THE ENZYMATIC METHYLATION OF RNA AND DNA. I*

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The methionine origin of the methyl group of the "trace bases" of Escherichia coli and ascites cells has been demonstrated by Borek et al (1) and Biswas et al (2). Fleissner and Borek (3) have recently reported that extracts of E. coli catalyse the transfer of the methyl group from C^{14}-methyl-labeled methionine to E. coli soluble RNA (S-RNA) provided this S-RNA was isolated from a methionine auxotroph which continues to synthesize RNA when deprived of its essential amino acid. Although the DNA dependent RNA polymerase system incorporated ribothymidylate from ribothymidine triphosphate into RNA specifically in place of uridylate (4), no demonstrable phosphorylation of ribothymidylate was detected in extracts of E. coli. These observations, and especially the report of Fleissner and Borek (3), suggest that the trace nucleotide ribothymidylate, is synthesized at the polynucleotide level.

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We wish to report that extracts of E. coli contain at least five RNA-methylating enzymes and in addition, DNA-methylating activity. Evidence will also be presented which indicates that S-adenosyl-methionine is the active methylating agent.

Materials and Methods. The various natural RNA and DNA preparations used in these studies were prepared by phenol extraction (5) and by the method of Marmur (6), respectively.

Identification of the products formed after methylation of RNA and DNA was performed in the following ways: a) degradation to bases with formic or perchloric acid followed by paper chromatography in different solvents (7), b) degradation to either the 2'(3') or 5' mononucleotides with alkali, or RNase and spleen phosphodiesterase or DNase and venom phosphodiesterase followed by electrophoresis, and c) degradation to nucleosides with crude snake venom followed by electrophoresis (8).

Both RNA and DNA capable of accepting methyl groups were prepared from a methionine requiring strain of E. coli grown in a limiting concentration of methionine in synthetic medium for approximately 15 hours. In such cultures, bacterial growth, as measured by either optical density or uptake of C^{14}-L-methionine, ceases after about 7 hours at 37°; RNA synthesis continues for 2 hours after growth stops, while DNA is made continuously throughout the incubation period. The nucleic acids from such cultures (termed "starved") were used in all routine assays.

Results. Studies with Purified Fractions. On purification of the methylating activity of crude extracts of E. coli W, it became evident that there were at least five RNA-methylating enzymes and a distinct

DNA-methylating activity. The details of the purification and characterization of these enzymes will be published elsewhere. The

TABLE I

Methylation of RNA

	Reaction Mixture	C^{14}-CH_3 Groups Incorporated
		mμmoles
1.	Complete	.380
2.	" + RNase (0.1 μg)	.030
3.	" + RNase (0.5 μg)	<.002
4.	" + DNase (0.5 μg)	.400
5.	" but C^{14}-methionine + ATP in place of S-adenosyl-methionine	<.002
6.	Omit starved E. coli S-RNA	<.002
7.	" + E. coli S-RNA (normal; 352)	.004
8.	" + E. coli Ribosomal RNA (normal; 249)	.003
9.	" + E. coli Ribosomal RNA starved; 200)	<.002
10.	" + E. coli DNA (normal; 100)	<.002
11.	" + E. coli DNA (starved; 200)	<.002
12.	" + Thymus DNA (100)	<.002
13.	" + M. lysodeikticus S-RNA (100)	.256

The complete reaction mixture (0.25 ml) contained 150 mμmoles of ribonucleotides as starved E. coli RNA, 10 mμmoles C^{14}-methyl-S-adenosyl-methionine (1.7×10^7 cpm per μmole), 10 μmoles triethanolamine buffer pH 8.8, 1 μmole $MgCl_2$, 2 μmoles of 2-mercaptoethanol and 1.8 μg enzyme protein. Other nucleic acids were substituted for starved E. coli S-RNA in the amounts indicated in parentheses, and in reaction mixture 5, 10 mμmoles C^{14}-methyl-methionine (1.1×10^7 cpm per μmole) and 2 μmoles of ATP were substituted for S-adenosyl-methionine. After incubation at 37° for 15 min, the reactions were stopped by the addition of cold 5% trichloroacetic acid (TCA), filtered through Millipore discs, washed with cold 1% TCA, dried and counted in a windowless gas-flow counter.

enzymes were distinguished by the following criteria: 1) differential fractionation and physical separation during purification, 2) differences in conditions for maximum activity such as pH, metal requirements, affinity for substrates, etc., 3) the rates and extent of loading of starved E. coli S-RNA with methyl groups, 4) the pattern of different bases methylated and 5) the type of nucleic acid methylated.

Table I summarizes some of the properties of the RNA-methylating enzyme which yields thymine as the only methylated base. This activity, purified approximately 200 fold, is completely dependent on the addition of starved E. coli S-RNA or S-RNA isolated from normal Micrococcus lysodeikticus. No other nucleic acid tested satisfied this requirement, nor did such synthetic polymers as poly A, poly C, poly U, poly I, alternating poly AU, all of which did not accept detectable methyl groups even in the presence of crude extract. ATP and methionine did not substitute for S-adenosyl-methionine. The incorporation was sensitive to relatively low concentrations of RNase, but completely resistant to DNase.

