Monoclonal antibodies targeted to α-oligonucleotides. Characterisation and application in nucleic acid detection

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ABSTRACT

The aim of the present study was to test the antigenicity of α-deoxyribonucleotides in order to develop a new tool for the detection of nucleic acid sequences for use in diagnostic applications. We describe four monoclonal antibodies (Mabs) which recognize α-deoxyribonucleotides. Two were raised against a poly(α-dT) sequence and specifically recognized the α-dT nucleotide. Two were raised against a sequence containing all four common nucleotides as α-nucleotides and, surprisingly, only recognized the α-dG nucleotide. For all four Mabs, no cross reactivity was observed with β-oligonucleotides. These Mabs were reactive with α-oligonucleotide sequences whether these sequences were single-stranded or hybridized to DNA or RNA. The four Mabs were tested in a sandwich hybridization assay that consisted of an α-oligonucleotide (for target sequence recognition), one of the four Mabs (for recognition of the hybridized α-oligonucleotide), and goat anti-mouse antibody conjugated to horse radish peroxidase (HRP) (for detection). One of the monoclonal antibodies, Mab 2E11D7, was directly conjugated to HRP and used in sandwich hybridization to detect PCR fragments of HPV 18 DNA. The sensitivity of this reaction was 1 pg of plasmid DNA containing the HPV 18 fragment. The specificity of the detection was demonstrated using HPV 6/11 and 16 DNA sequences.

INTRODUCTION

Nucleic acid hybridization is an analytical tool with great potential for the detection and characterization of microbial agents (1). The replacement of radioisotope labels with labels such as biotin (indirect labeling), or by conjugation to enzymes (direct labeling), has made this technique more attractive for use in clinical laboratories where the handling and disposal of radioisotopes is inconvenient and impractical. Another technical advance in assay design has been the development of a format adapted to large scale screening (2). The combination of non-isotopic labeling and the use of sandwich hybridization for detection on a solid phase has provided a new technology in diagnostic applications, both in microbial and genetic diseases (3–5).

The applicability of monoclonal antibodies as probes of nucleic acid structure has been demonstrated in several systems both in vivo and in vitro (6). The generation of antibodies to double-stranded DNA has not generally been very successful except for Z-DNA (7, 8). Other double-stranded polynucleotides such as RNA–RNA and DNA–RNA, however, are immunogenic. Diagnostic applications of monoclonal antibodies directed against DNA–RNA hybrids (9–11) and RNA–RNA hybrids (12) have been described. Monoclonal antibodies which recognize double-stranded RNA have been used in the detection and characterization of double-stranded RNA in nucleic acid extracts (13). Whereas antibodies to double-stranded nucleic acids appear to recognize structure, antibodies to single-stranded poly-nucleotides usually recognize bases or sequences of bases. These antibodies specifically react with single-stranded polynucleotides and not with double-stranded nucleic acids in which the antigenic bases are probably inaccessible (14). Antinucleoside antibodies have also been described (15) but this application too is limited to the detection of single-stranded DNA (16). The most utilized method for the detection of a nucleic acid sequence using antibodies is to label a nucleic acid probe with a hapten that is recognized by the antibody. Applications of this technology have been reviewed by Guesdon (17). These applications all describe uses in which the nucleic acid probe contains naturally occurring nucleosides or nucleotides in which the glycosidic linkage is in the β-anomeric form. A limitation to the use of β-oligonucleotides, however, is their susceptibility to degradation by nucleases which are ubiquitous in biological samples. Since nuclease activities are not necessarily destroyed during sample preparation, this becomes an important concern in diagnostic applications because nucleases can affect the sensitivity of an assay, especially if the target is RNA. On the other hand, α-oligodeoxyribonucleotides have been shown to be resistant to

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nucleolytic attack (18, 19). Moreover, α-oligodeoxyribonucleo-
tides are able to hybridize in a parallel orientation to
complementary β-oligodeoxyribonucleotide (20, 21) and β-oligo-
ribonucleotide (22), and these hybrids form complexes which are
highly resistant to nuclease attack (22–24), thus favoring the
use of α–β hybrids in diagnostic applications.

