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Antigen structural requirements for recognition by a cyclobutane thymine dimer-specific monoclonal antibody

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ABSTRACT

A monoclonal antibody (TDM-2) specific to a UV-induced cyclobutane pyrimidine dimer (T[*cis-syn*]T) has previously been established; however, the immunization had used UV-irradiated calf-thymus DNA containing a heterogeneous mixture of photoproduct sites. We investigated here the structural requirements of antigen recognition by the antibody using chemically synthesized antigen analogs. TDM-2 bound with *cis-syn*, but not *trans-syn* thymine dimer, and could bind strongly with four nucleotide analogs in which the *cis-syn* pyrimidine dimer was located in the center. Antigen analogs containing abasic linkers at the 5′- or 3′-side of the *cis-syn* cyclobutane pyrimidine dimer were synthesized and tested for binding to TDM-2. The results indicated that TDM-2 recognizes not only the cyclobutane ring but also both the 5′- and 3′-side nucleosides of the cyclobutane dimer. Furthermore, it was proved that either the 5′- or 3′-side phosphate group at a cyclobutane dimer site was absolutely required for the affinity to TDM-2. The antibody showed a strong binding to single stranded DNA but indicated little binding to double stranded DNA.

INTRODUCTION

Ultraviolet light (UV) induces mutations in DNA, which are detected predominantly in dipyrimidine sites as cyclobutane pyrimidine dimers, pyrimidine [6-4] pyrimidone and Dewar photoproducts. These lesions cause mutations during the replication process (1). There are *cis-syn* [*c*,*s*] and *trans-syn* [*t*,*s*] cyclobutane pyrimidine dimer isomers (Fig. 1). These are photoproducts from the photo [2+2] cycloaddition of the 5,6-double bond of two adjacent pyrimidine nucleotides. The *cis-syn* pyrimidine dimers have anti glycosyl conformations, and in contrast, the 5′-side thymidine of the *trans-syn* pyrimidine dimers is in the *syn* glycosyl conformation. Usually, the *cis-syn* pyrimidine dimers are the major photoproducts of UV-irradiation, although *trans-syn* type pyrimidine dimers are generated in single stranded DNA (ssDNA). These photoproducts induce mutations in complementary strands during replication. *Cis-syn* pyrimidine photoproducts (T[*c*,*s*]C) have the ability to cause C→T transitions derived from replicative bypass (2). We also reported that in *in vivo* experiments, *cis-syn* type cyclobutane thymine dimers (T[*c*,*s*]T) induced T→A transversions on the 3′-side thymine of the dimer and *trans-syn* type (T[*t*,*s*]T) T→A transversions on the 5′-side thymine of the dimer (3).

There are enzymes to repair thymine dimers (4) and [6-4] photoproducts (5). Antibodies specific for the cyclobutane or [6-4] photo products have been constructed to investigate the relationships between the generation of these UV-induced photo products and their biological effects (6–8). Baan and colleagues obtained a monoclonal antibody that binds DNA containing a cyclobutane thymine dimer by immunization of mice with the tetranucleotide, GpT[*c*,*s*]TpG (6). The antibody bound thymine dimer-containing tetranucleotides, but not those with a photo-dimer only d(T[*c*,*s*]T). Antibodies binding to [6-4] photoproducts were established using dinucleotides of [6-4] photoproducts as the hapten (9). Antibodies that catalyze the photo-induced reverse reaction of a uracil cyclobutane dimer to normal dipyrimidine have also been reported (10).

Nikaido and colleagues have established a variety of monoclonal antibodies that bind with either cyclobutane, [6-4] or Dewar pyrimidine dimers (7,8,11). These monoclonal antibodies were obtained by immunization with UV-irradiated oligodeoxy-nucleotides and were characterized using DNA irradiated under controlled UV-irradiation conditions. These monoclonal anti-bodies have proved to be powerful tools for investigating cellular repair systems (12).

