2224–2229 *Nucleic Acids Research, 1998, Vol. 26, No. 9*

Advantages of 2′-*O*-methyl oligoribonucleotide probes for detecting RNA targets

Mehrdad Majlessi, Norman C. Nelson and Michael M. Becker*

Gen-Probe Inc., 10210 Genetic Center Drive, San Diego, CA 91212-4362, USA

Received November 6, 1997; Revised and Accepted March 6, 1998

ABSTRACT

We have compared various kinetic and melting properties of oligoribonucleotide probes containing 2′-*O*-methylnucleotides or 2′-deoxynucleotides with regard to their use in assays for the detection of nucleic acid targets. 2′-*O*-Methyl oligoribonucleotide probes bound to RNA targets faster and with much higher melting temperatures (T_m values) than corresponding 2′-deoxy oligoribonucleotide probes at all lengths tested (8–26 bases). T_m values of both probes increased with length up to ~19 bases, with maximal differences in T_m between 2′-*O*-methyl and 2′-deoxy oligoribonucleotide probes observed at lengths of 16 bases or less. In contrast to RNA targets, 2′-*O*-methyl oligoribonucleotide probes bound more slowly and with the same T_m to DNA targets as corresponding 2′-deoxy oligoribonucleotide probes. Because of their greatly enhanced T_m when bound to RNA, 2′-*O*-methyl oligoribonucleotide probes can efficiently bind to double-stranded regions of structured RNA molecules. A 17 base 2′-*O*-methyl oligoribonucleotide probe was able to bind a double-stranded region of rRNA whereas the same 17 base 2′- deoxy oligoribonucleotide probe did not. Due to their enhanced T_m when bound to RNA targets, shorter 2′-*O*-methyl oligoribonucleotide probes can be used in assays in place of longer 2′-deoxy oligoribonucleotide probes, resulting in enhanced discrimination between matched and mismatched RNA targets. A 12 base 2′-*O*-methyl oligoribonucleotide probe had the same T_m as a 19 base 2′-deoxy oligoribonucleotide probe when bound to a matched RNA target but exhibited a much larger decrease in T_m than the 2′-deoxy oligoribonucleotide probe when bound to an RNA target containing either 1 or 2 mismatched bases. The increased T_m, faster kinetics of hybridization, ability to bind to structured targets and increased specificity of 2′-*O*-methyl oligoribonucleotide probes render them superior to corresponding 2′-deoxy oligoribonucleotides for use in assays that detect RNA targets.

INTRODUCTION

The use of oligonucleotides as potential therapeutic agents has gained widespread attention in the last decade (1). Limitations on the use of DNA or RNA oligonucleotides include their toxicity, expulsion from cells, sensitivity to nucleases and insufficient specificity for target nucleic acids *in vivo*. In an effort to overcome these limitations a wide variety of modifications to the bases, sugar ring and/or backbone of oligonucleotides have been examined (1,2). One promising group of oligonucleotides is the 2′-*O*-alkyl oligoribonucleotides. These oligonucleotides have been shown to be nuclease resistant (3–8) and exhibit high affinities for RNA targets (3,4,8–10). Many studies have been published regarding the potential *in vivo* therapeutic application of 2′-*O*-alkyl oligoribonucleotides (4,11–17), but the potential use of these oligomers as diagnostic probes has not been extensively investigated.

In this study we examined the use of oligoribonucleotides containing 2′-*O*-methyl groups as nucleic acid probes. The 2′-*O*-methyl group is a naturally occurring modification found in RNA that enhances affinity for RNA targets due to the preference of 2′-*O*-methyl-modified ribose sugars to adopt a C_3′-*endo* conformation (18 and references therein). Here we carefully characterized oligonucleotide probes containing 2′-*O*-methyl-nucleotides and 2′-deoxynucleotides with respect to a number of physical properties that are important for their use as diagnostic probes. Our measurements revealed that the faster hybridization kinetics, increased T_m, ability to bind structured targets and enhanced binding specificity of 2′-*O*-methyl oligoribonucleotide probes render them superior to corresponding 2′-deoxy oligoribonucleotide probes in diagnostic assays that detect RNA molecules. Similar improvements in probe performance are expected for other modifications that also enhance the ability of the sugar ring to adopt a C_3′-*endo* conformation.

MATERIALS AND METHODS

Oligonucleotide synthesis and purification

Oligonucleotides were synthesized using standard phosphoramidite chemistry and purified using standard polyacrylamide gel electrophoresis. 2′-*O*-methylphosphoramidites (A, G, C and U) were purchased from Glen Research (Sterling, VA).

