
Effect of LNA- and OMeN-modified oligonucleotide probes on the stability and discrimination of mismatched base pairs of duplexes

YING YAN^{†,‡}, JING YAN[†], XIANYU PIAO, TIANBIAO ZHANG and YIFU GUAN*

Department of Biochemistry and Molecular Biology, Key Laboratory of Medical Cell Biology of Minister of Education, China Medical University, Shenyang, Liaoning, 110001, China

**Corresponding author (Fax, +86-24-23255240; Email, yfguan55@sina.com)*

[†]These authors contributed equally.

[‡]Current address: Department of Radiotherapy, Shenyang Northern Hospital, Shenyang, Liaoning, 110016, China

Locked nucleic acid (LNA) and 2'-O-methyl nucleotide (OMeN) are the most extensively studied nucleotide analogues. Although both LNA and OMeN are characterized by the C3'-endo sugar pucker conformation, which is dominant in A-form DNA and RNA nucleotides, they demonstrate different binding behaviours. Previous studies have focused attention on their properties of duplex stabilities, hybridization kinetics and resistance against nuclease digestion; however, their ability to discriminate mismatched hybridizations has been explored much less. In this study, LNA- and OMeN-modified oligonucleotide probes have been prepared and their effects on the DNA duplex stability have been examined: LNA modifications can enhance the duplex stability, whereas OMeN modifications reduce the duplex stability. Next, we studied how the LNA:DNA and OMeN:DNA mismatches reduced the duplex stability. Melting temperature measurement showed that different LNA:DNA or OMeN:DNA mismatches indeed influence the duplex stability differently. LNA purines can discriminate LNA:DNA mismatches more effectively than LNA pyrimidines as well as DNA nucleotides. Furthermore, we designed five LNA- and five OMeN-modified oligonucleotide probes to simulate realistic situations where target-probe duplexes contain a complementary LNA:DNA or OMeN:DNA base pairs and a DNA:DNA mismatch simultaneously. The measured collective effect showed that the duplex stability was enhanced by the complementary LNA:DNA base pair but decreased by the DNA:DNA mismatch in a position-dependent manner regardless of the chemical identity and position of the complementary LNA:DNA base pair. On the other hand, the OMeN-modified probes also showed that the duplex stability was reduced by both the OMeN modification and the OMeN:DNA mismatch in a position-dependent manner.

[Yan Y, Yan J, Piao X, Zhang T and Guan Y 2012 Effect of LNA- and OMeN-modified oligonucleotide probes on the stability and discrimination of mismatched base pairs of duplexes. *J. Biosci.* **37** 233–241] DOI 10.1007/s12038-012-9196-4

Keywords. nucleotide analogue; hybridization; mismatch; discrimination

Abbreviations used: LNA, locked nucleic acid; MM, mismatch; OMeN, 2'-O-methyl nucleotide; PM, perfect match; RT-PCR, real-time polymerase chain reaction; SNP, single nucleotide polymorphism

Supplementary materials pertaining to this article are available on the *Journal of Biosciences* Website at <http://www.ias.ac.in/jbiosci/jun2012/supp/Yan.pdf>

1. Introduction

A great number of nucleotide analogues have been developed specifically in recent years (Wilson and Keefe 2006). These nucleotide analogues bearing different chemical modifications have offered many advantages to improve functionalities of synthetic oligonucleotides. Among these nucleotide analogues, β -L-locked nucleic acid (LNA) has attracted a great attention. LNA has a methylene bridge between O2' and C4' atoms of ribose to form a bicyclic ribosyl structure (figure 1). It is this linkage that 'locks' the ribose in the C3'-*endo* sugar pucker conformation, which is observed primarily in A-form DNA and RNA (Braasch and Corey 2001). In addition to the excellent properties of adequate aqueous solubility, low cellular toxicity and high resistance to nuclease hydrolysis, LNA nucleotides have demonstrated an enhanced binding affinity to their complementary oligonucleotides. Previous studies have shown that LNA-modified oligonucleotide probes can increase the duplex stability by 3.0°C to 9.6°C per modification (Braasch *et al.* 2002; Johnson *et al.* 2004; Kaur *et al.* 2007), thus improving the efficacy for medical diagnoses and therapeutic applications (Dominick and Jarstfer 2004; Johnson *et al.* 2004; Uguzzoli *et al.* 2004). It has also been observed that LNA site-specific modifications can regulate polymorphic DNA structures in different environments (Chou *et al.* 2005).

2'-O-methyl nucleotide (OMeN) is another 2'-O-modified nucleotide analogue. Due to attachment of a methyl group to the O2' atom, OMeN also adopts a C3'-*endo* sugar pucker conformation (figure 1). Previous investigations have concluded that OMeN incorporation can reduce the non-specific binding *in vitro* (Majlessi *et al.* 1998; Tsourkas *et al.* 2003; Kierzek *et al.* 2005). When incorporated in oligonucleotide probes, OMeN-modified oligonucleotide probes demonstrated a higher binding affinity towards RNA targets than towards DNA targets (Majlessi *et al.* 1998; Tsourkas *et al.* 2003; Kierzek *et al.* 2005). Additionally, OMeN can provide a faster hybridization dynamics than deoxyribonucleotides (Majlessi *et al.* 1998; Kierzek *et al.* 2005). Due to its excellent properties of *in vivo* stability, biocompatibility and

nuclease resistance, OMeN has been used as a key element in antisense-based therapeutic applications (Burmeister *et al.* 2005; Chen *et al.* 2009; Laursen *et al.* 2010).

