A Deoxyribonuclease of Diplococcus pneumoniae Specific for Methylated DNA*

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SUMMARY

A deoxyribonuclease specific for methylated DNA was isolated from Diplococcus pneumoniae. The enzyme, an endonuclease, degrades DNA from Escherichia coli to fragments of average molecular weight about half a million; it forms discrete fragments from phage λ DNA. Methyl-deficient E. coli DNA is not attacked, neither is DNA from Micrococcus radiodurans, which contains no methylated adenine or cytosine. Nor is DNA from D. pneumoniae or phage T7 attacked. However, DNA from M. radiodurans, D. pneumoniae, and T7 is attacked after methylation with an E. coli extract. Methylated T7 DNA is degraded to discrete fragments. Although the genetic transforming activity of normal DNA from D. pneumoniae is not affected by the enzyme, transforming activity of methylated DNA is destroyed. The enzyme is designated endonuclease R-Dpn I.

Under certain conditions another enzyme of complementary specificity can be isolated. This enzyme, designated endonuclease R-Dpn II, produces a similar pattern of fragments from the DNA of T7 without prior methylation of the DNA. It also degrades normal DNA from D. pneumoniae. It is suggested that this pair of enzymes plays a role in some unknown control process, which would involve a large fraction of the specific base sequences that are methylated in E. coli DNA and are present but not methylated in DNA from other sources.

A variety of deoxyribonucleases that recognize specific base sequences and make a limited number of cuts in DNA have been isolated from bacteria (reviewed in 1–3). These enzymes have been called restriction endonucleases because by destroying introduced DNA they can restrict viral infection. For some of these enzymes, at least, methylation of susceptible DNA at its recognition site prevents the endonucleolytic split (4). We report a new enzyme which, in contrast, appears to split DNA only at sites that have been methylated.

The novel activity was observed in the course of an investigation of the major endonuclease of Diplococcus pneumoniae, an enzyme that gives acid-soluble products (5) and is implicated in genetic transformation (6, 7). Mutant strains apparently devoid of this major DNase still contained an endonuclease able to cleave DNA from Escherichia coli to large fragments that were not acid-soluble. DNA from other sources, such as coliphage T7 and D. pneumoniae, was not attacked; however, these DNAs were rendered susceptible upon methylation by an extract of E. coli.

Cleavage of susceptible DNA yields fragments of discrete size, so the enzyme must be site-specific. Because it is similar to a restriction endonuclease, we will use the nomenclature suggested for such enzymes (8) and call the DNase specific for methylated DNA: endonuclease R-Dpn I. There is no evidence, however, that the enzyme functions to restrict foreign DNA. On the contrary, its mode of action and the occurrence in D. pneumoniae of another endonuclease of complementary specificity suggests a role in the cell other than restriction.

EXPERIMENTAL PROCEDURES

Materials

Bacterial Strains—Strains of Diplococcus pneumoniae that were used are listed in Table I. All were derived by mutation or transformation of strain R6, obtained from R. Hotchkiss, Rockefeller University, except for 533, which was obtained from M. Fox, Massachusetts Institute of Technology. The mutation end-1 greatly reduces the major DNase activity of D. pneumoniae (9) and the residual activity is abolished by nzo-9 or nzo-19 (6). The major exonuclease activity (6) is reduced by cwo 8 and cwo 3 (9). Strain 592 was isolated by us as a mutant deficient in the ATP-dependent DNase (10) and this mutation was designated add-1. The sul-d marker (11) confers resistance to sulfathiazole. Other markers listed have been described, but they are not relevant to this study. Micrococcus radiodurans and the methionine-requiring strain of Escherichia coli, 58-161, came from the American Type Culture Collection. E. coli B was obtained from the late M. Demerec, formerly of this laboratory, E. coli C from F. W. Studier, of this laboratory, and E. coli K1200, an endonuclease I-deficient mutant isolated by Dürwald and Hoffman-Berling (12), from J. Dunn, of this laboratory.

