# THE SEQUENCES OF 5-METHYLCYTOSINE IN THE DNA OF ESCHERICHIA COLI

J. Doskočil and Z. Šormová
Institute of Organic Chemistry and Biochemistry
Czechoslovak Academy of Science
Praha, Czechoslovakia

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Recent work on the methylation of DNA by bacterial cell-free extracts or purified DNA-methylases revealed that the methylation is species- and strain-specific (Gold, Hurwitz and Anders, 1963; Gold and Hurwitz, 1964). The factors determining the specificity are not known. Since two methylated bases, i.e. 6-methylaminopurine and 5-methylcytosine have been detected so far in the DNA of bacteria, the strain specificity could be due in some instances to qualitative differences. Strains containing the same methylated bases in their DNA may still display differences in the selectivity of methylation. To decide which of these factors actually determines the specificity of methylation, DNAs from several strains of E. coli were analysed with respect to the relative contents of 6-methylaminopurine and 5-methylcytosine and to the occurence of the latter base in polypyrimidine isopliths. The results were compared with similar, though less complete, data concerning the group of strains related to Bacillus subtilis (Doskočil and Šormová, 1965).

DNA labeled with $^{14}C$-methyl groups was prepared by growing the bacteria on glucose-mineral salts medium (Spizizen, 1958) containing $CH_3$-$^{14}C$-methionine (Radiochemical Centre, Amersham, England, specific activity 10 μc/μM). The relative amounts of methylated bases were determined after hydrolysis of DNA with formic acid at 170°C and chromatography of the hydrolysate in n-butanol-ammonia (Markham and Smith, 1952). The pyrimidine isopliths were obtained by partial hydrolysis of DNA with diphenylamine and formic acid (Burton and Petersen 1960). The isopliths were separated by chromatography on DEAE-cellulose column, using a linear concentration gradient of ammonium formate buffer, pH 5.5, with 0.08M and 0.4M as limiting concentration. Ammonium formate was removed by sublimation in vacuo. The radioactivity of the fractions was measured on aluminium planchettes on a Frieseke-Hoepfner gas flow counter.

The levels of radioactivity found in the hydrolysates of DNAs from various strains of E. coli are given in Tab.I. No distinct radioactive zones besides those of 5-methylcytosine and 6-methylaminopurine were present. The data confirm the earlier finding of the presence of both 6-methylaminopurine and 5-methylcytosine in the DNA of most strains except E. coli B, where only 6-methylaminopurine occurs. There is less 5-methylcytosine than 6-methylaminopurine in the DNA of all strains. The strains ATCC 9663 and $K_{12}$ show lower content of 5-methylcytosine with respect to 6-methylaminopurine; in other strains the relative amount of 5-methylcytosine is higher and the values are very similar. No strain of E. coli has been found containing only 5-methylcytosine but not 6-methylaminopurine.

TABLE I

Occurence of 6-methylaminopurine and 5-methylcytosine in the DNA of different strains of Escherichia coli

| Bacterial strain | Counts/min | | Ratio |
|---|---|---|---|
| | 6-methyl-aminopurine | 5-methyl-cytosine | 5-methylcytosine :6-methylamino-purine |
| B (Hershey) | 635 | 38 | 0.06 |
| B (Delbrück)-streptomycine-resistant | 235 | 5 | 0.02 |
| C (Sinsheimer) | 680 | 443 | 0.65 |
| $K_{12}$ lambda-sensitive | 472 | 275 | 0.58 |
| ATCC 9663 (methionine auxotroph) | 920 | 530 | 0.58 |
| $K_{12}$ $T^-$ (thymine auxotroph) | 322 | 233 | 0.72 |

About 300 g of the DNA labeled with $^{14}C$ in the methyl groups of methylated bases was hydrolysed with formic acid and the hydrolysate was chromatographed in n-butanol-ammonia.

Further experiments were designed to show whether differences in sequential arrangement of 5-methylcytosine could be detected in strains containing this base. The occurence of 5-methylcytosine in different classes of polypyrimidine isopliths is shown on Fig. 1. It is evident that the distribution pattern of 5-methylcytosine is nearly the same in all strains. Very little 5-methylcyto-

sine is found in the monopyrimidine fraction. In the dipyrimidine class more 5-methylcytosine occurs, but most of it is found in the tripyrimidine class. In higher isopliths the contents of 5-methylcytosine per cytosine content of these fractions decrease.