These antibodies were elicited by UV-irradiated DNA, which is thought to contain heterogeneous antigens. The regions of the photoproducts recognized by these antibodies have not been well characterized. We now report here the recognition size of the antigen for the monoclonal antibody (TDM-2), determined by measuring the binding affinities of the antibody by competitive ELISA, using antigen analogs in which a chemically synthesized cyclobutane dimer was specifically introduced. This information is important not only for understanding the antibody recognition of the photoproducts, but also for the elicitation of antibodies using photoproducts.

MATERIALS AND METHODS

Oligonucleotides

All oligodeoxynucleotides were synthesized using phosphor-amidite units purchased from Perkin-Elmer Applied Biosystems

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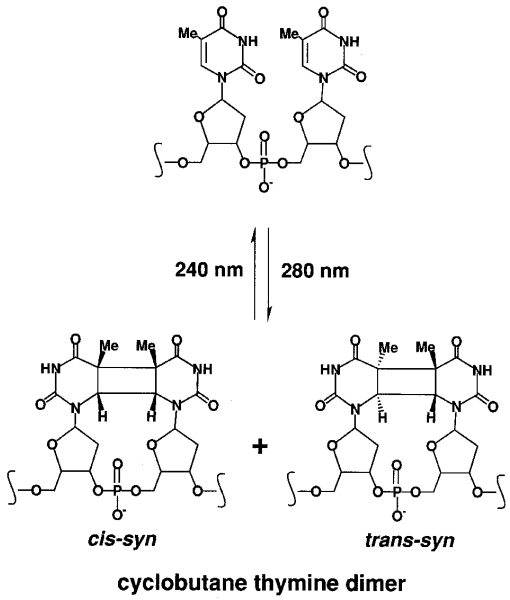


Figure 1. Structures of cyclobutane thymine dimers (*cis-syn* and *trans-syn* types). The cyclobutane thymine dimer is converted to the thymine bases with 240 nm irradiation.

Inc. *Cis* and *trans* cyclobutane thymine dimers were inserted in oligodeoxynucleotides by coupling the cyclobutane dimer amidite blocks as reported (13,14). Oligodeoxynucleotides containing the biotin or propyl phosphate group at the 3′-ends were synthesized using Biotin TEG CPG (GLEN research) or 3′-spacer C3 CPG (GLEN research) as the 3′-support. For the propyl phosphate group at the 5′-terminus, a propandiol phosphoroamidite block was synthesized as reported (15). pro·pT[*c*,*s*]T and T[*c*,*s*]Tp·pro were prepared from UV-irradiation of prop·TpT and TpTp·pro. All oligodeoxynucleotides were purified by reverse phase HPLC using columns of Inertsil ODS-3, 8 mm i.d. × 300 mm (GL Sciences) or μ-Bondasphere 4.6 mm i.d. × 250 mm (Waters). If necessary, anion-exchange HPLC was carried out using columns of TSK gel DEAE-2SW, 4.6 i.d. × 250 mm (Tosoh).

Sequences of oligonucleotides

cs23, 5′d(ATCGCGACGAAT[*c*,*s*]TAAGCAGCGCT)3′; ts23, 5′d(ATCGCGACGAAT[*t*,*s*]TAAGCAGCGCT)3′; competitors consisting of four and two bases, 5′d(AT[*c*,*s*]TA)3′, 5′d(GT-[*c*,*s*]TG)3′, 5′d(CT[*c*,*s*]TC)3′, 5′d(TT[*c*,*s*]TT)3′, 5′d(T[*c*,*s*]T)3′. AA23, 5′d(AGCGCTGCTTAATTCGTCGCGAT)3′; mis2, 5′d(A-GCGCTGCTTGGTTCGTCGCGAT)3′; mis4, 5′d(AGCGCTGC-TAGGATCGTCGCGAT)3′; mis6, 5′d(AGCGCTGCAAGGAA-CGTCGCGAT)3′. AA23, mis2, mis4 and mis6 form duplexes with cs23; however, underlined sequences are not matched with cs23.