Labeling and purification of acridinium ester-labeled probes

An amine-terminated linker arm was incorporated at a pre-determined position in each oligomer during synthesis using an abasic linker arm chemistry (19). The oligomers were then labeled with acridinium ester as previously described (20) and purified by reversed phase HPLC (Vydac C4 column; Western Analytical Products, Hisperia, CA) with a binary solvent system consisting of 0.1 M triethylammonium acetate, pH 7.0, and

*To whom correspondence should be addressed. Tel: +1 619 410 8832; Fax: +1 619 410 8871; Email: mickb@gen-probe.com

acetonitrile. The acetonitrile was increased from 5 to 25% (v/v) over 40 min at a flow rate of 1.0 ml/min and the desired fractions collected and precipitated with ethanol.

$C_o t$ analysis

An acridinium ester-labeled probe (0.25 fmol) was hybridized to 0.25–300 fmol target sequence in 60 µl hybridization buffer (0.1 M lithium succinate, pH 5.1, 8.5% w/v lithium lauryl sulfate, 125 mM LiOH, 1.5 mM EDTA and 1.5 mM EGTA) at 60°C for 45 min. Following hybridization, acridinium ester associated with unhybridized probe was destroyed *in situ* by adding 150 µl hydrolysis buffer (0.19 M $Na_2B_4O_7$, pH 7.6, and 5% v/v Triton X-100) and heating at 60°C for 10 min. Chemiluminescence of the solution was measured during a 2 s interval in a Leader I luminometer with automatic injection of 0.1% (v/v) H_2O_2 and 1 mM HNO_3 followed by 1 N NaOH. A plot of log percent maximum chemiluminescence versus log $C_o t$, where C_o is the initial concentration of the target in nucleotides per liter and t is the hybridization time in seconds, was used to calculate the second order rate constant, k_o, where percent maximum chemiluminescence = $100 \times [1 - \exp(-k_o \times C_o t)]$.

Melting analysis

Equimolar concentrations of oligonucleotide probes and targets (1.6 µM) were dissolved in 60 µl hybridization buffer (see above) and annealed at 70°C for 30 min, followed by slow cooling to room temperature for 3 h. The samples were then diluted into 320 µl of the same buffer and absorbance versus temperature profiles measured at 260 nm over the range 20–100°C at 0.5°C/min with a Beckman DU-640 melting apparatus. For the melting data in Figure 4 and Table 1, a 26 base target was used for probes 26 and 19 bases long, a 16 base target was used for probes 16 and 15 bases long and a 14 base target was used for probes 14 bases or under.

Hybridization to structured RNA

An acridinium ester-labeled probe (50 fmol) was hybridized to 5 fmol *Escherichia coli* rRNA in the presence or absence of 1 pmol helper probes (21) in 15 µl hybridization buffer at 60°C for 1 h. Following hybridization, acridinium ester associated with unhybridized probe was destroyed *in situ* by adding 150 µl hydrolysis buffer and heating at 60°C for 10 min. The 17 base probe was 5′-GCTCGTTG(AE)CGGGACTTA-3′, while the 26 base probe was 5′-GCTCGTTGCGGGACTT(AE)AACCCAA-CAT-3′, where AE denotes the site where acridinium ester was inserted.

RESULTS

Melting behavior

Previous studies showed that 2′-*O*-methyl-modified oligoribonucleotide probes exhibit higher affinity for RNA targets than corresponding 2′-deoxy oligoribonucleotide probes (3,4,8–10), resulting in higher melting temperatures. In order to systematically compare the melting behavior of 2′-*O*-methyl and 2′-deoxy oligoribonucleotides, probes of varying length were derived from a ‘parental’ 26 base sequence so as to constrain the GC content of each probe to ~50% (Fig. 1A, oligonucleotide series 1). The

A

Oligonucleotide series 1 Length

```
5′-GCTCGTTGCGGGACTTAACCCAACAT-3′  26
      GCGGGACTTAACCCAACAT         19
        CGGGACTTAACCCA            14
    TTGCGGGACTTA                  12
      CGGGACTTAA                  10
       GGGACTTA                    8
```

Oligonucleotide series 2

```
5′-TCCGGTGTACTCACCGGTTCCGTAGA-3′  26
      GGTGTACTCACCGGTTCCGT        20
    GGTGTACTCACCGGT               15
     GTGTACTCACCG                 12
       TACTCACCGG                 10
```

B

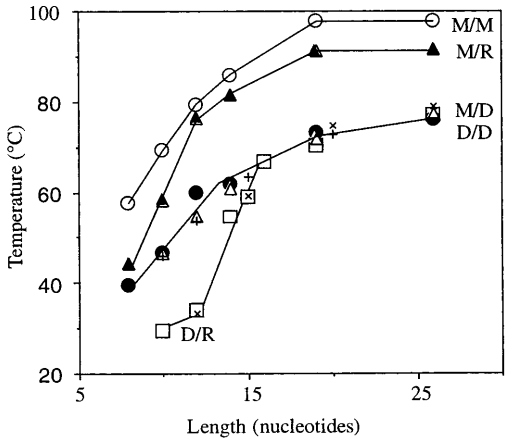

Figure 1. Dependence of T_m on oligonucleotide length. (**A**) Oligonucleotide probes of varying length were derived from two different 26 base long parental sequences (oligonucleotide series 1 and 2) whose complement served as the target sequence. (**B**) Plot of T_m versus length of the oligonucleotide probe. The identity of each probe and target strand is denoted as probe/target, where M = 2′-*O*-methyl, R = RNA and D = DNA. Data points denoted ○ (M/M), ● (M/D), △ (D/D), ▲ (M/R) and □ (D/R) were derived from oligonucleotide series 1, while data points denoted + (D/D) and × (D/R) were derived from oligonucleotide series 2.

probes were synthesized to contain either all 2′-*O*-methyl- or all 2′-deoxynucleotides and the T_m of each probe bound to a complementary DNA, RNA or 2′-*O*-methyl target was determined by UV spectroscopy (Fig. 1B).