In this study we report for the first time on the antigenicity
of α-deoxyribonucleotides and describe four monoclonal
antibodies specific to these molecules. We also describe an
immunological assay for the detection of α–β hybrids. The Mabs
against the α-oligodeoxyribonucleotides were used in a sandwich
hybridization assay to detect the human papillomavirus (HPV)
DNA sequence.

MATERIALS AND METHODS

β-oligodeoxyribonucleotides were synthesized on an automated,
solid phase DNA synthesizer (ABI 394, Applied Biosystems)
using standard cyanogenyl-N, N-diisopropylamino phosphite
chemistry and were purified by reversed phase HPLC. 
α-oligodeoxyribonucleotides were synthesized as previously
described (25, 26). Aminolink2 (Applied Biosystems) was added
at the 5’ end of α-oligodeoxyribonucleotide sequences (a) and
(b) for conjugation (see below).

Preparation of antigens: α-oligodeoxyribonucleotide–BSA
conjugates

To conjugate α-oligonucleotides to bovine serum albumin (BSA),
400 µg of vacuum-dried 5-aminocetyl-α-oligodeoxyribonu-
cleotide were mixed first with 25 µl of 0.1 M sodium borate
buffer (pH 9.3), and then with 500 µl of 20mg/ml 1,4 phenylene
diisothiocyanate (DITC, Sigma) prepared in dimethylformamide.
The mixture was incubated in the dark for 2 hours at room
temperature, then 3 ml of H2O were added. After 3 extractions
with equal volumes of 1-butanol, the aqueous phase was vacuum
dried and activated oligonucleotides resolubilized in 150 µl of
0.1 M sodium borate buffer (pH 9.3) containing 1 mg of BSA
(Sigma). After an overnight incubation at room temperature, α-
oligonucleotide–BSA conjugates were purified by gel permeation
chromatography using a 30 cm X 21.5 mm TSK 3000 SW column
(Tosoh Corporation) and 50mM Na phosphate (pH 6.0)/150 mM
Na2SO4 as the elution buffer.

Two α-oligodeoxyribonucleotide–BSA conjugates were prepared:
one containing sequence (a), 5’-d(ACCCCGAG-
ATTACGTTAG)-3’, and the other sequence (b), 5’-dT3O-3’.

Preparation of antibodies

Four Balb/c mice were immunized by intraperitoneal injections
of 30 µg of α-oligonucleotide–BSA conjugate emulsified in an
equal volume of Freund’s complete adjuvant (Difco Labs). These
injections were followed on days 28, 60 and 82 with injections
containing the same quantity of conjugate in Freund’s incomplete
adjuvant. Spleen cells isolated from immunized mice were fused
with sp2/0-Ag14 myeloma cells as described (27) using
polyethylene glycol. Culture supernatants, diluted from 1/10 to
1/106 in 1 XPBST, were screened using a ‘direct’ protocol assay
(described below) in which a panel of single-stranded α- and β-
oligonucleotides, and double-stranded α–β, β–β, and α–α
oligonucleotide hybrids were immobilized on microtitre plates
(Table I). Positive colonies were subcloned twice by limiting
dilution. Ascitic fluids were obtained from mice primed with 0.5
ml intraperitoneal injection of pristane and then injected with 106
hybridoma cells.

Monoclonal antibodies (Mabs) were purified by Protein A
column chromatography (Pharmacia). Two Mabs were obtained
against sequence (a), 2E11D7 and 5B7C7, and two Mabs were
obtained against sequence (b), 4H4F5 and 5H11H10. The specificity
of these purified Mabs was determined and characterized using the direct and competition assays described
below. All four Mabs belonged to the IgGl class.

Specificity of monoclonal antibodies

Two procedures were used to characterize the antigenic specificity
of monoclonal antibodies. In the first procedure, the ‘direct’
assay, different nucleic acid antigens were immobilized on
microtiter plates and reacted (directly) with Mabs. In the second
procedure, the ‘competition’ assay, the specificity of Mabs was
determined by competition with α-oligonucleotides in solution
prior to reacting with bound nucleic acid sequences. Oligonucleotides used in these assays are described in Table I.