Enzyme-linked immunosorbent assay (ELISA) with a protamine coated plate

The ELISA was performed according to a standard procedure. A flat-bottom 96 micro well plate (Nunc) was precoated by incubation with 1% protamine sulfate (50 μl/well) at 37°C for 2 h (11). After the wells were washed with sterile water and dried, 50 μl (10 ng/μl) oligodeoxynucleotide solution (cs23, ts23 or dsDNAs) were added to the wells, and the plate was incubated at 37°C for 20 h. The plate was dried and washed with PBS containing 0.05% Tween20 (Nakarai; 100 μl/well ×5) (PBS-T). PBS containing 2% skim milk (Yukijirushi Nyugyo) was added to the plate (200 μl/well), and the plate was incubated at 37°C for 1 h to prevent non-specific binding of the antibody. The plate was washed with PBS-T (200 μl/well ×5) and the monoclonal antibody, which was diluted in PBS containing 2% skim-milk, was added to the wells (100 μl/well). The plate was incubated at 37°C for 1 h. The plate was washed with PBS-T (150 μl ×5) and then was incubated with goat anti-mouse immunoglobulin G (IgG; H+L) conjugated with peroxidase (100 μl/well) at 37°C for 1 h. After the plate was washed with PBS-T (150 μl ×3) and citrate-phosphate buffer, pH 5.0 (150 μl ×2), the substrate solution (0.04% *o*-phenylene diamine, 0.007% H_2O_2 in citrate-phosphate buffer) was added to the plate (100 μl/well), and the plate was incubated at room temperature for 15 min. To stop the reaction, 2 M sulfuric acid was added (50 μl/well), and the absorbance at 492 nm was measured using an Immuno Reader Model 2550 EIA (BioRad).

ELISA with an avidin coated plate

The 96 microwell plates were prepared as described previously (9,16). Avidin (Organon Teknika Co.) dissolved in PBS was dispensed into a 96 microwell plate (5 μg/50 μl per well), which was covered with parafilm and incubated at 4°C overnight. After the plate was washed with PBS (200 μl/well ×3), 3′-biotinylated cs23 dissolved in PBS (20 nM) was added to the plate (50 μl/well), which was covered and incubated at 4°C for 2 h. The plate was washed with PBS-T (100 μl/well ×5). The antibody binding was measured as described above.

Competitive ELISA using the avidin coated plates

TDM-2 was dissolved in PBS (4 ml), at the concentration at which TDM-2 bound to 3′-biotinylated cs23 to indicate ~0.5 A_{492} unit, and competitors were dissolved in PBS (180 μl). An equal volume (180 μl) of the antibody solution was added to the competitor solution (180 μl) and mixed gently. Aliquots of 100 μl were added to wells of immobilized cs23-Bio. The binding of TDM-2 was measured using the same procedure as the ELISA with the protamine coated plate. Percent inhibitions were calculated as follows (7). Percent inhibition = $A - A_1/A° - A_1 \times 100$, where A° is the absorbance at 492 nm without competitors, A is the absorbance with competitors, and A_1 is the background absorbance without antibody and competitors.

Binding of TDM-2 to duplexes with mispairs using gel mobility shift assay

Gel mobility shift assays were carried out as previously reported (17). The 5′-end labeled cs23 (0.1 μM) and complementary strand (AA23, mis2, mis4 or mis6; 0.2 μM) were dissolved in a PBS buffer solution (1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, 140 mM NaCl, 25 μl), denatured at 90°C for 2 min, and then immediately transferred to an ice bath. The solution containing dsDNAs was heated at 80°C for 2 min and was annealed by cooling to room temperature. The solution (10 μl) was removed and mixed with TDM-2 and calf thymus DNA. The PBS solution contained 50 nM DNA duplexes, 0.05 μg calf thymus DNA and 0.51 μM TDM-2. After the solution was incubated at 37°C for