DNA Preparations—Previously described procedures were used to grow cultures and prepare DNA from D. pneumoniae (13) and from E. coli (14). Methyl-deficient E. coli DNA was prepared by growing E. coli 58-161 in medium containing 20 μg L-methionine per ml. For growth of K1200 the E. coli medium was supplemented with a mixture of vitamins added to the pneumococcal medium (13). DNA was prepared from M. radiodurans according to Moseley and Setlow (15). DNA from Micrococcus lysodeikticus and Clostridium perfringens was obtained from the Sigma Chemical Co. and bovine thymus DNA from the Worthington Biochemical Corp. DNA from phages T7 (grown on E. coli B) and λ26 (grown on E. coli W3110) was prepared by phenol extractions (17) of phage kindly provided by F. W. Studier.

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and M. Simon. Phage T4 DNA was a gift of J. Dunn. Phage 434 DNA, T7 [\(^{32}\)P]DNA, and fragments of T7 DNA produced by endonuclease RHI were gifts of M. Simon, of this laboratory.

**Methylation of DNA**—A crude extract of DNA-methylating enzyme was prepared from *E. coli* K1200 according to Marinos and Morris (19). Reaction mixtures contained 1 to 4 mg of extract protein, 100 to 200 \( \mu \)g of DNA, 4 mmol of \( S\)-adenosyl-L-[methyl-\(^{14}\)C]methionine (17.5 \( nCi/nmol \)), 0.5 mmol of dithiorthreitol, 5 mmol of EDTA, 50 \( \mu \)mol of Tris-HCl, and 0.6 mmol of glyceral in a total volume of 0.8 ml at pH 8.0. Samples were incubated at 30° or 37° and chilled. After adding 50 \( \mu \)l of 2 M NaCl, DNA was precipitated with addition of 1.5 ml of ethanol. The centrifuged precipitate was dissolved in 1.0 ml of 0.1 M NaCl and 0.1 M EDTA and passing them through a Sephadex column. Cell debris was removed by centrifugation. 2 M EDTA was added, and 15 \( \mu l \) were placed in sample wells of a polyacrylamide slab gel. DNA in the mixtures was precipitated with 1.2 ml of ethanol and dissolved in 10 or 20 \( \mu l \) of 10 mm Tris-HCl, pH 7.5. One-half volume of bovine serum albumin in 0.2 ml were incubated at 37° for 2 hours. The equation of Studier (21) was used for the calculation of \( \eta_{sp} \), and assuming that distances sedimented were proportional to the square of the sedimentation coefficient, specific viscosities were calculated as

\[
\eta_{sp} = \left( \frac{t_{f} - t_{0}}{t_{0}} \right) / \eta_{0}
\]

where \( t_{f} = \) flow time. Solvent flow times were generally about 165 s and were not affected by addition of the enzyme extract. Reduced viscosities of the DNA preparations used were calculated by dividing the initial specific viscosity \( (\eta_{0}/\eta_{sp}) \) by the concentration of DNA in grams/ml.

**Results**

**Action of Extracts on Viscosity of Various DNAs**—Crude extracts of strains that contain the end-1 mutation, which results

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>533</td>
<td>RF645KNE</td>
<td>sul-a, sul-d, str, bry, non, ery</td>
</tr>
<tr>
<td>562</td>
<td>T6tritile4x4</td>
<td>end-1, exo-2, mal-596, trt-1, hex-4</td>
</tr>
<tr>
<td>592</td>
<td>Mcenedlex2sex1addl</td>
<td>end-1, exo-2, mal-518, hex-2, add-1</td>
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<tr>
<td>681</td>
<td>R6endlex42</td>
<td>end-1, exo-2, nov</td>
</tr>
<tr>
<td>606</td>
<td>T6tritile2ends</td>
<td>exo-2, mal-596, trt-1, hex-4</td>
</tr>
<tr>
<td>621</td>
<td>T6tritile2sex4two9not6</td>
<td>end-1, exo-2, nov, trt-1, hex-5, mal-569, trt-1, hex-4</td>
</tr>
<tr>
<td>641</td>
<td>R6endlex3noiz19</td>
<td>end-1, exo-5, noiz-19</td>
</tr>
<tr>
<td>649</td>
<td>R6endlex2SFNZNKA</td>
<td>end-1, exo-2, sul-d, str, nov, azu, ery, ami</td>
</tr>
</tbody>
</table>

