rat liver, E. coli and P. shermanii t-RNA, using enzyme from rat liver supernatant and enzyme from P. shermanii supernatant.

TABLE II

Specificity of Rat Liver Enzyme in Transferring Methyl From $C^{14}H_3B_{12}$

	cpm
MeB_{12} + s-RNA rat	1,204
MeB_{12} + Enz. rat + s-RNA rat	6,590
MeB_{12} + Enz. rat + s-RNA *E. coli*	6,400
MeB_{12} + —— + s-RNA *P. shermanii*	1,340
MeB_{12} + Enz. *P. shermanii* + s-RNA *P. shermanii*	3,144
MeB_{12} + Enz. *P. shermanii* + s-RNA rat	5,660

The data in Table III give the relative amounts of radioactivity incorporated into six different methylated bases of rat liver t-RNA. Other minor methylated compounds were found, but they have not been identified.

TABLE III

Pattern of Labeling of Methylated Bases

Rat liver s-RNA	Total cpm
3-Methyl guanine	166
7-Methyl guanine	84
9-Methyl guanine	340
7-Methyl adenine	362
3-Methyl adenine	838
dimethyl 6-aminopurine	1376

DISCUSSION

By examination of Tables I and II, it is readily apparent that there is a considerable amount of non-enzymatic methylation of t-RNA; however, it is also apparent that the addition of enzyme increases this by 2 to 5 times, thus, it does appear to be an important enzymatic reaction and it does seem quite clear that methyl cobamide will serve as a methyl donor for the enzymatic methylation of t-RNA. From the data in Table I, it is apparent that S-adenosylmethionine does not significantly dilute the amount of radioactivity, that is transferred from methyl cobamide to t-RNA, indicating either that there are two pathways of methylation, one involving direct methylation from S-adenosylmethionine and the other involving methylation from methyl cobamide or that S-adenosylmethionine serves as a methyl donor for t-RNA methylation indirectly by serving as a methyl donor for the methylation of vitamin B_{12}, which then transfers its methyl group to t-RNA. It would appear that, if there are two independent pathways of methylation of transfer RNA, one using S-adenosylmethionine as donor and the other methyl cobamide, the sites of methylation may be different and that there is no competition since no dilution was obtained. From the data given in Table II, it appears

that there is little specificity with regard to the crude enzyme preparations used. The greater methylation of rat liver t-RNA as compared to P. shermanii t-RNA may merely reflect a greater amount of prior methylation in the case of transfer RNA from P. shermanii. The data in Table III show that of the methylated bases characterized, dimethyl-6-aminopurine was most labeled followed by 3-methyl adenosine. Since it does appear that methyl cobamide is a good methyl donor for the methylation of t-RNA, it is perhaps permissible to postulate regarding the possibility that this might explain earlier data suggesting a possible role of vitamin B_{12} in protein synthesis (Wagle 1958), (Makowski J. 1960),(Zalta and Meyer 1965) and (Venkataraman and Sreenivasan 1964). The work of Wagle (1958), indicated that in vitamin B_{12} deficiency, t-RNA was less active in accepting amino acids than was t-RNA from normal animals. Recent work of Peterkofsky (1964) has shown that the various amino acid transfer RNA's obtained from methionine deficient E.coli mutant have lowered activities with regard to transport of most of the amino acids. Since the paper of Hurwitz et al., (1964), does not give the details regarding their experiments with methyl cobamide, it is not possible at the present time to discuss possible reasons for the differences between their data and those presented in this paper.

The isolation of methyl vitamin B_{12} from human liver has recently been reported by Stralberg (1965), and this gives further support to the suggestion that this is a naturally occurring methyl donor.

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REFERENCES

Fleissner, E., and Borek, E. Biochemistry 2, 1093 (1963).
Gold, M., Hurwitz, J. Fed. Proc, 22, 230 (1963).
Hurwitz, J., Gold, M., Anders, M. J. Biol. Chem. 239, 3474 (1964).
Kirby, K. S., Biochem. J. 64, 405 (1956).
Littlefield, T. W. and Dunn, D. B. Biochem. J. 70, 642 (1958).
Makowski J., Tryuka B. Acta Physiol Polon. II, 821 (1960).
Peterkowsky A. Proc. Natl. Acad. Sci. 52, 1233 (1964).
Stralberg, G. Scand. J. Haematol. 21, 80 (1965).
Venkataraman, S. and Sreenivasan, A. Bull. Natl. Inst. Sci. India 25, 11 (1964).
Zagalak, B. Acta Biochim. Polon. X, 387 (1963).
Zalta, J. P. and Meyer, F. Experientia 21, 519 (1965).