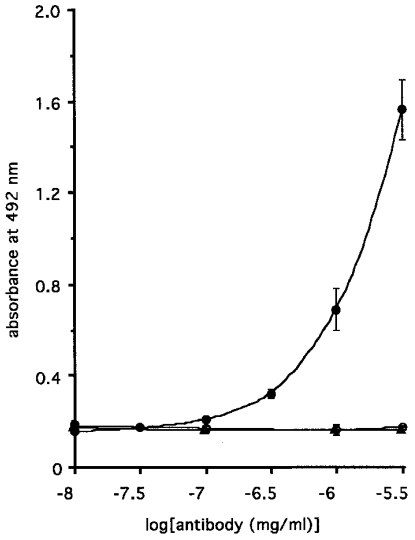
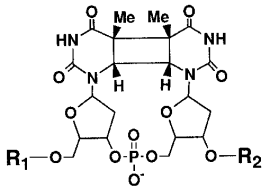


Figure 2. Analysis of binding of TDM-2 to cs23 (solid circles), ts23 (solid triangles) and non-UV irradiated 23 mer (open circles).



	R_1	R_2
ApT[*c,s*]TpA	A(3')P(O)(O⁻)–	–P(O)(O⁻)(5')A
pro·pT[*c,s*]Tp·pro	$CH_3(CH_2)_2OP(O)(O^-)$–	$CH_3(CH_2)_2OP(O)(O^-)$–
ApT[*c,s*]Tp·pro	A(3')P(O)(O⁻)–	$CH_3(CH_2)_2OP(O)(O^-)$–
pro·pT[*c,s*]TpA	$CH_3(CH_2)_2OP(O)(O^-)$–	–P(O)(O⁻)(5')A
pro·pT[*c,s*]T	$CH_3(CH_2)_2OP(O)(O^-)$–	H
T[*c,s*]Tp·pro	H	$CH_3(CH_2)_2OP(O)(O^-)$–
T[*c,s*]T	H	H

Figure 3. Structures of competitors. Adenosine or propandiol phosphates are covalently attached to R1 or R2.

1 h, 30% glycerol (5 µl) was added to the aliquot, and 8% native polyacrylamide gel (acrylamide:bisacrylamide, 29:1) electrophoresis was carried out at 24°C. The gel was dried and the percentages of complex formation were measured by a bio-imaging analyzer (FUJIX BAS2000).

The 5′-end labeled cs23 (1 pmol) was mixed with TDM-2 (10 pmol) and calf thymus DNA (0.05 µg) in PBS buffer (18 µl). After the solution was incubated at 37°C for 50 min, the complementary strand (AA23; 0.5 pmol/2 µl), dissolved in PBS,

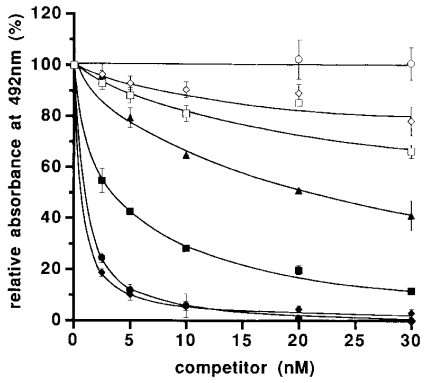


Figure 4. Effects of nucleosides and phosphate groups neighboring a cyclobutane dimer on binding to TDM-2. Plots of relative absorbance (%) versus concentrations of competitors. ApTpTpA (open circles), ApT[*c,s*]TpA (solid circles), pro·pT[*c,s*]TpA (solid diamonds), ApT[*c,s*]Tp·pro (solid squares), pro·pT[*c,s*]Tp·pro (solid triangles), pro·pT[*c,s*]T (open diamonds), T[*c,s*]Tp·pro (open squares).

was added to the aliquot. The reaction mixture (20 µl) was incubated again at 37°C for 15 min. For analysis, 5 µl of 30% glycerol was added to the solution, and the complexes were separated by 8% native polyacrylamide gel (29:1) electrophoresis at 25°C.