Although the T_m of an oligonucleotide will depend upon its sequence, the series of probes used in this study was designed to have very similar base compositions, thereby minimizing sequence effects on T_m. Furthermore, a probe series derived from an entirely different parental sequence (Fig. 1A, oligonucleotide series 2) yielded nearly identical melting behavior (Fig. 1B). Thus, the results in Figure 1 largely reflect the effect of length rather than sequence on T_m. Several features of these data are noteworthy. First, the T_m of all probes increased with length up to 19 nt, at which point T_m reached a plateau. Thus, when binding to a single-stranded target no significant increase in thermal stability was gained by increasing the length of the probes beyond 19 nt in length. Second, hybrid stability followed the order: 2′-*O*-methyl/2′-*O*-methyl > 2′-*O*-methyl/RNA > 2′-*O*-methyl/DNA, 2′-deoxy/DNA ≥ 2′-deoxy/RNA. Thus, 2′-*O*-methyl-nucleotides increased the affinity of oligonucleotide probes for the RNA and 2′-*O*-methyl targets but not the DNA target. Third,

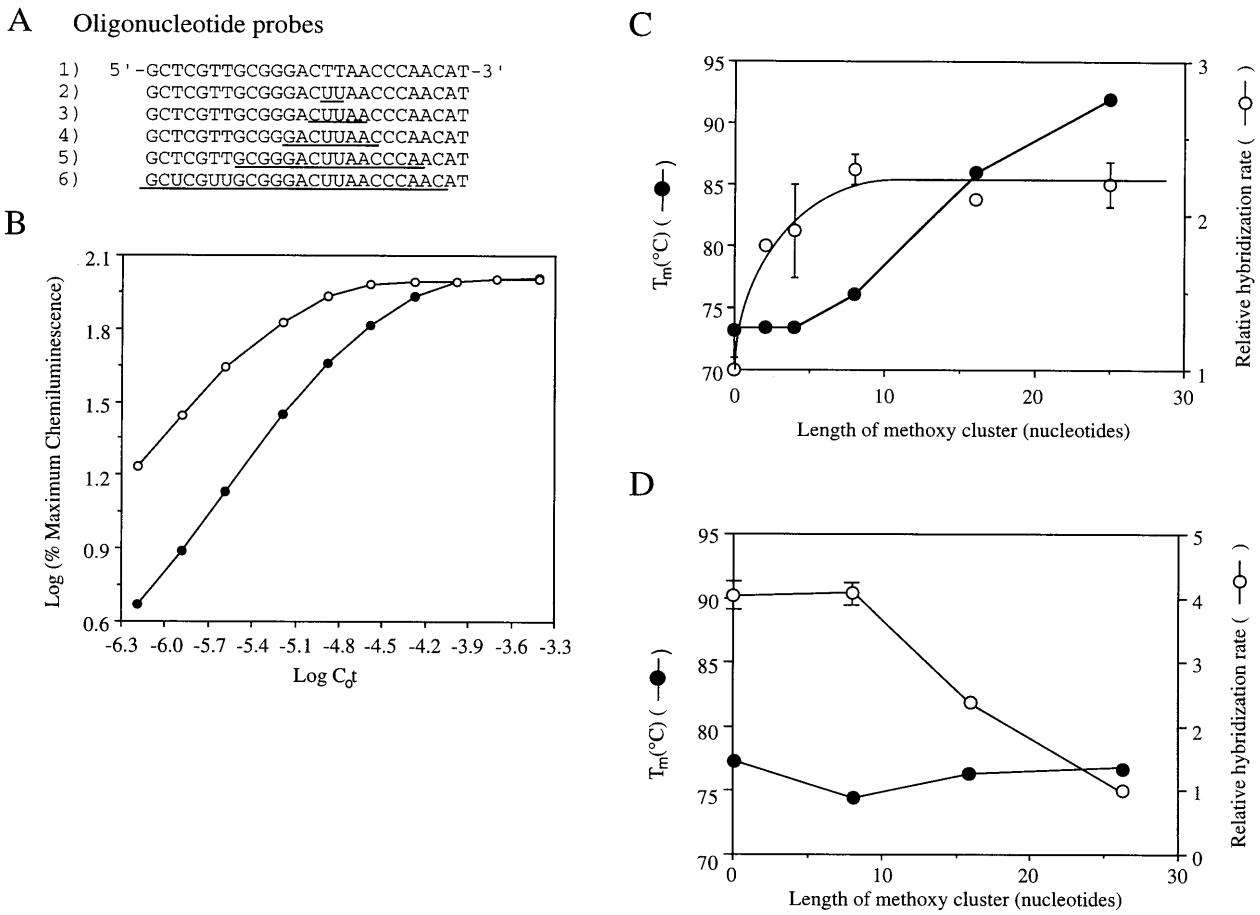

Figure 2. Hybridization kinetics of 2′-*O*-methyl or 2′-deoxy oligoribonucleotide probes binding to RNA or DNA. (**A**) Sequence of oligonucleotides used in this study. Underlined bases denote 2′-*O*-methyl bases. Acridinium ester was inserted into the TT(AE)AA site. (**B**) C_0t curves showing hybridization of 2′-deoxy oligoribonucleotide probe 1 (○) or 2′-*O*-methyl oligoribonucleotide probe 6 (●) to a complementary DNA target. (**C** and **D**) Dependence of the relative rates of hybridization or T_m of oligonucleotide probes in (A) on the length of the 2′-*O*-methyl cluster within the probe. Targets were complementary RNA (C) or DNA (D). Error bars denote one standard deviation. The second order rate constants observed for hybridization of the 2′-deoxy ribonucleotide probe containing no 2′-*O*-methyl ribonucleotides to RNA and DNA targets were 2.9×10^4 and 1.5×10^5/M/s, respectively.

when bound to RNA very short 2′-*O*-methyl oligoribonucleotide probes (e.g. 12 bases) exhibited the same T_m as much longer (e.g. 25 bases) 2′-deoxy oligoribonucleotide probes.