**Assay of DNA Transforming Activity**—Transforming DNA was prepared from strains 533 and 645 (Table I). These DNAs or their methylated derivatives were treated with varying amounts of the methylated DNA-specific endonuclease in 0.4 ml of a sterile solution containing 20 \( \mu \)mol of Tris-HCl, pH 7.6, 16 \( \mu \)mol of NaCl, 2 \( \mu \)mol of MgCl\(_2\), 1.2 \( \mu \)mol of 2-mercaptoethanol, 160 \( \mu \)g of bovine serum albumin, and 0.05 \( \mu \)g of DNA. The reaction mixtures were held at 37° for 2 hours. Recipient cultures of strain 606 were prepared by thawing a culture which had been grown to \( A_{590} = 0.15 \) and frozen with 10% glycerol, diluting it 250-fold into fresh semi-synthetic medium (13) to give approximately 3 \( \times 10^5 \) colony forming units/ml, and incubating it at 30° for 30 min. To each reaction tube were then added 1.6 ml of recipient culture. The tubes were incubated for 20 min at 30°, 2 \( \mu \)g of pancreatic deoxyribonuclease 1 (Worthington) were added to terminate DNA uptake, and the tubes were held for 30 min at 37°. The number of transformants was determined by plating samples in agar medium containing molenicamide at 100 \( \mu \)g/ml. One unit of methylated DNA-specific endonuclease activity is defined as the amount that reduces the transforming activity of a standard methylated pneumococcal DNA preparation (A or C in Table II) to 25%.
in a deficiency of the major pneumococcal DNase (9), are still able to degrade DNA from *Escherichia coli*, as determined by reduction in viscosity (Fig. 1 and b). Although end-I mutants contain residual activity corresponding to the major DNase (6), that is, an RNA-inhibitable endonuclease that produces short oligonucleotides (5, 9), this residual activity is not manifest in crude extracts not previously subjected to RNase or high concentrations of salt. Furthermore, extracts of nos mutants, which lack the residual activity (6), can still degrade *E. coli* DNA (Fig. 1c). Exonucleases are present in the extracts (6), but they yield far too little acid-soluble product under these conditions to account for the observed reduction in viscosity.

The action on *E. coli* DNA, therefore, suggests the presence of another endonuclease distinct from the major pneumococcal DNase or its mutant derivative. The new enzyme is more discriminating with respect to the source of its substrate. DNA from *Micrococcus radiodurans* is degraded, but DNAs from other sources, including *Clostridium perfringens*, coliphages T4 and T7, bovine thymus, and *Diplococcus pneumoniae*, are not attacked (Fig. 1).

**Size of Degradation Product—Sedimentation of treated DNA**

- **A**, *Clostridium perfringens*, 13.8; **X**, bovine thymus, 17.2; **A**, *Micrococcus lysodeikticus*, 22.0; **A**, *Clostridium perfringens*, 13.8; **X**, phage T4, 54.8; **A**, phage T7, 33.2.

**TABLE II**

**Methylation of DNA**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Preparation</th>
<th>Source of DNA</th>
<th>Hours of incubation</th>
<th>Extent of methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Methyl groups per 10^8 daltons of DNA</td>
</tr>
<tr>
<td>I</td>
<td>A</td>
<td><em>Diplococcus pneumoniae</em></td>
<td>3</td>
<td>1.98</td>
</tr>
<tr>
<td>II</td>
<td>B</td>
<td><em>D. pneumoniae</em></td>
<td>1</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td><em>D. pneumoniae</em></td>
<td>4</td>
<td>2.13</td>
</tr>
<tr>
<td>III</td>
<td>D</td>
<td><em>Micrococcus radiodurans</em></td>
<td>5</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>T7 phage</td>
<td>5</td>
<td>0.22</td>
</tr>
</tbody>
</table>

were not attacked by the enzyme, we were led to the hypothesis that the susceptibility of sites in *E. coli* DNA depended on their specific methylation rather than on the absence of such modification.

**Action on Normal and Methylated Transforming DNA**—An extract of *E. coli* K1200 was used to methylate DNA because this strain lacks the major DNase of *E. coli* (12). Table II shows the extent of methylation obtained in representative experiments with DNA from *D. pneumoniae*, *Micrococcus radiodurans*, and...
phage T7 grown on *E. coli*. Appreciable numbers of methyl groups, giving an extent of methylation comparable in magnitude to the frequency of susceptible sites in *E. coli* DNA, were introduced into pneumococcal and *M. radiodurans* DNA by the *E. coli* methylating extract. Less methylation was obtained with phage T7 DNA, but, as will be seen below, the number of methyl groups introduced is comparable to the number of susceptible sites formed.