RESULTS AND DISCUSSION

Binding of TDM-2 to synthesized oligodeoxynucleotides containing a *cis-syn* or *trans-syn* thymine dimer

Cis-syn and *trans-syn* type cyclobutane thymine dimer blocks were chemically synthesized by the reported procedures (13,14), and the oligodeoxynucleotides cs23 and ts23 were synthesized (sequences shown in Materials and Methods). These oligonucleotides contained the cyclobutane dimer at the center. The binding to the antibody (TDM-2) was assayed by the enzyme-linked immuno-assays (ELISAs) (7). TDM-2 bound with cs23 in an antibody concentration dependent manner, but did not recognize ts23 as the antigen (Fig. 2). UV irradiation of d(TpT) induces almost equal amounts of *cis-syn* and *trans-syn* type cyclobutane dimers as the photoproducts. On the other hand, *cis-syn* type pyrimidine dimers are generated as the major product in oligonucleotides exposed to UV. Since the haptens were prepared by UV-irradiation of oligonucleotides, they contained the *cis-syn* type of cyclobutane dimers as the major products, and so probably the antibody specific to the *cis-syn* type dimer was elicited.

To investigate the base preference in the antigen recognized by TDM-2, four antigen analog bases (5′NpT[*c,s*]TpN3′; N = A, G, U, C) were synthesized. These had the *cis-syn* type photodimer at the center, and a competitive ELISA against cs23 was performed. The 3′-end of cs23 was covalently attached to the biotin and was immobilized on the plate coated with avidin (9,17). All of the tetramers, NpT[*c,s*]TpN, showed the same inhibitory effects as cs23. However, the cyclobutane dimer, d(T[*c,s*]T) only had a slight inhibitory effect (Table 1). These results suggest that the recognition size of TDM-2 ranges in the four bases containing the cyclobutane dimer, and the composition of the nucleotide flanking the photodimer does not affect the binding of the antibody. Furthermore, the various antigen analogs

shown in Figure 3 were synthesized to investigate whether the antibody recognizes the nucleosides and the phosphates on each side of the dimer, T[*c*,*s*]T. Analogs of NpT[*c*,*s*]TpN were tested, in which either N was replaced with propandiol (pro) phosphate as an abasic analog but retained the phosphate diester, which has the same negative charge as that in an oligodeoxynucleotide. The results are shown in Figure 4 and Table 1.

Table 1. Concentrations required for 50% inhibition (IC_{50}) of binding of TDM-2 to cs23

Competitor	IC_{50} (nM)
cs23	0.7
ApT[*c*,*s*]TpA	1.0
GpT[*c*,*s*]TpG	0.5
CpT[*c*,*s*]TpC	0.8
TpT[*c*,*s*]TpT	1.2
pro·pT[*c*,*s*]TpA	0.9
ApT[*c*,*s*]Tp·pro	3.4
pro·pT[*c*,*s*]Tp·pro	20
T[*c*,*s*]Tp·pro	1.7×10^2
pro·pT[*c*,*s*]T	3.0×10^3
d(T[*c*,*s*]T)	1.6×10^4

The analog pro·pT[*c*,*s*]TpA, in which only the 5′-side nucleoside was replaced with propandiol, inhibited the binding of TDM-2 to cs23 to the same extent as ApT[*c*,*s*]TpA. The analog ApT[*c*,*s*]Tp·pro, which had a propandiol at the 3′-side of the cyclobutane dimer, also competed with cs23 for the binding to the antibody; however, the inhibition by ApT[*c*,*s*]Tp·pro was ~3-fold lower than that of pro·pT[*c*,*s*]TpA. These results suggest that the antibody seems to bind to the 3′-side nucleoside to the cyclobutane dimer more tightly than the 5′-side nucleotide. *Escherichia coli* DNA photo-lyase also reversed T[*c*,*s*]TpT more efficiently than TpT[*c*,*s*]T (18). The thymine dimer {d(T[*c*,*s*]T)} adopts a different structure from that in DNA (19). Therefore, when the 3′-side nucleoside is attached to the thymine dimer, rather than the 5′-side nucleoside, the dimer may form a structure similar to that in oligonucleotides. The analog, pro·pT[*c*,*s*]Tp·pro, which has propandiols at both ends, showed an ~20-fold weaker binding ability than ApT[*c*,*s*]TpA or pro·pT[*c*,*s*]TpA, and was ~6-fold weaker than ApT[*c*,*s*]Tp·pro. The 5′-side nucleoside might also be important in maintaining the shape of the thymine dimer for binding to TDM-2. It is not clear why pro·pT[*c*,*s*]Tp·pro was poorly recognized, as compared with pro·pT[*c*,*s*]TpA and ApT[*c*,*s*]Tp·pro. The structure of T[*c*,*s*]T may affect the recognition, and the deoxyriboses of the nucleosides flanking T[*c*,*s*]T may also be recognized by TDM-2.