Hybridization kinetics

In order to characterize the hybridization kinetics of 2′-*O*-methyl oligoribonucleotide probes, a model system based on the 26 base probe shown in Figure 2A was designed. A series of probes, each labeled with a chemiluminescent acridinium ester (22), was synthesized with an increasing number of contiguous 2′-*O*-methyl modifications. Each probe was then hybridized to a complementary RNA or DNA target and hybridization kinetics measured by a chemiluminescent method described in Materials and Methods. Figure 2B shows a typical analysis.

As shown in Figure 2C, as few as eight contiguous 2′-*O*-methylnucleotides were sufficient to enhance hybridization rates >2-fold to a single-stranded RNA target. Similar enhancements (2- to 4-fold) were seen for probes of different sequence (results not shown). Even greater rate enhancements were observed for highly structured RNA targets (results not shown and results below).

In contrast to the results observed with an RNA target, increasing the numbers of contiguous 2′-*O*-methylnucleotides within the probe slowed hybridization to a DNA target. A fully substituted 2′-*O*-methyl oligoribonucleotide probe hybridized 4-fold slower to a DNA target than the corresponding 2′-deoxy oligoribonucleotide probe (Fig. 2D). A similar decrease in hybridization rate (7-fold) was seen for a second probe of different sequence (results not shown).

The ability of 2′-*O*-methylnucleotides to alter the hybridization rate of a probe to an RNA or DNA target could be related to the ability of 2′-*O*-methylnucleotides to alter the melting properties of the hybridized duplexes. However, as shown in Figure 2C and D, the ability of 2′-*O*-methylnucleotides to alter hybridization rates did not correlate with their ability to alter the T_m of the hybridized duplex.

Hybridization to structured RNA

A novel property of 2′-*O*-methyl oligoribonucleotide probes is their ability to efficiently bind to double-stranded regions of RNA molecules (23; unpublished results). To characterize this reaction in more detail, probes 17 and 26 bases long consisting entirely of