Direct assay protocol. Nucleic acid antigens (single- or double-
stranded) were passively adsorbed to microtiter plate wells
(Maxisorb, Nunc) at 0.2 nmole/ml in 3 X PBS (0.15 M phosphate,
0.45 M NaCl, pH 7.0) for 2 hours at 37°C. Plates were washed
three times with 1 X PBS made 0.05% in Tween 20 (PBST), and
then blocked with 1% lyophilyzed milk extract (REGILAIT) in
3 X PBS made 1% in BSA. After incubation for 1 hour at 37°C,
plates were washed and the detection substrate, a solution of 2

Table I. *Oligodeoxyribonucleotides used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence 5’–3’ Nucleotide</th>
<th>Length</th>
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<tr>
<td>a</td>
<td>ACACCGAGATTTAGTATG</td>
<td>ALPHA 21</td>
</tr>
<tr>
<td>b</td>
<td>TGTTTTTTTTTTTTTTTTTTTTT</td>
<td>ALPHA 20</td>
</tr>
<tr>
<td>c</td>
<td>GTCACACCGAGCTGTGGA</td>
<td>ALPHA 20</td>
</tr>
<tr>
<td>d</td>
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<td>ALPHA 20</td>
</tr>
<tr>
<td>e</td>
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<td>ALPHA 20</td>
</tr>
<tr>
<td>f</td>
<td>GGGGGGGGGGGGGG</td>
<td>ALPHA 20</td>
</tr>
<tr>
<td>g</td>
<td>GGCCGCGGTGTCGCGCGGGG</td>
<td>ALPHA 19</td>
</tr>
<tr>
<td>h</td>
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<td>ALPHA 19</td>
</tr>
<tr>
<td>i</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>ALPHA 17</td>
</tr>
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<td>ALPHA 17</td>
</tr>
<tr>
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<td>ALPHA 17</td>
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<tr>
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<tr>
<td>z</td>
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Sequences with lower case letter designations are synthesized with alpha nucleotides. The sequences with upper case letter designations are synthesized with beta nucleotides.
mg/ml p-nitrophenyl phosphate (Sigma) in diethanolamine buffer (1.29 M diethanolamine, 0.56M MgSO4, 0.38 mM NaN3, pH 9.8), was added. After incubation for 20 minutes, enzymatic activity was blocked with 100 µl of 1N NaOH and absorbance measured at 405 nm with an AXIA MICROREADER (BioMérieux).

Direct assay protocol using polydA/polyrA—α(dT)20 hybrids. Poly dA or poly rA (Pharmacia) were adsorbed to microtiter plate wells at 1 µg/ml in 3×PBS made 0.2% in diethyl pyrocatecholate (DEPC; Sigma) for 2 hours at 37°C. After washing, plates were incubated with the poly dTα-oligonucleotide sequence (b) at 0.02 nmole/ml in 3×PBS/0.2% DEPC/10 µg/ml salmon sperm DNA (Sigma) for 1 hour at 37°C. The β-oligonucleotide (F) (Table I) was used as negative control. After washing, plates were reacted with either Mab 4H4F5 or 3H11H10 (diluted to between 0.05 µg/ml and 5 µg/ml in 1×PBST) for 1 hour at 37°C. Nucleic acid—Mab complexes were detected using goat anti-mouse IgG conjugated to AKP. The enzymatic substrate was added and absorbance measurements taken as described above.

Competition assay protocol. Microtiter plates were coated with α-oligodeoxyribonucleotides (a) at 0.2 n mole/ml in 3×PBS for 2 hours at 37°C. Purified Mabs at 10−5 mg/ml in 1×PBST were preincubated with 0.2 nM of the competitive nucleic acid sequence for 1 hour at 37°C, and then added to precoated microtiter plates. Subsequent steps were identical to those in the direct assay protocol.

Detection of nucleic acid by the sandwich hybridization protocol

DNA amplification. PCR was performed on purified plasmid carrying cloned HPV DNA of types 6, 11, 16 and 18 using primers chosen from the E6—E7 region (28). Amplification products were denatured with NaOH (0.2 mole/l) and then neutralized with acetic acid (0.2 mole/l) before detection.

