Antigenicity of Methylated Deoxyribonucleic Acid

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IGARASHI, J. and OKUHARA, E. *Antigenicity of Methylated Deoxyribonucleic Acid*. Tohoku J. exp. Med., 1976, 120 (1), 25-30 — Methylated salmon sperm deoxyribonucleic acid (Me-ssDNA) prepared by using dimethyl sulfate was used to elicit antibodies in rabbits. The antisera obtained reacted specifically with Me-ssDNA but not with thermally denatured Me-DNA, native DNA, thermally denatured DNA or ribonucleic acids (RNAs). Antibody activity was demonstrable in both IgG and IgM fractions which were obtained by Sephadex G-200 column chromatography. A single precipitating line was formed by either of IgG and IgM fractions in the double diffusion test with Me-ssDNA. — anti-Me-DNA antibody; immunoglobulin

Our previous studies on the antigenic determinants of denatured DNA reactive with rabbit anti-DNA antisera suggested that some three-dimensional structural units within the denatured DNA molecule act as the antigenic determinants and that the minimum chain length required for an antigenic determinant of DNA is the pentanucleotide chain (Wakizaka and Okuhara 1975). In addition, the inhibition studies implied the importance of the base compositions or base sequences of the oligonucleotides (Wakizaka and Okuhara 1975). To investigate further this problem, we planned to use a chemically modified DNA as the antigen. If DNA is chemically modified, specifically modified bases as well as changes in the conformation of the DNA molecule caused by the modification procedures may alter the antigenicity of DNA. Accordingly, the modified DNA may become a useful tool for the study of DNA antigenicity. Methylated DNA was employed as antigen in the immunization procedures and the specificities of the antibodies for methylated DNA elicited in rabbits were investigated. The present paper is a report of the results of these studies.

MATERIALS AND METHODS

DNA preparation. The DNA was prepared in our laboratory by the usual method from salmon sperm (*Oncorhynchus keta*) collected from the Tama River, Akita. The detailed procedures for the preparation and analysis of DNA have been described in a previous paper (Okuhara et al. 1975).

Yeast rRNA was obtained from Sigma, St. Louis, and yeast tRNA, *E. coli* rRNA and tRNA were purchased from Miles, Elkhart, Ind.. *Candida utilis* tRNA was kindly

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supplied by Jujo Paper Manufacturing Co., Akita, and was further purified by Sephadex G-200 column chromatography. *Candida utilis* 5S RNA was isolated from tRNA by the method of Morell et al. (1967).

Methylation. Methylation of salmon sperm DNA was performed with dimethyl sulfate in the presence of tri-n-butylamine according to the method of Pochon and Michelson (1967), and with methylmethane sulfonate according to the method of Strauss and Robbins (1968). All procedures for the reaction, dialysis, precipitation and drying were conducted in accordance with the above methods.

For the determination of base composition, a sample of methylated DNA (1.0 mg) was hydrolyzed in a sealed tube with formic acid (0.5 ml of 88%) at 175°C for 30 min and analyzed by a modification of the method of Murakami et al. (1970) using cation-exchange chromatography.

C'-fixation. The quantitative C'-fixation measurements were made by a modification of Kolmer's micromethod as previously described (Matsumoto and Okuhara 1974). Double diffusion tests were carried out at room temperature in Petri dishes containing 0.9% agar in 0.05 M phosphate buffer (pH 7.0)-saline, containing 0.01% sodium azide.

RESULTS

Physicochemical properties of methylated DNA

The optical properties and the extent of methylation of the DNA guanine residues are shown in Tables 1 and 2.

There were no significant optical differences in the two methylated DNA preparations obtained by using dimethylsulfate and methylmethane sulfonate, respectively (Table 1). As seen in Table 2, the percent content of 7-methylguanine rose proportionally with the increase of dimethylsulfate added.

Production of anti-Me-DNA antibodies in rabbits and reactivities of various nucleic acids with antibodies

Rabbits were immunized with Me-DNA containing 20% 7-methylguanine using

TABLE 1. *Optical properties of methylated DNA*

	Me-DNA (I) (methylated by dimethyl sulfate)	Me-DNA (II) (methylated by methyl- methane sulfonate)	DNA (intact)
$\lambda_{\max}$ (nm)	260	260	260
$\lambda_{\min}$ (nm)	232	231	230
$\epsilon(P)$ 260 nm	6196	5035	6195
230 nm/260 nm	0.453	0.418	0.431
280 nm/260 nm	0.657	0.604	0.541

TABLE 2. *Contents of 7-methylguanine*

	Volume of dimethyl sulfate (I) used for methylation (ml)	7-Methylguanine (%)
Me-DNA (I) a	3.0	20.0
" b	0.3	7.0
" c	0.15	3.0
" d	0.03	2.0

poly (A)·poly(U) as adjuvant, according to the procedures previously described (Matsumoto and Okuhara 1974; Okuhara et al. 1975). The titers for 50% maximal C'-fixation were in the range of 256 to 32. The antiserum of rabbit No. 105 with a titer of 256 was used.