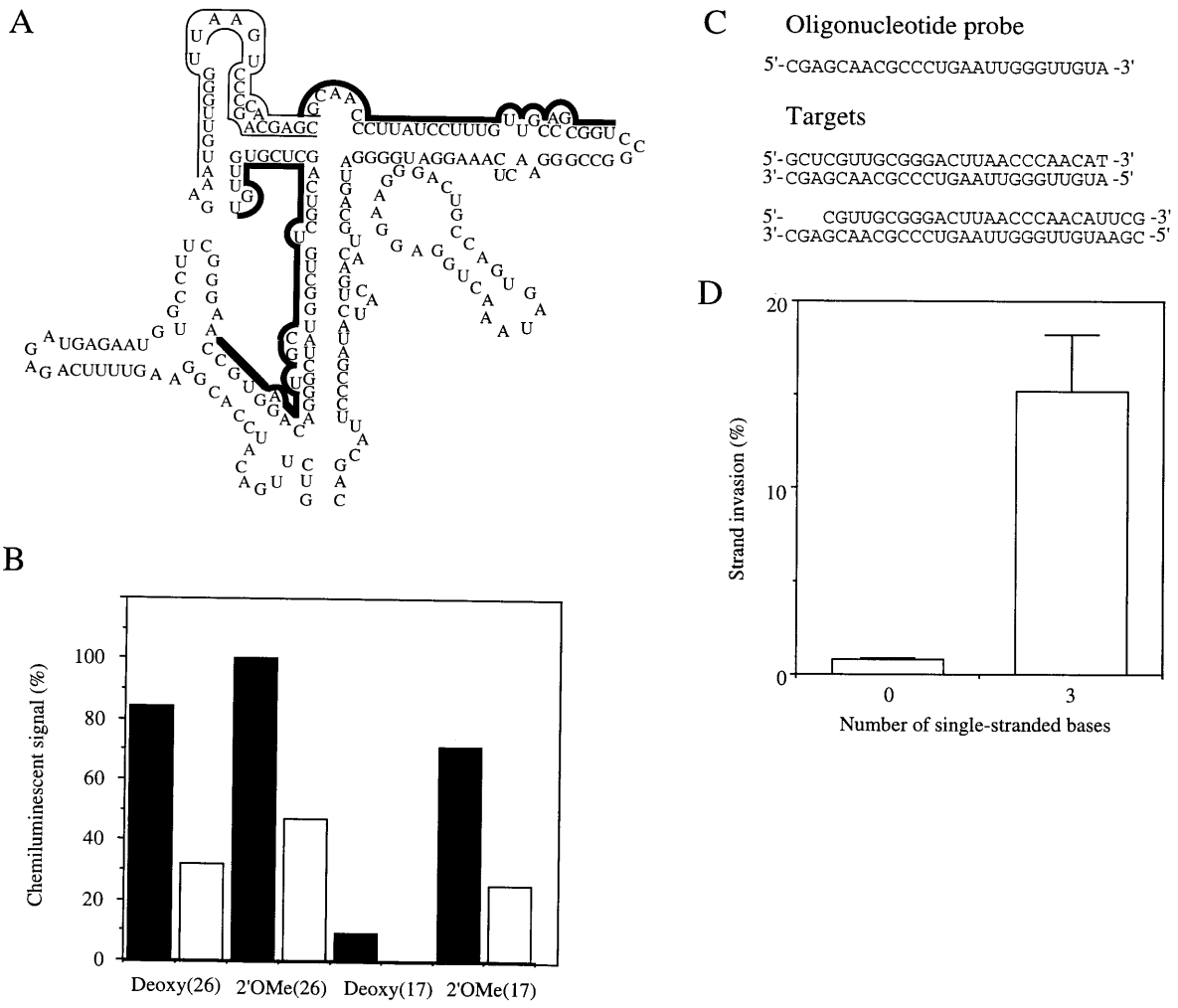

Figure 3. Hybridization of 2′-*O*-methyl and 2′-deoxy oligoribonucleotide probes to a double-stranded region of rRNA. (**A**) Sequence of a portion of 16S rRNA used in these experiments. The location of the 17 and 26 base long oligonucleotide probes (thin lines) and two longer helper probes (thick lines) are denoted. (**B**) Chemiluminescent signal (expressed as a percentage of the maximum observed signal) for hybridization of 17 and 26 base long 2′-deoxy and 2′-*O*-methyl oligoribonucleotide probes to rRNA in the presence (filled bars) or absence (open bars) of two helper probes. (**C**) Oligonucleotide probe and target sequences used in strand invasion experiments. (**D**) Dependence of strand invasion on the number of single-stranded bases adjacent to a double-stranded helix. The probe and target sequences from (C) were hybridized to one another and the extent of binding measured as described in the text.

2′-*O*-methylnucleotides or 2′-deoxynucleotides were labeled with a chemiluminescent acridinium ester label and hybridized to a double-stranded region of rRNA in the absence or presence of helper probes (Fig. 3A). Large concentrations of helper probes assist hybridization of probes to structured RNA molecules by hybridizing to double-stranded regions and displacing sequences complementary to the probe (21). Following hybridization, acridinium ester associated with unhybridized probe was destroyed *in situ* by alkaline hydrolysis and the chemiluminescent signal from hybridized AE probe measured (20). As summarized in Figure 3B, the short 17 base 2′-deoxy and 2′-*O*-methyl oligoribonucleotide probes hybridized to rRNA very differently from one another. In the absence of helper probes the 2′-deoxy oligoribonucleotide probe did not hybridize, whereas the 2′-*O*-methyl oligoribonucleotide probe did. Addition of helper probes resulted in weak hybridization of the 2′-deoxy oligoribonucleotide probe but strong hybridization of the 2′-*O*-methyl oligoribonucleotide probe. Similar results were also observed when a 17 base 2′-deoxy or a 17 base 2′-*O*-methyl oligoribonucleotide probe of different sequence was hybridized to a different double-stranded region of rRNA (results not shown). Since adjacent helper probes allowed binding of the 2′-deoxy oligoribonucleotide probe, the inability of the 2′-deoxy oligoribonucleotide probe to bind in the absence of helper probes was due to its inability to efficiently open and bind to double-stranded rRNA.