The DNA methylating activity has not been as extensively purified as the RNA methylating enzymes, and is still contaminated with some RNA-methylating activity. The major contaminant appears to be the thymine-forming RNA-methylase. The properties of the DNA methylating system are summarized in Table II. This reaction is RNase resistant but DNase sensitive. Methylation occurs with starved E. coli DNA, but not with DNA isolated from the same auxotroph grown in the presence of excess methionine. However, this DNA methylating fraction transfers methyl groups to a number of normal DNA preparations, e.g. human bone marrow, Mycobacterium phlei,

TABLE II

Methylation of DNA

	Reaction Mixture	C^{14}-CH_3 Groups Incorporated
		mμmoles
1.	Complete	.064
2.	" + RNase (0.5 μg)	.060
3.	" + DNase (0.1 μg)	<.001
4.	" but with heated DNA	<.001
5.	Omit starved E. coli DNA	<.001
6.	" + E. coli DNA (normal; 100)	<.001
7.	" + E. coli S-RNA (normal; 100)	<.001
8.	" + E. coli S-RNA (starved; 88)	.070
9.	" + E. coli Ribosomal RNA (normal; 125)	<.001
10.	" + E. coli ribosomal RNA (starved; 100)	<.001
11.	" + Thymus DNA (100)	.032
12.	" + Wheat germ DNA (100)	<.001
13.	" + Wheat germ DNA polymerase product (80)	.012

The reaction mixture (0.25 ml) contained 10 μmoles Tris buffer pH 8.0, 1 μmole $MgCl_2$, 2 μmoles 2-mercaptoethanol, 10 mμmoles C^{14}-methyl-S-adenosyl methionine (1.7×10^7 cpm per μmole) and 5 μg of enzyme protein. The complete system contained 100 mμmoles of deoxynucleotides as starved E. coli DNA and in other cases the amounts as indicated in parentheses. The heated DNA was prepared by heating a solution of the DNA (4.5 μmoles of deoxynucleotides per ml) at 100° for 5 minutes and then rapidly cooling.

After 30 min at 37°, the reaction mixtures were treated as described in Table I.

M. lysodeikticus, and calf thymus. On the other hand, DNA preparations from phages T2, SP 2[1/], SP 8[1/], α[1/], and φX 174[2/] are not methylated, nor are the dAT copolymer and dGdC homopolymer. While wheat germ DNA itself is not methylated, the product formed during a two-fold replication of this DNA by the enzyme DNA polymerase, is methylated to a significant extent.

Starved E. coli DNA, heated at 100° and rapidly cooled is completely inactive as an acceptor of methyl groups, and the binding of actinomycin or proflavin to the starved DNA also markedly reduced methylation.

Degradation of Methylated Products. When products formed after methylation of RNA were degraded by the procedures outlined in Methods, it was found that each of the enzymes thus far purified leads to methylation of a different base. There appear to be two activities leading to guanine methylation which can be separated during purification. The enzyme described in Table I gives only thymine, while other enzymes yield products containing methylated adenine and cytosine derivatives. The further isolation and identification of the methylated bases is in progress.

The partially purified DNA-methylating activity produces 5-methylcytosine and 6-methylaminopurine. We have found that with starved E. coli DNA and calf thymus DNA, the relative amounts of these two methylated bases are reversed, there being more 5-methyl-

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cytosine produced in the former and more 6-methylaminopurine in the latter.

Discussion. The direct methylation of DNA and RNA is another example of changes in nucleic acids which occur at the polymeric level (10). In such changes, the base sequence and/or the general structure of the polymer is presumably responsible for the specificity of the reaction. The studies reported here indicate that not only is there specificity at the polynucleotide level, but also species specificity of the methylating enzymes.

At present it is difficult to determine whether the trace bases are involved in the adapter role of S-RNA. While recent reports (11) indicate that this may not be the case, the high degree of specificity of the methylation reaction is suggestive of an important role for these bases in the information encoded in S-RNA.

REFERENCES

Borek, E., Mandel, L.R., and Fleissner, E., Fed. Proc., 21, 379 (1962).

Biswas, B.B., Edmonds, M., and Abrams, R., Biochem. Biophys. Res. Comm., 6, 146 (1961).

Fleissner, E., and Borek, E., Proc. Nat. Acad. Sci. U.S., 48, 1199 (1962).

Kahan, F.M., and Hurwitz, J., J. Biol. Chem., 237, 3778 (1962).

von Ehrenstein, G., and Lipman, F., Proc. Nat. Acad. Sci. U.S., 47, 941 (1961).

Marmur, J., J. Mol. Biol., 3, 208 (1961).

Dunn, D.B., and Smith, J.D., Biochem. J., 68, 627 (1958).

Markham, R., and Smith, J.D., Biochem. J., 52, 552 (1952).

Hurwitz, J., Furth, J.J., Anders, M., and Evans, A., J. Biol. Chem., 237, 3752 (1962).

Kornberg, S.R., Zimmerman, S.B., and Kornberg, A., J. Biol. Chem., 236, 1487 (1961).

Starr, J.L., Biochem. Biophys. Res. Comm., 10, 181 (1963).