Nucleic acid detection. As capture probe, sequence (I) (Table I), an HPV18-specific sequence, was adsorbed to microtiter plate wells at 0.2 n mole/ml in 100 µl of 3×PBS for 2 hours at 37°C. After washing plates, 50 µl of a 1/10 dilution of denatured PCR product prepared in 3×PBS was added with an equal volume of detection probe, α-oligonucleotide (a)-conjugated to horse radish peroxidase (HRP) (4), at 15 pmole/ml in 3×PBS containing 10 µg/ml salmon sperm DNA. Plates were incubated for 1 hour at 37°C, washed, then incubated for 20 min with the detection solution, 4 mg/ml ortho-phenylenediamine (OPD; Cambridge Medical Biotechnology) prepared in 0.05 M citric acid/0.1 M NaH2PO4/H2O2 0.03 vol/pH4.9. Enzymatic activity was stopped with 100 µl 1N H2SO4 and absorbance read at 492 nm.

The sensitivity of this direct detection assay was compared to that of a protocol in which the α-oligonucleotide (a) was detected either by anti α-nucleotide antibody conjugated to HRP or a secondary antibody conjugated to HRP that recognized the bound anti-α nucleotide. In the latter case, detection was performed with α-oligonucleotide (a) for target recognition, Mab for recognition of the hybridized α-oligonucleotide and a goat anti-mouse antibody conjugated to HRP for the detection reaction. After incubation of precoated microtiter plates with target and α-oligonucleotide for 1 hour at 37°C, plates were washed and then Mab (100 µl) added at a concentration of 0.5 µg/ml in 1×PBST/10 µg/ml salmon sperm DNA/10% horse serum (BioMérieux). Plates were incubated for 1 hour at 37°C. The plates were then washed three times with 1×PBST. As secondary antibody, goat anti-mouse IgG (Sigma Immuno Chemicals) conjugated to HRP was used at a 10−4 dilution in 1×PBST made 1% in BSA. Plates were incubated for 1 hour at 37°C, washed three times and the detection assay performed as described above.

To eliminate one step of antibody—antibody interaction in the sandwich protocol, Mab 2E11D7 was conjugated directly to HRP (29). In this modified protocol, after incubation of precoated microtiter plates with target and α-oligonucleotide for 1 hour at 37°C, plates were washed and then the 2E11D7-HRP conjugate (100 µl) was added at a concentration of 0.1 µg/ml in 1×PBST/10 µg/ml salmon sperm DNA/10% horse serum (BioMérieux). Plates were incubated for 1 hour at 37°C, washed three times and the detection assay performed as described above.

The specificity of HPV 18 DNA detection was demonstrated using PCR fragments of HPV 6, 11 and 16.

RESULTS AND DISCUSSION

Specificity of monoclonal antibodies

The aim of the present study was to test the antigenicity of α-deoxyribonucleotides in order to provide a new tool for the detection of nucleic acid sequences in diagnostic applications. Monoclonal antibodies were obtained in Balb/c mice for two α-oligodeoxyribonucleotide sequences covalently coupled to BSA as hapten. The two sequences, sequence (a), α-5’-[dACCCCI-GAGATTACGTATAG-3’], and sequence (b), α-5’-dT20-3’, were modified at their 5’-ends with Aminolink2 to generate a reactive amino function that could be activated with DITC for coupling to BSA. Conjugated BSA—oligonucleotides were purified by gel permeation chromatography to remove unreacted BSA and uncoupled oligonucleotides. The determined molar ratio of conjugated α-oligonucleotide to BSA was 5.6 for sequence

Figure 1. Specificity of monoclonal antibodies raised against the α-dT20 sequence (b). Black columns represent Mab 3H11H10 and striped columns Mab 4H4F5. Single stranded (b, c, h, A, F) or double stranded (b—G, h—H, A—B, F—G) oligonucleotides were adsorbed to microtiter plates and reacted with Mabs using the direct assay protocol. The relative binding of Mabs was measured as absorbance at 405 nm.
(a) and 2.0 for sequence (b). Immunizations with non purified antigen at a molar ratio < 0.5 of oligonucleotide to BSA gave poor results with respect to antigenicity.