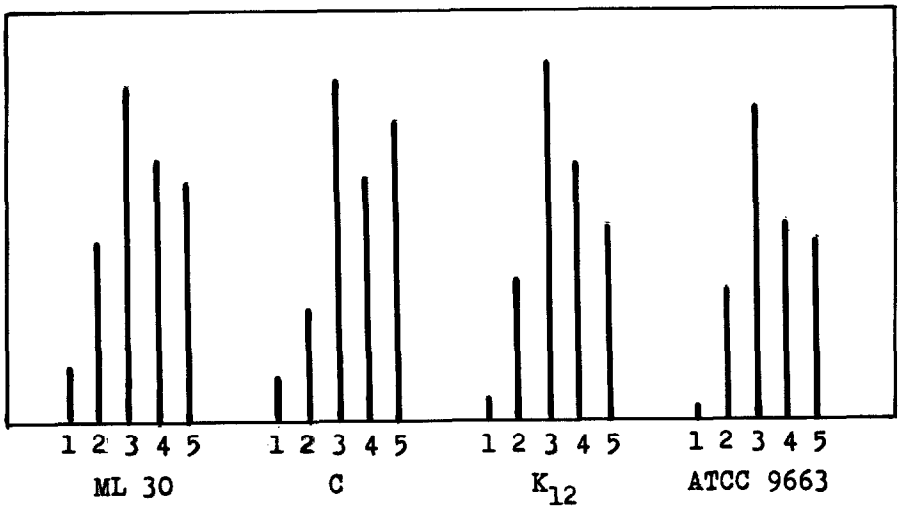


Fig. 1. Distribution of 5-methylcytosine in mono- to pentapyrimidine isopliths from the DNA of different strains of E. coli. Abscissa: Class of isopliths; ordinata: counts/min/cytosine content of the fraction. Negligible radioactivity was found in hexapyrimidines and higher isopliths.

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The present experiments show that more than one methylated base may occur in the DNA of a particular bacterial strain; the simultaneous occurence of 6-methylaminopurine and 5-methylcytosine is a common property of many strains, whereas E. coli B containing only 6-methylaminopurine in its DNA is rather exceptional. Comparing this situation with that encountered previously in the group of B. subtilis we find that the strains B. subtilis 168 (wild type) and B. niger also contain both methylated

bases in their DNA, but 5-methylcytosine only is found in the DNA from B. subtilis v. aterrimus (Doskočil and Šormová, 1965).

The sequential specificity of the deoxycytidylate methylase seems to be complex. For the methylation to occur the vicinity of a pyrimidine nucleotide is required. But this condition being fulfilled the methylation is further enhanced if the sequence is a part of a tripyrimidine sequence. Evidently more than one neighbour nucleotide is involved in the determination of methyl acceptor activity of deoxycytidylic acid. The location of 5-methylcytosine in bacterial DNA is entirely different from that found in either mammalian or plant DNA, where solitary 5-methylcytosine prevails (Sinsheimer, 1955; Doskočil and Šorm, 1960; Shapiro and Chargaff, 1960; Vanyushin et al., 1962).

The striking similarity in the distribution of methylated bases among various strains of E. coli strongly suggests that the DNA-deoxycytidylate methylases of these strains have the same sequential specificity. It should be noted, however, that a similar distribution, including the nearly absence of 5-methylcytosine in monopyrimidine and the prevalence in the tripyrimidine isopliths has been found in an unrelated strain, namely B. subtilis v. aterrimus (Doskočil and Šormová, 1965b). The difference between this strain and E. coli ATCC 9663 became apparent when the di- and tripyrimidine isopliths were further fractionated by paper electrophoresis. 5-Methylcytosine was found to be linked with neighbour deoxycytidylic acid in E. coli, but in B. subtilis v.aterrimus the vicinity of thymidylic acid was most frequent. This type of analysis

has not yet been performed with the DNA of other strains of *E. coli* and it cannot be excluded that a more detailed analysis could reveal differences which were not detected in the present experiments.

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