Although pro·pT[*c*,*s*]Tp·pro was recognized by TDM-2 less efficiently, as compared with the tetramer analogs, it has a higher affinity than the dimer {d(T[*c*,*s*]T)}. To investigate the effect the phosphate groups neighboring the cyclobutane dimer on the binding to TDM-2, T[*c*,*s*]Tp·pro and pro·pT[*c*,*s*]T lacking either the 5′- or 3′-side phosphate were synthesized (Fig. 3). In the competitive ELISA, neither T[*c*,*s*]Tp·pro nor pro·pT[*c*,*s*]T could inhibit TDM-2 binding sufficiently (Fig. 4 and Table 1). The lack of either the 5′- or 3′-phosphate group drastically decreased the affinity to the antibody. However, these competitors harboring a phosphate group still maintained a larger affinity than the dimer d(T[*c*,*s*]T) alone. These results indicate that both the 5′- and 3′-end phosphates participate in recognition of the epitope by TDM-2. Perhaps there are weak electrostatic interactions between the negatively charged phosphate groups and the positively charged amino acid residues in the antibody. The antibody is specific for the cyclobutane ring of T[*c*,*s*]T, because it binds neither a normal thymidine dinucleotide nor a *trans-syn* thymine dimer. The presence of the phosphate groups at both ends of the cyclobutane dimer seems to enhance the affinity of the antigen with the antibody, while the dimer d(T[*c*,*s*]T) without the flanking phosphates, might wobble in the binding pocket of the antibody.

The antibodies that were elicited by using only thymine dimers without flanking sequences {[6-4] photoproducts (9) or *trans-syn* thymine dimer analogs (10)} were able to bind directly with the thymine dimers in the absence of neighboring nucleotides. However, TDM-2 was obtained from immunization with UV irradiated calf thymus ssDNAs, and it has been proved by ELISA experiments that not only the cyclobutane ring but also the neighboring nucleoside and phosphate groups enhance the affinity of the hapten to the antibody. The tertiary structure of the epitope binding site of TDM-2 might be different from that of other antibodies obtained from immunization with the smaller molecules.

Binding of TDM-2 to double stranded DNAs

TDM-2 was obtained by immunization using UV-irradiated ssDNAs, and it has been proved that the antibody recognized oligonucleotides with three or four base spans containing one *cis-syn* cyclobutane thymine dimer. Phosphate groups on both sides of these oligonucleotides were required for recognition by TDM-2. It was important to investigate whether TDM-2 was able to bind to the thymine dimer in a double stranded DNA (dsDNA).