In contrast to the 17 base probes, the longer 26 base 2′-deoxy and 2′-*O*-methyl oligoribonucleotide probes efficiently hybridized to rRNA in the absence of helper probes. Helper probes enhanced hybridization of both probes only 2- to 2.5-fold. We conclude that short 2′-deoxy oligoribonucleotide probes lack sufficient affinity for RNA to open and bind double-stranded regions. In contrast, the higher affinity of long 2′-deoxy or short and long 2′-*O*-methyl oligoribonucleotide probes for RNA allows them to efficiently bind to double-stranded regions.

Although these and previous experiments (23) demonstrated that 2′-*O*-methyl oligoribonucleotide probes can efficiently bind to double-stranded regions of RNA, they did not address the mechanism of this reaction. To investigate the mechanism we

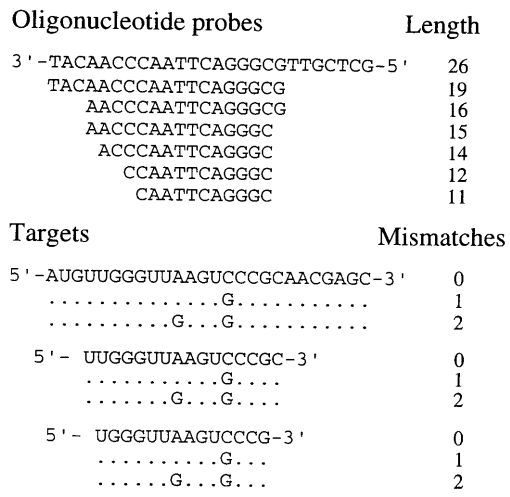

Figure 4. Comparison of the specificity of 2′-*O*-methyl and 2′-deoxy oligoribonucleotide probes for RNA targets. The sequence of the oligonucleotide probes (upper) and RNA targets (lower) used in the study are shown.

compared hybridization of a 2′-*O*-methyl oligoribonucleotide probe labeled with acridinium ester to two nearly identical RNA targets (Fig. 3C), one which was fully double-stranded (T_m = 89°C) and a second which was nearly fully double-stranded but contained a protruding single-stranded tail three bases long (T_m = 87°C). Following hybridization the chemiluminescent signal from hybridized AE probe was measured as before. To establish the chemiluminescent signal corresponding to 100% hybridization the probe was hybridized to a complementary single-stranded RNA target and the chemiluminescent signal from the hybridized probe measured.

Since the T_m values of the two RNA duplexes were nearly identical to one another, significant differences in the binding of the 2′-*O*-methyl oligoribonucleotide probe to these double-stranded molecules cannot be attributed to differences in the thermal stabilities of the RNA duplexes. Furthermore, because the two 2′-*O*-methyl/RNA hybrids which can form in this experiment are identical to one another, differences in binding of the 2′-*O*-methyl oligoribonucleotide probe to these molecules cannot be attributed to differences in the thermodynamic stabilities of the resultant 2′-*O*-methyl/RNA hybrids. As shown in Figure 3D, the 2′-*O*-methyl oligoribonucleotide probe bound the RNA duplex containing the short single-stranded tail 20-fold better than it bound the RNA duplex lacking the tail. We conclude that binding of a 2′-*O*-methyl oligoribonucleotide probe to double-stranded RNA is greatly accelerated by the presence of single-stranded bases adjacent to the double-stranded RNA duplex, which presumably facilitate a strand invasion mechanism.

Specificity

When bound to RNA targets, short 2′-*O*-methyl oligoribonucleotide probes exhibited T_m values equivalent to longer 2′-deoxy oligoribonucleotide probes (Fig. 1). Since mismatches will lower the T_m of a short duplex more than a longer duplex, a short 2′-*O*-methyl oligoribonucleotide probe should discriminate between matched and mismatched RNA targets better than a longer 2′-deoxy oligoribonucleotide probe of equivalent T_m.

Table 1. Melting properties of matched and mismatched 2′-*O*-methyl and 2′-deoxy oligoribonucleotides[a]

Length	2′-*O*-Methyl			2′-Deoxy		
	T_m (°C)	ΔT_m (°C)		T_m (°C)	ΔT_m (°C)	
	Match	Single	Double	Match	Single	Double
26	91.3	9.4	10.4	76.8	5.9	7.8
19	88.9	10.9	15.4	69.8	8.8	11.8
16	89.5	13.9	19.9	63.3	8.0	14.0
15	81.6	14.0	20.0	59.1	12.5	17.5
14	78.8	15.4	24.9	55.4	13.1	19.4
12	71.9	18.7	27.3	nd	nd	nd
11	64.9	20.3	29.9	nd	nd	nd

[a]Oligonucleotide and target sequences are shown in Figure 4. Melting temperatures were determined as described in Materials and Methods. The concentrations of each oligomer used were 1.6×10^{-6} M (26 and 19 base oligomers), 3.2×10^{-6} M (16 and 15 base oligomers) and 4.8×10^{-6} M (14, 12 and 11 base oligomers). T_m values for the 11 and 12 base 2′-deoxy probes were not determined (nd).