Treatment of pneumococcal transforming DNA with the crude *E. coli* extract in the absence of *S*-adenosylmethionine, so that no methylation occurred, appeared to degrade the DNA somewhat because transforming activity was reduced approximately 30% (data not shown). Methylation of the DNA did not reduce the transforming activity below this level (Fig. 3, null enzyme preparations). The lack of effect of methylation on transforming activity was striking because the frequency of susceptible sites in *E. coli* DNA, were introduced into pneumococcal and *M. radiodurans* DNA by the *E. coli* methylating extract. Less methylation was obtained with phage T7 DNA, but, as will be seen below, the number of methyl groups introduced is comparable to the number of susceptible sites formed.

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TABLE III

Requirements of endonuclease specific for methylated DNA

An agarose column fraction containing the enzyme was dialyzed against 500 volumes of 10 mm Tris-HCl, pH 7.5. The complete reaction mixture contained, in 0.4 ml: 2 µl of the dialyzed fraction, corresponding to 0.5 µg of protein, 0.05 µg of methylated DNA (preparation C of Table II), 100 µg of bovine serum albumin, 0.05 mm Tris-HCl, pH 7.6, 0.04 mm NaCl, and MgCl₂ as indicated. After incubation for 2 hours at 37°, transforming activity of the treated DNA was assayed as described under "Methods." The number of <i>sal</i>-<i>d</i> transformants obtained per milliliter without enzyme treatment was 5100.

<table>
<thead>
<tr>
<th>Conditions of treatment</th>
<th>[Mg²⁺]</th>
<th>Relative transforming activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No enzyme</td>
<td>5.0</td>
<td>(1.00)</td>
</tr>
<tr>
<td>Complete</td>
<td>5.0</td>
<td>0.058</td>
</tr>
<tr>
<td>Complete</td>
<td>1.0</td>
<td>0.962</td>
</tr>
<tr>
<td>Complete</td>
<td>0.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Complete</td>
<td>0.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Complete + 0.5 mm EDTA</td>
<td>0.0</td>
<td>0.93</td>
</tr>
<tr>
<td>Complete + 4.0 mm 2-mercaptoethanol</td>
<td>5.0</td>
<td>0.049</td>
</tr>
<tr>
<td>Complete + 0.1 mm NaCl</td>
<td>5.0</td>
<td>0.25</td>
</tr>
<tr>
<td>Complete + 0.2 mm NaCl</td>
<td>5.0</td>
<td>1.04</td>
</tr>
</tbody>
</table>

negatively charged at pH 7.5 and has been fractionated on DEAE-cellulose columns.

Requirements for the action of the methylated DNA-specific enzyme are indicated in Table III. Magnesium ions are essential. Although 2-mercaptoethanol was routinely used in the buffers, it does not seem to be necessary. High concentrations of salt are inhibitory.

Analysis by Gel Electrophoresis of Products of Enzyme Action on Various DNA Preparations—Polyacrylamide gel electrophoresis of DNA treated with the enzyme was useful in examining the susceptibility of DNA of various origins in a semiquantitative manner. Fig. 5 shows the result of one such electrophoresis in which pairs of DNA samples, untreated and treated with enzyme, were compared. In this system only DNA smaller than 5 million daltons will migrate perceptibly further than the high molecular weight starting material. It is clear from Fig. 5, a, b, and d (and Fig. 6) that DNA from various strains of <i>E. coli</i>, including B, C, and K, are all equally susceptible. Methyl-deficient DNA from <i>E. coli</i> grown with limiting methionine, however, was not attacked (Fig. 5c). Normal DNA from <i>D. pneumoniae</i> was not degraded (c), nor was it attacked after treatment with an <i>E. coli</i> methylating extract in the absence of a methyl donor (f). Methylated DNA from <i>D. pneumoniae</i> was attacked (g and h) and the extent of degradation depended on the extent of methylation. <i>M. radiodurans</i> DNA, which is not normally methylated at all (24), was resistant to the enzyme (i) but was rendered susceptible by methylation (j). <i>M. lysodeikticus</i> DNA was degraded but not so extensively as <i>E. coli</i> DNA or methylated DNA from <i>D. pneumoniae</i> or <i>M. radiodurans</i> (k). A fraction of the <i>M. lysodeikticus</i> DNA may be refractory to the enzyme because it is unmethylated either as a consequence of the normal methylation pattern in this species or because the commercial preparation was partly made up from cells that had been starved for methionine. Thymus DNA was not degraded (l).