In a previous report, protamine coated plates were used for the ELISA (7) and it is not clear whether the dsDNA dissociated into ssDNA. Such a dissociation of dsDNA could be detected in a gel mobility shift assay. The 5′-end labeled 23mer, cs23, was prepared and combined with a complementary strand (AA23) to form a dsDNA (cs23·AA23), and gel mobility shift assays were performed. The results of the gel shift assays showed that TDM-2 could bind with a ssDNA, but not with a dsDNA, in the presence of large excess of antibody (Fig. 5a). It was reported that both thymine bases in the cyclobutane dimer form T·A base pairs with the opposing adenines, although the hydrogen-bonding interactions were reduced (19,20). TDM-2 might not recognize a cyclobutane dimer in the DNA helix because of the base pairs. To investigate the effect of base pairs at the cyclobutane dimer site, two adenine bases in the complementary strand were changed to guanines (mis2), and four and six bases in the neighboring positions were also changed (mis4, mis6, respectively). When cs23 was combined with mis2, TDM-2 showed slight binding with the cs23·mis2 double strands. However, TDM-2 bound with a higher affinity to all double strands containing four and six base mismatches (cs23·mis4, cs23·mis6, Fig. 5b). The hydrogen bonds between the cyclobutane thymine dimer and the opposite adenines in the cs23·mis2 duplex seemed to be disrupted, and the cyclobutane dimers in the cs23·mis4 and cs23·mis6 duplexes may be exposed. Internal loops (bubble structures) seemed to be formed in these duplexes, and TDM-2 may be able to access the cyclobutane thymine dimers in these duplexes. TDM-2 showed

high affinity with ApT[*c*,*s*]TpA but a low affinity with d(T[*c*,*s*]T) in the competitive ELISAs. Since TDM-2 bound to double strands containing four or six mispairs more efficiently than to a double strand in the presence of two mismatched pairs (cs23·mis2), the results from the gel mobility shift assay can be explained. TDM-2 recognizes a narrow region of DNA containing the cyclobutane thymine dimer. After cs23 was incubated with TDM-2, the complementary strand (AA23) was added to the solution containing the cs23–TDM-2 complex. Then, the cs23–TDM-2 complex was disrupted and cs23·AA23 duplex was formed (data not shown). This result suggests that TDM-2 could not bind to a dsDNA in the presence of the completely complementary strand.

DNA binding antibodies arise in the human autoimmune disorder, systemic lupus erythematosus and in a similar condition that occurs in some strains of inbred mice. The Hed 10 (21,22) and BV04-01 (23,24) antibodies were shown to bind to ssDNA with a preference for thymine bases. In contrast, the Jel 241 (25) and Jel 72 (26) antibodies bind to dsDNAs. The three-dimensional structure of Fab BV04-01, which is specific to ssDNA, in complex with a trinucleotide [d(pT)3] revealed that the N-3 and O-4 atoms of the central thymine nucleotide form hydrogen bonds with a serine residue of the Fab fragment (25). However, the N-3 and O-4 atoms are involved in Watson–Crick hydrogen bonds with the adenine base in the complementary strand. TDM-2 showed specificity to ssDNA containing a T[*c*,*s*]T, and this feature is similar to BV04-01. Furthermore, there is a direct charge–charge interaction between a phosphate group of d(pT)3 and an arginine residue in the CDR-H2 of BV04-01, and Hed 10 also interacts with only two phosphate groups. Since the present competitive ELISA suggests that two phosphate groups flanking a cyclobutane dimer are important for recognition by TDM-2, it may also have a recognition mechanism for DNA containing cyclobutane dimer similar to Hed10 and BV04-01, specific to ssDNA. Antibodies specific to dsDNAs recognize bases and lengths of duplexes, because these antibodies contact the dsDNA at the major or minor groove. Perhaps the mechanisms for antibody recognition of dsDNA are different from those for ssDNA. Since the *cis-syn* cyclobutane thymine dimer bends dsDNA (27,28), dsDNAs containing a thymine dimer may be immunogenic. Screening of monoclonal antibodies using dsDNAs containing a thymine dimer seems to be required for the establishment of antibodies specific to dsDNA containing a thymine dimer.