Two fusion experiments were carried out and hybrid cells colonies were screened for antibody synthesis using an ELISA (Enzyme Linked Immuno Sorbent Assay) test. The wells of the microtiter plate were coated with the sequences (a) and (b) and the β equivalents (A) and (F). Four hybrid cells colonies were retained for additional work and cloned at limiting dilution (twice). Two Mabs were obtained against sequence (a), 2E11D7 and 5B7C7, and two Mabs were obtained against sequence (b), 4H4F5 and 3H11H1O. The specificity of Mabs were analyzed first, with the direct protocol assay, using a panel of single-stranded α- and β-oligonucleotides, and double stranded α−β, β−β and α−α oligonucleotides.

Mabs, 3H11H10 and 4H4F5, demonstrated the same reactivity to α-oligodeoxyribonucleotides containing α-dT, independently of whether these sequences were single- or double-stranded (Figure 1). No cross reactivity was observed to the α-oligonucleotides lacking α-dT [e.g. sequence (h) in Figure 1] or to β-oligonucleotides.

Mabs 5B7C7 and 2E11D7 reacted with a large panel of α-oligonucleotides, independently of whether these oligonucleotides were hybridized or not to α- or β- oligonucleotides (Figure 2). These two Mabs did not react with antigens f−F, b−G, h−H and f, which contain homopolymers of α-nucleotides; i.e., α−dA in (f), α−dT in (b) and α−dC in (h). No cross reactivity was observed with β-oligonucleotides. This included sequence (A) which is a beta sequence in opposite orientation as compared to (a). α−β DNA duplexes are formed with parallel strands, in contrast to β−β DNA duplexes which are formed with anti-parallel strands, and both sequences (a) and (A) can hybridize with sequence (B).

In the direct assay, antigens, whether in alpha or beta form or single- or double-stranded, were passively adsorbed to microtiter plate wells. Although the mechanism of this adsorption is not known, adsorbed oligonucleotides, though only 13 to 20 bases in length, are available for hybridization (4). A concern in oligonucleotide adsorption is that the conformation of bound oligonucleotides might be modified, which could affect the reactivity of Mabs. To overcome this problem, a competition assay was designed. In this assay, sequence (a), which contains both α-dT and α-dG, was passively adsorbed to microtiter plate wells. Then, after preincubating oligonucleotide sequences in

![Figure 2](https://academic.oup.com/nar/article-abstract/22/15/2951/1087209)

**Figure 2.** Specificity of monoclonal antibodies raised against sequence (a). Black columns represent Mab 5B7C7 and striped columns Mab 2E11D7. Single stranded (a, c, d, f, A, C) or double stranded (a−B, c−D, d−E, c−F, h−H, e−d, A−B, D−E) oligonucleotides were adsorbed to microtiter plates and reacted with Mabs using the direct assay protocol. The relative binding of Mabs was measured as absorbance at 405 nm.

![Figure 3](https://academic.oup.com/nar/article-abstract/22/15/2951/1087209)

**Figure 3.** Competition assay for the specificity of monoclonal antibodies against sequence (a-dT20) (b). Black columns represent Mab 3H11H10 and striped columns Mab 4H4F5. Sequence (a) was adsorbed to microtiter plates and reacted with Mab that was preincubated with the competing single-stranded oligonucleotide. Column (−): no competitive oligonucleotide adding during preincubation of Mabs. The relative binding of Mabs was measured as absorbance at 405 nm.

![Figure 4](https://academic.oup.com/nar/article-abstract/22/15/2951/1087209)

**Figure 4.** Competition assay for the specificity of monoclonal antibodies against sequence (a). Black columns represent Mab 5B7C7 and striped columns Mab 2E11D7. Sequence (a) was adsorbed to microtiter plates and reacted with Mab preincubated with the competing single-stranded oligonucleotide. Column (−): no competitive oligonucleotide added during preincubation of Mabs. The relative binding of Mabs was measured as absorbance at 405 nm.
solution with Mabs, the preincubation mixtures were reacted with the adsorbed sequence.