To examine this hypothesis, a series of 2′-*O*-methyl and 2′-deoxy oligoribonucleotide probes of varying lengths were hybridized to RNA targets containing 0, 1 or 2 mismatches (Fig. 4) and the T_m values of the resulting duplexes (Table 1) determined by UV spectroscopy. As anticipated, mismatches in shorter duplexes lowered the T_m more than in longer duplexes, yielding larger ΔT_m values between matched and mismatched duplexes for both 2′-*O*-methyl and 2′-deoxy oligoribonucleotide probes. However, for probes of similar T_m, the ΔT_m values between matched and mismatched duplexes were much greater for 2′-*O*-methyl than 2′-deoxy oligoribonucleotide probes. In fact, 2′-*O*-methyl oligoribonucleotide probes of significantly higher T_m than 2′-deoxy oligoribonucleotide probes still yielded larger ΔT_m values. Surprisingly, even for probes of equivalent length the 2′-*O*-methyl oligoribonucleotide probes yielded consistently higher ΔT_m values.

DISCUSSION

The results presented here demonstrate that, regardless of length, 2′-*O*-methyl oligoribonucleotide probes exhibit much higher T_m values when bound to RNA targets than do 2′-deoxy oligoribonucleotide probes of the same length. This statement is true for all probe lengths examined (8–26 bases) and the difference in T_m was most dramatic when probe lengths were 16 bases or less. For example, the T_m of a 12 bp 2′-*O*-methyl/RNA hybrid was 76.6°C, while the T_m of the corresponding 2′-deoxy/RNA hybrid was much lower (33.9°C) (Fig. 1B). T_m values eventually reached a plateau for both 2′-*O*-methyl oligoribonucleotide and 2′-deoxy oligoribonucleotide probes, after which point there was no further increase in T_m afforded by increasing probe length. Therefore, short 2′-*O*-methyl oligoribonucleotide probes, which have equivalent or higher stability when bound to RNA targets than much longer 2′-deoxy oligoribonucleotide probes, yielded the largest advantage over 2′-deoxy oligoribonucleotide probes.

In contrast to the increased T_m observed with RNA targets, 2′-*O*-methyl oligoribonucleotide probes exhibited the same T_m values with DNA targets as the corresponding 2′-deoxy oligoribonucleotide probes. As a result, the length of a 2′-*O*-methyl

oligoribonucleotide probe can be adjusted such that it binds tightly to an RNA target but not to a corresponding DNA target.

In addition to enhanced T_m values when bound to RNA targets, our measurements revealed that 2′-*O*-methyl oligoribonucleotide probes hybridized 2- to 4-fold faster to RNA than 2′-deoxy oligoribonucleotide probes. Enhanced hybridization rates did not correlate with the effect of 2′-*O*-methyl nucleotides on the T_m of the resultant duplex. Even greater rate enhancements were exhibited by 2′-*O*-methyl oligoribonucleotide probes when the target sequence contained double-stranded base pairs. Surprisingly, as few as eight contiguous 2′-*O*-methylnucleotides were sufficient to maximally enhance hybridization rates to an RNA target. Since 2′-*O*-methylnucleotides greatly enhanced the T_m values of oligoribonucleotide probes bound to RNA (Table 1), yet yielded only modest increases in hybridization rates, the dissociation of 2′-*O*-methyl nucleotides from RNA must be very slow (see also 23).

In contrast to RNA targets, 2′-*O*-methyl oligoribonucleotide probes hybridized to DNA targets 4- to 7-fold more slowly than 2′-deoxy oligoribonucleotide probes. The decrease in hybridization rate did not correlate with the effect of 2′-*O*-methyl nucleotides on the T_m of the resultant hybrid.

In addition to binding tighter and faster to RNA, our measurements revealed that 2′-*O*-methyl oligoribonucleotide probes bound double-stranded regions of structured RNA molecules more efficiently than 2′-deoxy oligoribonucleotide probes. Binding of a 2′-*O*-methyl oligoribonucleotide probe to a double-stranded RNA target required that the probe/target duplex be more stable than the double-stranded region (results not shown) and was greatly accelerated by the presence of a single-stranded region flanking the double-stranded region of the target.

Because of their greatly enhanced T_m when bound to RNA, one can utilize 2′-*O*-methyl oligoribonucleotide probes in diagnostic assays that are considerably shorter than 2′-deoxy oligoribonucleotide probes. An important property of short 2′-*O*-methyl oligoribonucleotide probes is that they discriminate between matched and mismatched RNA targets much better than longer 2′-deoxy oligoribonucleotide probes. For example, although a 12 base 2′-*O*-methyl and a 19 base 2′-deoxy oligoribonucleotide probe had the same T_m for a matched RNA target, the 2′-*O*-methyl oligoribonucleotide probe exhibited a much larger decrease in T_m when bound to a RNA target containing either one or two mismatches than did the 2′-deoxy oligoribonucleotide probe.

