Further Evidence for the Involvement of Base and Base Sequence in the Antigenic Determination of Thermally Denatured DNA

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WAKIZAKA, A. and OKUHARA, E. *Further Evidence for the Involvement of Base and Base Sequence in the Antigenic Determination of Thermally Denatured DNA.* Tohoku J. exp. Med., 1977, 121 (2), 121-126 — The antigenic determinant of thermally denatured salmon sperm (ss) DNA was extensively investigated with a radioimmunoassay technique using the ssDNA immune system. It was demonstrated that mixed sequences of purines and pyrimidines play a key role in the antigenic determination of DNA. On the other hand, the importance of the bases in the manifestation of DNA antigenicity was demonstrated by using brominated, methylated and/or ultraviolet irradiated ssDNA preparations. — DNA; antigenic determination; radioimmunoassay

The inhibition studies described in the previous paper implied the importance of the base composition or base sequences of the oligonucleotides from DNase digests (Wakizaka and Okuhara 1975a). To elucidate the role of the bases in the manifestation of DNA antigenicity, we planned to use chemically modified and ultraviolet irradiated DNA as antigen. In this paper, we report the results of inhibition studies on the involvement of bases in antigenic determination using the salmon sperm DNA immune system.

MATERIALS AND METHODS

The preparation of [³H]-labeled-DNA, oligonucleotides and antisera to DNA and the measurement of the [³H]-DNA binding to antibody were performed by the methods described in the previous papers (Matsumoto and Okuhara 1974; Wakizaka and Okuhara 1975a).

Bromination of DNA. The bromination of DNA was carried out according to the method of Yu and Zamecnick (1963): One ml aliquot of thermally denatured ssDNA solution (2 mg/ml of saline), 0.25 ml of 0.4 M bicarbonate buffer (pH 9.0) and 0.75 ml of bromine water in a variety of concentrations were mixed. After 10 min at room temperature, the mixture was applied to a Sephadex G-100 column (1×30 cm) and eluted with 0.1 M borate buffer (pH 8.5). The decrease in absorption at 260 nm was taken as a measure of bromination. Oligonucleotides were also brominated in the same manner. Base composition was analyzed using cation exchange column chromatography (Wakizaka and Okuhara 1975a).

Methylation of DNA. Exhaustively methylated ssDNA (Me-DNA) was prepared with

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dimethyl sulfate according to the method of Pochon and Michelson (1967). The physico-chemical properties of the Me-DNA have been described in the previous paper (Igarashi and Okuhara 1976).

Ultraviolet irradiation. Thermally denatured ssDNA solution (1.0 mg/ml of saline) in Petri dishes was irradiated by a germicidal lamp (Toshiba GL 15 W) at a distance of 5 cm for various periods.

RESULTS

Inhibition by various oligonucleotides. First, the inhibitory activity of oligonucleotides from the DNase digests was confirmed by employing five anti-DNA rabbit sera. The haptenic binding inhibition by various oligonucleotides was carried out with these five antisera. As shown in Table 1, the oligonucleotides from DNase digests (D-oligo) which contained mixed purine and pyrimidine nucleotide sequences were more highly potent inhibitors than pyrimidine- (Py-oligo) or purine- (Pu-oligo) oligonucleotides.

TABLE 1. *Inhibition of [^3H]DNA binding to antibodies by oligonucleotides*

Antiserum No.	Inhibition (%)		
	D-oligo	Py-oligo	Pu-oligo
R72	47.2 $\pm$ 8.0	14.6 $\pm$ 3.3	14.4 $\pm$ 3.4
R73	38.3 $\pm$ 4.5	18.1 $\pm$ 4.2	0.6 $\pm$ 0.3
R74	62.9 $\pm$ 2.1	5.2 $\pm$ 0.6	14.5 $\pm$ 4.8
R116	56.4 $\pm$ 5.2	35.8 $\pm$ 1.2	31.1 $\pm$ 3.9
R117	51.7 $\pm$ 1.3	18.3 $\pm$ 2.9	22.8 $\pm$ 7.6

The assay medium contained 1.25 μg of thermally denatured mouse embryo [^3H]DNA (5,000 dis./min/ μg) and 50 μl of a 1:50 dilution of antisera in 0.2 ml of 0.1 M borate buffer (pH 8.5), and 2.5 A_{260} units of each oligonucleotide inhibitor was added to the assay medium. Results represent the mean $\pm$ S.E. of three experiments.