Careful examination of Fig. 5 reveals discrete bands in the broad smears of degraded DNA. (The bands were more distinct in the original photographic negatives.) These bands formed from bacterial DNA indicate that the endonuclease hydrolyzes at specific sites to give discrete fragments. Furthermore, the methylating enzymes from <i>E. coli</i> that render other DNAs susceptible must also methylate at specific sites. The discrete nature of the enzyme products can be seen more readily from the action of the enzyme on viral DNA and the analysis of products by electrophoresis in 1% agarose, which gives better resolution of bands of high molecular weight as shown in Fig. 6.

Untreated phage T7 DNA was placed in well c. When this DNA was treated with the methylated DNA-specific endonuclease <i>R.Dpn I</i>, no appreciable degradation was observed (Fig. 6a). Endonuclease <i>R.Dpn II</i>, however, did attack normal T7 DNA to give five heavy bands (b). Five minor bands were also produced. Fragments of T7 DNA produced by endonuclease <i>R.Hpa I</i> and calibrated with respect to molecular weight were run in a; molecular weights corresponding to the positions of intact T7 DNA and a number of the fragments are indicated at the left of Fig. 6. The major bands formed by the <i>Dpn II</i> enzyme correspond to a large fragment that migrates slightly faster than intact T7 DNA and fragments approximately 4.4, 2.4, 2.0, and 1.8 million in molecular weight. By difference, the large fragment would be 14.4 million. In that it cleaves DNA at specific sites and does not require specific methylation the <i>Dpn II</i> enzyme behaves like a typical restriction endonuclease. It attacks unmethylated DNA from <i>M. radiodurans</i> and normal pneumo-

1 M. Simon, personal communication.
T7 DNA treated with 1.0 pg of endonuclease R-Dpn I was a fraction from an agarose column. (See “Methods” for other details.) The entire amounts of DNA, 0.5 pg in a to d and 1.0 pg in e to h, and j to m, were subjected to electrophoresis at 30 volts for 16 hours (anodal direction downward in the figure). The DNA was stained with ethidium bromide, and its fluorescence on exposure to ultraviolet light was photographed. a, phage T7 DNA treated with endonuclease R-Hpa I; b, T7 DNA treated with endonuclease R-Dpn II; c, T7 DNA; d, T7 DNA treated with 1.0 μg of endonuclease R-Dpn I; e, methylated T7 DNA; f and g, methylated T7 DNA treated with 0.25 μg of endonuclease R-Dpn I; h, methylated T7 DNA treated with 1.0 μg of endonuclease R-Dpn I; i, phage λ DNA; j, λ DNA treated with 0.25 μg of endonuclease R-Dpn I; k, λ DNA treated with 1.0 μg of endonuclease R-Dpn I; l, E. coli K1200 DNA treated with 1.0 μg of endonuclease R-Dpn I; m, E. coli K1200 DNA.

ccocal DNA as well; methylated pneumococcal DNA is degraded to a lesser extent and E. coli DNA is not degraded at all (data not shown).

endonuclease R-Dpn I attacked only methylated T7 DNA (Fig. 6d to h). The methylated DNA sample (e) was already somewhat degraded, in a random fashion, by treatment with the methylating extract. Exposure of the methylated DNA to endonuclease R-Dpn I, however, gave rise to discrete bands (f to h). The greatest exposure (h) corresponded to that given unmethylated T7 DNA in d. In h, four heavy bands, two intermediate bands, and three light bands are visible. Curiously, all of these bands correspond in size to fragments produced by endonuclease R-Dpn II on unmethylated DNA. It is possible that a 14.4 million fragment is also formed, but that its presence is obscured by the random degradation of the methylated DNA starting material. The methylated DNA-specific enzyme produced discrete fragments from phage λ DNA without prior methylation (i to k). Phage 434 DNA is similarly attacked (data not shown). Degradation of E. coli DNA by the enzyme is illustrated in l and m.