The specificity of Mabs, 4H4F5 and 3H11H10, was tested against a large panel of single stranded α-oligonucleotides (Figure 3). No competition was observed with β-oligonucleotides A and F.

For Mab 4H4F5, high competition was shown with oligonucleotides (a), (b), (i) and (l), all of which are rich in α-dT residues. The reactivity of 4H4F5 was not competed by oligonucleotides (g) and (n); the poor affinity to sequence (g) may be explained by the absence of α-dT nucleotides, and the poor affinity to sequence (n) by the low content of α-dT nucleotides (only one α-dT in the sequence). The reactivity of 4H4F5 with sequence (c), which contains four isolated α-dT residues, shows clearly that this antibody recognizes isolated α-dT nucleotides rather than an array of contiguous α-dT nucleotides. The correlation between immuno-reactivity and the number of α-dT nucleotides in the sequence is shown in Figure 5. An explanation for the high reactivity of 4H4F5 to sequence (m) could be the formation of an hairpin with a loop of 5 nucleotides containing 3 α-dT. The α-dT nucleotide is more exposed in this secondary structure.

The reactivity of Mab 3H11H10 (Figure 3), on the other hand, was not competed by α-oligonucleotides (c), (d), (e), (g), (n), (o) and (p). This Mab was highly competed only by α-oligonucleotide (b), which was the antigen used to generate the antibody. If we decreased the concentration of Mab from $10^{-5}$ to $5 \times 10^{-6}$ mg/ml, a slight inhibition occurred with sequences containing α-dT. Thus, it also appears, using the competitive assay, that this antibody too recognized the α-dT nucleotide, but with weaker affinity compared to Mab 4H4F5.

For Mabs 2E11D7 and 5B7C7, the competition assay (Figure 4) indicated that these Mabs only recognized α-oligodeoxyribonucleotides containing α-dG nucleotides. For example, no competition was observed with sequences (b), (i) and (l), all of which are devoid of α-dG residues (Figure 4). These two antibodies were competed by sequences (o) and (a), both of them containing separated α-dG nucleotides and as described for Mab 4H4F5, Mabs 2E11D7 and 5B7C7 recognized individual α-dG nucleotide rather than an array of adjacent α-dG nucleotides. For example, oligomers (d) and (e) which contain adjacent G’s were worse competitor than oligomers (p) and (a) which contain isolated G’s. The higher antigenicity of sequences (o) and (a) suggested that the α-dG nucleotide was the main epitope in these oligonucleotides. These results are consistent with those previously reported by Traincard et al. (15) for the antigenicity of β-dG nucleotides. Figure 6 shows a correlation between reactivity and the presence of dG nucleotides for Mab 2E11D7.

Figure 5. Correlation between the number of α-dT nucleotides in a sequence and reactivity of Mab 4H4F5. Sequence (a) was adsorbed to microtiter plates and, using the competitive assay format, Mab 4H4F5 was preincubated with the indicated α-oligonucleotide sequence. The relative binding of 4H4F5 was measured as absorbance at 405 nm.

![Figure 5](https://example.com/figure5.png)

Figure 6. Correlation between the number of α-dG nucleotides in a sequence and reactivity of Mab 2E11D7. Sequence (a) was adsorbed to microtiter plates and, using the competitive assay format, Mab 2E11D7 was preincubated with the indicated α-oligonucleotide sequence. The relative binding of 2E11D7 was measured as absorbance at 405 nm.

![Figure 6](https://example.com/figure6.png)

Figure 7. Reactivity of Mab 4H4F5 to α-dT$_{20}$ hybridized to poly rA (O) and poly dA (A). Poly dA and poly rA were first adsorbed to microtiter plates, then hybridized to α-dT$_{20}$ and reacted with Mab. The negative control used was β-dT$_{20}$ hybridized to poly rA (C) and poly dA (■). The relative binding of Mabs was measured as absorbance at 405 nm.

![Figure 7](https://example.com/figure7.png)
Sequence (g) was an exception to this rule. This oligonucleotide contains eight adjacent ω-dG residues and it is well known that such a string of G residues have the potential to self-associate to form stable G-tetrad (30, 31). Thus it is likely that the formation of such a stable structure might preclude recognition of ω-oligonucleotide (g) by antibody.