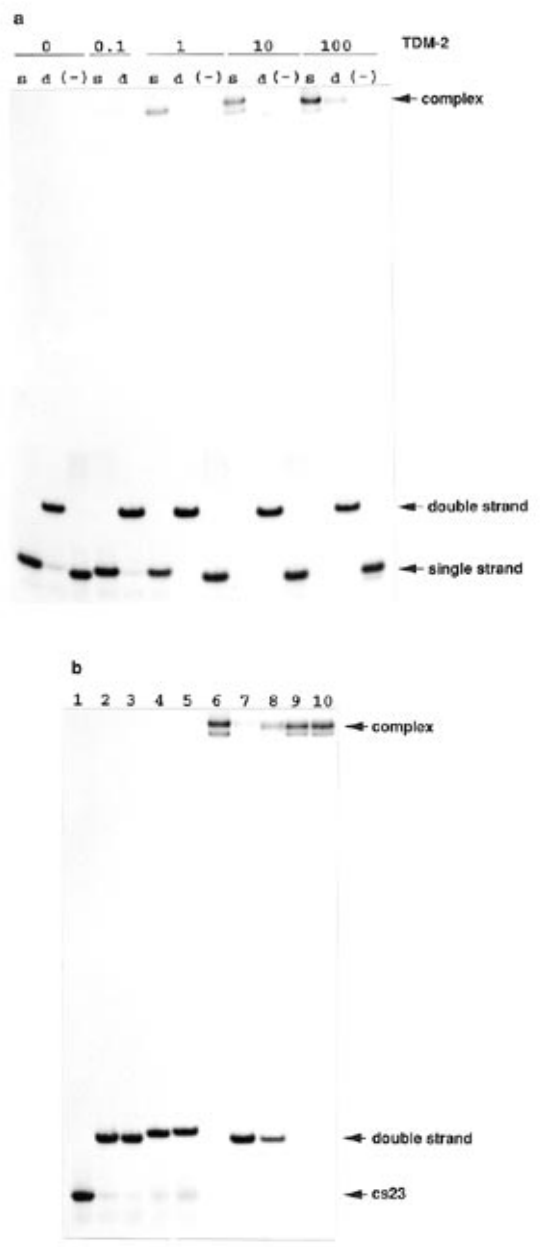


Figure 5. Analyses of binding to double stranded DNAs by native gel electrophoresis. (**a**) Binding of TDM-2 (0, 1, 10 and 100 equivalents) to cs23, cs23·AA23, or non-UV-irradiated 23mer. s, d and (–) on the top of the gel indicate cs23, cs23·AA23 and non-UV-irradiated 23mer, respectively. (**b**) Binding of TDM-2 to duplexes containing mispairs Lanes 1, 6, cs23; lanes 2, 7, cs23·AA23; lanes 3, 8, cs23·mis2; lanes 4, 9, cs23·mis4; lanes 5, 10, cs23·mis6; lanes 1–5, in the absence of TDM-2, and lanes 6–10, in the presence of TDM-2.

CONCLUSION

TDM-2, obtained by immunization using UV-irradiated DNA, indicated specific binding to the *cis-syn* type cyclobutane thymine dimer, but not to the *trans-syn* type cyclobutane dimer. For TDM-2, binding requires not only the cyclobutane dimer but also the neighboring nucleosides. TDM-2 could bind with NpT[*c*,*s*]TpN efficiently. Either the 5′- or 3′-side nucleoside of NpT[*c*,*s*]TpN was changed to a propandiol linker as an abasic analog while maintaining a phosphate diester. From competitive ELISA using these analogs, it was proved that the 5′- and 3′-side nucleosides were important for the recognition by TDM-2. Particularly, the existence of the 3′-side nucleoside affected the binding to TDM-2. Both the 5′ and 3′-phosphates of the cyclobutane dimer were important for the binding to TDM-2; the cyclobutane dimers lacking a phosphate at either end were almost inactive. In a gel mobility shift assay, TDM-2 did not indicate binding to a dsDNA. However, the antibody could bind to duplexes with mispairs near the cyclobutane dimer.

TDM-2 has been shown to specifically recognize a certain span of DNA containing a *cis-syn* thymine cyclobutane dimer. The procedures for using the antigen analogs described here will be important for recognition studies of photo damaged DNA by

various enzymes and for the construction of new antibodies with various functions.

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