**DISCUSSION**

The formation of discrete fragments from bacterial DNA (Fig. 5) and, more clearly, from viral DNA (Fig. 6) indicates that endonuclease R-Dpn I acts at specific sites presumably determined by specific base sequences. It appears that one or more bases at this site must be methylated. Table IV, which summarizes the data on specificity of action of the enzyme, supports this view. M. radiodurans DNA, which is devoid of N-6-methyladenine and 5-methylcytosine (24), is not susceptible, whereas E. coli DNA and phage λ DNA which contain these methylated bases (25, 26) are susceptible. Inasmuch as phage T7 infection does not alter E. coli methylating enzyme activity (27), it might be expected that the phage DNA is also methylated, but we have not been able to find data in the literature on the content of methylated bases in T7 DNA. Although not normally susceptible to the enzyme, T7 DNA is attacked, as are other normally unsusceptible DNAs, after *in vitro* methylation (Table IV). And the number of breaks produced in methylated T7 DNA (five), as deduced from the six distinct bands seen in Fig. 6h, is close to the number of methyl groups (5.5) introduced per phage genome (Table II).

E. coli K has both N-6-methyladenine and 5-methylcytosine in its DNA, but E. coli B has only N-6-methyladenine (28), yet DNA from either strain is equally susceptible. Although action at sites containing 5-methylcytosine cannot be ruled out, the enzyme must act at sites containing N-6-methyladenine. In DNA from E. coli B the mole frequency of this base is 0.5% (25). If the enzyme caused a break at every such base, the average molecular weight of the limit product could be as low as 130,000. Inasmuch as the observed limit product was about 500,000, at least one-quarter of the methylated adenine residues represent susceptible sites. Phage λ DNA contains approximately 100 N-6-methyladenine residues (26); it contains at least 25 susceptible sites, judging from the number of discrete fragments produced by the enzyme (Fig. 6h). Although a substantial proportion of N-6-methyladenine residues may correspond to cleavage sites, not all sequences containing this base are cleaved. This is certainly true for *D. pneumoniae* and bovine thymus DNA, which contain N-6-methyladenine at mole frequencies of 0.13% and 0.05%, respectively (25), yet are not attacked by the enzyme.

What function the enzyme serves in the cell is not known. It seems unlikely that its role is restriction of foreign DNA. *In vivo* it does not destroy methylated transforming DNA as it does *in vitro*. The immunity of transforming DNA might be due to its single-stranded structure after entry (29) or to its compartmentalization; and the intended victim of the restriction activity might be injected viral DNA. However, endonuclease R-Dpn I, unlike the endonuclease of restriction-modification systems, does not have the optimal specificity for such restriction activity. Of the five species of foreign DNA tested (Table IV), only two were degraded by the enzyme.

Another enzyme, endonuclease R-Dpn II, appears to have complementary specificity in that it attacks unmethylated DNA. The fact that it forms fragments from normal T7 DNA that are the same size as those formed by R-Dpn I from methylated T7 DNA suggests that the two enzymes act at the same site: enzyme I when the site is methylated; enzyme II when it is not methylated. Their complementary specificity suggests a possible role for the two enzymes in a cellular regulatory process. Although conditions for the isolation of enzyme II have not been defined, in the extracts from which it was isolated enzyme I was
not present. Inasmuch as the DNA sites can exist in two forms and the enzyme can also exist in two forms, the elements of a control system are present. But we have no inkling of the process under control. E. coli DNA contains a large number of such sites in methylated form. The reason for their methylation may reside in a similar control system in E. coli. The absence of such methylation in T7 DNA may result either from interference by the virus with this control system or from inaccessibility of the replicated viral DNA to the requisite methylating system.

It is conceivable that these endonucleases play a role in the genetic transformation of D. pneumoniae. Morrison and Guild (30) reported that transforming DNA undergoes double-stranded cuts at the surface of the pneumococcal cell prior to entry, as had been found in Bacillus subtilis transformation (31). Such cleavage could be carried out by these enzymes.

Whatever may be its role in the cell, we hope that the methylated DNA-specific endonuclease may be useful in the study of DNA, as have been the restriction endonucleases of opposite specificity.

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