Mab 4H4F5 and 3H11H10 exhibited identical reactivities against ω-dT20 (sequence b) hybridized to poly rA or to poly dA (Figure 7). These antibodies, however, did not react with hybridized ω-dT20. From these reactivities to hybridized ω-dT-containing sequences we assume that Mabs raised against sequence (a) would probably also recognize ω-oligodeoxyribonucleotides containing ω-dG when hybridized to RNA.

Detection of nucleic acids

Mabs were tested for their ability to detect target nucleic acid sequences. For this detection we chose the Human Papillomavirus (HPV) DNA sequence. HPV is known to induce benign and malignant epithelial proliferations in skin and mucosal tissues (32). Among the 66 types of HPV known, HPV 16 and 18 are considered to be high risk types because of their association with malignant lesions (33).

The sandwich hybridization assay involved the use of an adsorbent capture probe and a labeled detection probe. The capture probe was the β-oligonucleotide sequence (I) (Table I) and the detection probe was the ω-oligonucleotide sequence (a) conjugated to HRP; both of these sequences are complementary to the E6–E7 region of HPV 18. The detection probe (a) was coupled to HRP via Aminolink2. In the standard sandwich protocol, a one step incubation was used which included target DNA (an amplified region of HPV DNA) and detection probe.

In a modified version of this protocol, unconjugated ω-oligonucleotide (a) was used as the detection probe. After hybridization to target sequence and washes to remove unbound detection probe, reactions were incubated with each of the four Mabs; all of these Mabs were able to recognize hybridized ω-oligonucleotide (a), which contains nucleotides ω-dT and ω-dG. HRP-labeled goat anti-mouse was used to detect bound Mabs.

To determine the sensitivity and specificity of these two protocols, variable quantities of cloned HPV 18 DNA (1 ng to 1 fg of plasmid) was amplified by PCR to generate a 201 bp fragment. As control for specificity, PCR-amplified fragments of HPV6, HPV11 and HPV16 (100 pg of HPV-containing plasmid) were used.

In the HPV18 DNA detection Mab 2E11D7 gave the best sensitivity using the double antibody protocol (Figure 8). Therefore, this antibody was conjugated directly to HRP in order to eliminate the need for antibody–antibody recognition and thereby simplify the protocol. In this format, we were able to detect HPV 18 PCR products at a signal-to-noise ratio of greater than 3 to 1 for 1 pg of HPV plasmid (Figure 9). However, a higher background was observed (0.5 unit of absorbance), which may explain the lower sensitivity. This increased background could be attributed to non-specific adsorption of the labeled Mab to the microtiter dish wells. This problem could possibly be resolved by an improvement of Mab coupling to HRP and by an optimization of the assay, particularly with respect to the buffer conditions. In comparison, the sensitivity of detection using the direct assay in which HRP was conjugated to the detection ω-oligonucleotide (a) was 0.1 pg of HPV-containing plasmid (Figure 9). Specificity for HPV 18 DNA detection was demonstrated using PCR fragments of HPV 6/11 and 16. Optical densities obtained from hybridization to these sequences were at the level of background, i.e. at that of the control where no target sequence was added.

CONCLUSION

We have demonstrated the antigenicity of ω-deoxyribonucleotides. With sequence (b), an ω-oligothymidylate, we obtained two Mabs directed against ω-dT. With sequence (a),
we expected to obtain Mabs directed against the glycosidic linkage in the α-anomeric form, but instead obtained anti α-DG Mabs. This might be due to the higher antigenicity of the α-DG nucleotide. We have shown that in both cases, the generated Mabs recognize a single α-nucleotide species, whether α-dT or α-dG, and that oligonucleotides containing these residues can be single-stranded, or hybridized to α-oligodeoxyribonucleotides, β-oligodeoxyribonucleotides, or β-oligoribonucleotides and be detected. Furthermore, we have shown that when conjugated to enzymes these antibodies can be used to detect α-oligonucleotides used as detection probes need not be enzymatically labeled after synthesis.

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