Inhibition studies by the use of Br-DNA. Fig. 1 shows the results of base analyses of DNA preparations brominated at various concentrations in the range of 0 to 10 mM of bromine. When the concentration of bromine in the reaction mixture was increased, the ultraviolet absorption of DNA gradually decreased. In the recovery of the bases from DNA brominated at 10 mM of bromine, that of adenine increased remarkably, whereas thymine, cytosine and guanine contents decreased compared with those from unbrominated DNA. Fig. 2 shows that the inhibitory activity of brominated DNA on [^3H]-DNA binding to antibody decreased markedly with the increase of added bromine, and at the concentration of 2.5 mM of bromine the inhibitory activity was completely lost. Similarly, oligonucleotides from DNase digests were brominated and the inhibition activity was examined and the results are shown in Table 2.

Inhibition by Me-DNA. Fig. 3 compares the inhibition activity of Me-DNA with that of non-Me-DNA. In the control assay, 3.0 μg of denatured DNA was required for 50% inhibition, whereas ten times more Me-DNA than non-Me-DNA

Fig. 1. Base composition of DNA brominated at various concentrations of bromine. (▲), adenine; (○), thymine; (△), cytosine; (●), guanine.

Fig. 2. Inhibition of [^3H]-DNA binding to antibody by Br-DNA. Ten μg of each Br-DNA were added to the assay medium.

TABLE 2. Inhibition of [^3H]-DNA binding to antibody by brominated oligonucleotides

Deoxynucleotide	Bromination	Inhibition (%)
Pentanucleotide	No	44.7 $\pm$ 7.2
Pentanucleotide	Yes	18.8 $\pm$ 3.1*
Hexanucleotide	No	52.5 $\pm$ 4.5
Hexanucleotide	Yes	30.3 $\pm$ 2.6†

* † Statistically significant difference between control and brominated experiments: * $p < 0.05$; † $p < 0.01$.

Penta- and hexadeoxynucleotide (20.0 A_{260} units/ml respectively) from DNase digests of ssDNA were brominated with 0.3 mM bromine in 50 mM bicarbonate buffer (pH 9.0). Two A_{260} units of the respective oligonucleotide were added to the assay medium. The results represent the mean $\pm$ s.e. of three experiments.

Fig. 3

Fig. 4

Fig. 3. Inhibition of [^3H]-DNA binding to antibody by exhaustively methylated DNA. After thermal denaturation, the Me-DNA was added to the assay medium. (o), inhibition by thermally denatured Me-DNA; (●), by denatured non-Me-DNA.

Fig. 4. Inhibition of [^3H]-DNA binding to antibody by ultraviolet irradiated DNA. Ten μg of irradiated DNA were added to the assay medium.

was required to obtain the same degree of inhibition.

Inhibition by ultraviolet-irradiated DNA. Thermally denatured ssDNA (1.0 mg/ml of saline) was irradiated with ultraviolet and aliquots withdrawn at various times were added to the assay medium for the inhibition tests. As shown in Fig. 4, no significant inhibitory activity was measurable although the DNA irradiated for 30 min showed a slight increase of inhibition.

DISCUSSION

As can be seen from the results obtained in the inhibition study, oligonucleotides with mixed purine and pyrimidine sequence showed the highest inhibition activity with the five anti-DNA sera used. The antisera examined were not very susceptible to the inhibition by pyrimidine oligonucleotides, while potent inhibition by pyrimidine nucleotide clusters was observed in human systemic lupus erythematoses sera by Stollar and Levine (Stollar 1968; Levine and Stollar 1968) and by us (Wakizaka and Okuhara 1975b).

According to the proposed mechanism of bromination (Yu and Zamecnik 1963; Tung et al. 1974) and methylation (Pochon and Michelson 1967), it is known that the bases are modified in both cases without any destruction of the secondary structure. In the bromination of uridylic acid, the bromine is added to C-5 to form 5-bromo-6-hydroxy-5,6-dihydrouridylic acid. The bromination of other pyrimidine nucleotides appears to proceed in a similar manner. The purine bases are also brominated resulting in the formation of 8-bromoderivatives. Actually we observed that all of the four bases of DNA are brominated to a different extent.

On the other hand, methylation occurred almost exclusively at the 7-position of the guanine residue on the DNA molecule. In contrast, ultraviolet irradiation of DNA forms several types of photoproducts (Wacker 1963; Setlow 1968), such as the dimers between adjacent pyrimidine residues which result from the creation of a cyclobutane ring joining two sets of 5,6 double bonds. Practically, such dimers are not formed within the DNA molecule as the antigenic structure is highly affected.

Clearly, the modes of modification are different among these three methods. However, it may be thought that bromination has an effect similar to that of methylation on the structure of the antigen. The extent of inhibitory activity of the modified DNA may be related to the numerical increase in modified bases within the DNA molecule.

Thus the present results provide strong evidence for the involvement of both base and base sequence in the antigenic determination of DNA. Incidentally, it can be said that the chemical modification extensively changes the antigenic structure. The production of antibodies specific for methylated DNA seems to support this (Igarashi and Okuhara 1976).

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