In summary, 2′-*O*-methyl oligoribonucleotide probes afford multiple advantages over 2′-deoxy oligoribonucleotide probes for detecting RNA targets, including greatly increased T_m, which allows use of shorter probes, faster kinetics of hybridization, ability to bind to structured targets under conditions where 2′-deoxy oligoribonucleotide probes will not and significantly improved specificity. These advantages render 2′-*O*-methyl oligoribonucleotide probes superior to 2′-deoxy oligoribonucleotide probes for use in assays that detect RNA targets.

REFERENCES

1 Crooke,S.T. and Lebleu,B. (1993) *Antisense Research and Applications*. CRC Press, Boca Raton, FL.
2 DeMesmackera,R.A., Altman,K.-H., Waldner,A. and Wendeborns,S. (1995) *Curr. Opin. Struct. Biol.*, **5**, 343–355.
3 Inoue,H., Hayase,Y., Imura,A., Iwai,S., Miura,K. and Ohtsuka,E. (1987) *Nucleic Acids Res.*, **15**, 6131–6149.
4 Iribarren,A.M., Sproat,B.S., Neuner,P., Sulston,I., Ryder,U. and Lamond,A.I. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 7747–7751.
5 Inoue,H., Hayase,Y., Iwai,S. and Ohtsuka,E. (1987) *FEBS Lett.*, **215**, 327–330.
6 Cummins,L.L., Owens,S.R., Risen,L.M., Lesnik,E.A., Freier,S.M., McGee,D., Guinosso,C.J. and Cook,P.D. (1995) *Nucleic Acids Res.*, **23**, 2019–2024.
7 Sproat,B.S., Lamond,A.I., Beijer,B., Neuner,P. and Ryder,U. (1989) *Nucleic Acids Res.*, **17**, 3373–3386.
8 Lamond,A.I. and Sproat,B.S. (1993) *FEBS Lett.*, **325**, 123–127.
9 Ecker,D.J., Vickers,T.A., Bruice,T.W., Freier,S.M., Jenison,R.D., Manoharan,M. and Zoundes,M. (1992) *Science*, **257**, 958–961.
10 Lesnick,E.A., Guinosso,C.J., Kawasaki,A.M., Sasmor,H., Zounes,M., Cummins,L.L., Ecker,K.J., Cook,P.D. and Freier,S.M. (1993) *Biochemistry*, **32**, 7832–7838.
11 Monia,B.P., Lesnick,E.A., Gonzalez,C., Lima,W.F., Mcgee,D., Guinosso,C.J., Kawasaki,A.M., Cook,P.D. and Freier,S.M. (1993) *J. Biol. Chem.*, **268**, 14514–14522.
12 Larrouy,B., Boiziau,C., Sproat,B. and Toulme,J.-J. (1995) *Nucleic Acids Res.*, **23**, 3434–3440.
13 Dominski,D. and Kole,R. (1994) *Mol. Cell. Biol.*, **14**, 7445–7454.
14 Dean,N.M., Mckay,R., Condon,T.P. and Bennett,C.F. (1994) *J. Biol. Chem.*, **269**, 16416–16424.
15 Chiang,M.Y., Chan,H., Zounes,M.A., Freier,S.M., Lima,W.F. and Bennett,C.F. (1991) *J. Biol. Chem.*, **266**, 18162–18171.
16 Johansson,H.E., Belsham,G.J., Sproat,B.S. and Hentze,M.W. (1994) *Nucleic Acids Res.*, **22**, 4591–4598.
17 Cotten,M., Oberhauser,B., Brunar,H., Holzer,A., Issakides,G., Noe,C.R., Schaffner,G., Wagner,E. and Birnstiel,M.L. (1991) *Nucleic Acids Res.*, **19**, 2629–2635.
18 Thibaudeau,C., Plavec,J., Garg,N., Papchikhin,A. and Chattopadhyaya,J. (1994) *J. Am. Chem. Soc.*, **116**, 4038–4043.
19 Arnold,L.J.,Jr, Reynolds,M.A. and Bhatt,R.S. (1989) European patent application, Bulletin 89/17, Publication no. 0 310 312.
20 Nelson,N.C., Reynolds,M.A. and Arnold,L.J.,Jr (1992) In Kricka,L.J. (ed.), *Nonisotopic DNA Probe Techniques*. Academic Press, San Diego, CA, pp. 275–310.
21 Hogan,J. and Milliman,C. (1991) US patent no. 5,030,557.
22 Mazumder,A., Majlessi,M. and Becker,M.M. (1998) *Nucleic Acids Res.*, **26**, 1996–2000.
23 Hou,Y.-M. and Gamper,H.B. (1996) *Biochemistry*, **35**, 15340–15348.