Only Me-DNA reacted intensely with the antiserum; thermally denatured Me-DNA, native DNA, thermally denatured DNA, poly(A)·poly(U), poly(A), poly(U), rRNA (*E. coli* and yeast), tRNA (*E. coli*, yeast and *Candida utilis*) and 5S RNA (*Candida utilis*) did not react at all. The amount of Me-DNA required for 50% C'-fixation with the 1:100 diluted antiserum was 10.2 ng.

The relationship between the extents of C'-fixation and methylation of DNA at 7-position of the guanine residues is shown in Fig. 1. The reactivities of Me-DNA preparations with the serum are shown in Fig. 2. Although the four Me-DNAs shown in Table 2 [(I) a, b, c, d] used as antigen differed in the extent of methylation, all of them reacted with the antiserum. On the other hand, the reactivity of Me-DNA methylated by methylmethane sulfonate with the antiserum was relatively low; no C'-fixation occurred even when 30 ng of this Me-DNA were used as antigen.

Fig. 1

Fig. 1. The reactivities of Me-DNA preparations methylated by dimethyl sulfate to various extents with the antiserum. The amount of each Me-DNA preparation used for C'-fixation was 20.0 ng.

Fig. 2

Fig. 2. The reactivities of Me-DNA preparations methylated respectively by dimethyl sulfate and methylmethane sulfonate with the antiserum. The physicochemical properties of Me-DNA (I) a and (II) are shown in Tables 1 and 2.

Immunoglobulin classes

The antisera were fractionated by Sephadex G-200 column chromatography (Killander and Hogman 1969; Matsumoto and Okuhara 1974) into IgG and IgM, and the antibody activity of each immunoglobulin class was examined by C'-fixation. As shown in Fig. 3, antibody activities in antisera No. 102 and No. 104 were demonstrable in both IgG and IgM fractions, whereas antisera No. 112 and No. 113 were exclusively IgG type. The pooled IgG and IgM fractions were

Fig. 3. Antibody activities in classes of immunoglobulins from the antisera.
—, protein amount measured by absorbance at 280 nm; ○- - -○, antibody activity.

Fig. 4. The reactivities between Me-DNA and IgG and IgM by double diffusion tests. The upper well contained 10 μ g of Me-DNA (I) a and the lower wells contained arbitrary amounts of the purified IgG and IgM fractions, respectively.

further purified by salting out with ammonium sulfate followed by Sephadex G-200 column chromatography. The purified IgG and IgM fractions for Me-DNA were examined by a double diffusion test, and precipitating antibody was demonstrated by the formation of a single precipitating line. The results were the same as those obtained by C'-fixation analysis.

The reactivities between Me-DNA and IgG and IgM were examined by double

diffusion tests and the precipitating antibodies were demonstrated by the formation of a single precipitating line, respectively (Fig. 4). The precipitation line for IgG joined with that for IgM.

DISCUSSION

The present experiments clearly indicated that antibody specific for methylated ssDNA was elicited in rabbits. The reason for the antigenic specificity manifested by methylated DNA is unclear at present and studies are in progress in our laboratory to obtain further insight into the relationship between immunogenicity and antigenic structure. However, it may be said that guanine residues methylated at N⁷ on the DNA molecule play a key role in the manifestation of immunogenicity and antigenic specificity. In the previous paper on the antigenic determinants of denatured DNA (Wakizaka and Okuhara 1975), it was stated that some structural units within the denatured DNA molecule act as the antigenic determinants. Moreover, detailed inhibition studies implied the importance of the base compositions or base sequences (Wakizaka and Okuhara 1975). With regard to the role of bases in antigenic determination, Levine and his co-workers (Murakami et al. 1962) reported that antibodies to T-even phage DNA were directed in part toward the glucosylated 5-hydroxymethylcytosine (HMC) of T-even phage DNA. The present report also indicates that methylated guanine residues on DNA can play a major role in antigenic determination. It has already been made clear that thermally denatured DNA is immunogenic whereas native DNA is not. Therefore, one may expect that antibody to thermally treated Me-DNA could be formed in similarity with the case of thermally denatured DNA. In the present study, however, the antibodies in rabbits were elicited by thermally untreated Me-DNA and not by thermally treated Me-DNA. It is interesting that thermally treated Me-DNA did not show any antigenicity. It should be noted in this connection that the methylated products is labile to heat and they can be degraded by heat treatment into small oligonucleotide fragments (Pochon and Michelson 1967). Probably these oligonucleotides may not be immunogenic. In addition, Pochon and Michelson stated that DNA could be methylated without loss of secondary structure. However, as Michelson's methylation is carried out at alkaline pH, it can be thought that the base pairings between the two strands of thoroughly methylated DNA molecule were destroyed to a great extent. Accordingly, we believe that the determinant groups, the methylated guanine residues, can be easily recognized by immune cell lines. Also, as shown in Fig. 1, all of four methylated DNAs reacted with the antiserum, although their reactivities differed according to the extents of methylation. This means that methylation of even a small portion of the DNA would give rise to a conformational change which is effective to expose determinant groups.

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