Analyses of reported data have revealed that, although LNA and OMeN share the same C3'-*endo* sugar pucker conformation, they do have different impact on the DNA and RNA duplex stabilities. It could be attributed to the intrinsic properties of these 2'-O-modified nucleotides, such as electrostatic interaction, hydration potential, sugar pucker rigidity, glycosidic conformational preference, etc. Literature analyses also revealed that the effects of mismatched hybridization on the duplex stability have been much less explored, particularly for LNA:DNA and OMeN:DNA mismatches. When LNA- or OMeN-modified oligonucleotide probes are used for genetic information analysis, they might hybridize accidentally with the non-target DNA or RNA fragments in the same sample solution; these mismatches will interfere unavoidably with the complementary hybridization and devalue the utilities of these intentionally designed oligonucleotide probes. It is the situation most likely to occur in hybridization-based analyses and diagnoses.

In the current study, we first examined how a single LNA:DNA or OMeN:DNA mismatch influences the hybridization stability, and utilized real-time PCR experiments to confirm our observations. We then simulated a real scenario where LNA-modified oligonucleotides were used as probes to hybridize with a group of DNA targets. These duplexes were designed specially so that each of these duplexes contained a complementary LNA:DNA or OMeN:DNA base pair and a DNA:DNA mismatch. Their collective effect on the duplex stability was evaluated and their usefulness in diagnostic and analytic applications was assessed. Although LNA and OMeN share the C3'-*endo* sugar pucker conformation, they do demonstrate different impacts on the duplex stability and mismatch discrimination. These results and conclusions provide valuable information on how to improve the LNA- and OMeN-modified oligonucleotide probes to achieve a better binding affinity towards their targets and a better capability of discriminating not-targeted nucleotide fragments for gene detection and analysis.

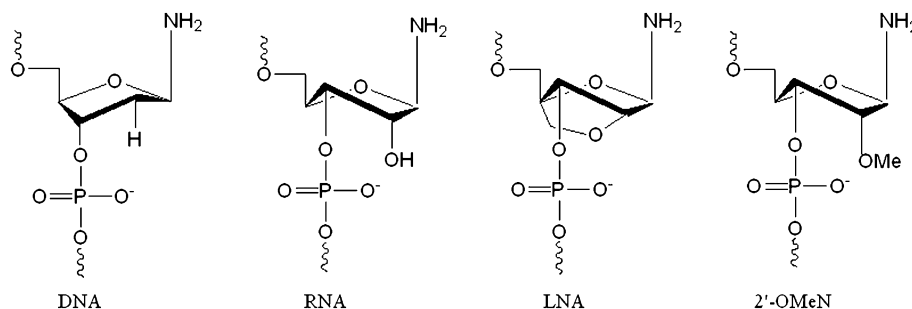


Figure 1. Structures of DNA, RNA, LNA and 2'-OMeN.

2. Materials and methods

2.1 Preparation of oligonucleotides

The nt772-nt791 region of *E. coli* 16S rRNA (access number J01859) was considered the target sequence, and a complementary 20-mer DNA probe was synthesized accordingly. To study the impact of the LNA- and OMeN-modification on the recognition of the non-perfect hybridization, the nucleotide at the selected points on DNA probe was modified with either LNA or OMeN analogue once a time. In addition to the perfectly matched LNA:DNA and OMeN:DNA base pairs, LNA:DNA and OMeN:DNA mismatches were created intentionally for the purpose to examine the mismatched LNA:DNA and OMeN:DNA stability (see supplementary tables 1a, 1b and S1c).

To study the collective effect of a LNA:DNA perfect match and a DNA:DNA mismatch on the duplex stability, 5 LNA-modified oligonucleotide probes were prepared, each having one LNA modification at different positions. Another 17 oligonucleotide strands were also synthesized, each of which were different from the target nucleotide sequence by one nucleotide. Thus, five groups of duplexes were constructed which contain a complementary LNA:DNA base pair at the selected position and a mismatched DNA:DNA at other position. Cytosine substitution was used to create the C:N mismatches considering the fact that the cytosine-containing mismatches are the most unstable ones (Piao *et al.* 2008). The same procedure was also applied to the study of OMeN:DNA hybrids.

Normal oligonucleotides (OPC-grade), LNA-modified oligonucleotides (HPLC-grade) and OMeN-modified oligonucleotides (HPLC-grade) were purchased from TaKaRa Biotech Ltd. (Dalian China). Their stock solutions were prepared with Tris-HCl buffer (0.1 M/L NaCl, pH7.4) to the final concentration of 0.05 mM/L, and stored at -20°C . An equal volume of the probe stock solution and the target stock solution were mixed to the final concentration of a duplex solution of 1 $\mu\text{M/L}$. Each duplex solution was prepared freshly prior to each measurement.

2.2 Melting temperature measurement

Melting curves of oligonucleotide duplexes were recorded on a UV-VIS spectrophotometer (Cary 100, Varian). Eighty microliters of duplex solution in a capped quartz cuvette of 1 cm path length was used in each measurement. To make parallel comparison of the experimental data, a multicell thermal block was used to take temperature measurement of up to six samples simultaneously. The optical density at 260 nm was measured versus temperature in the range from 30°C to 90°C with 1°C increment at a rate of 1°C/min . To

ensure the complementary duplex formation, three denaturation-annealing cycles were performed prior to T_m measurements. Each curve for data analysis was an average of three measurements. Melting temperatures were determined in terms of the peak position of the first-derivative of melting curves. The accuracy of T_m values was within $\pm 0.2^{\circ}\text{C}$ for perfect matches and $\pm 0.5^{\circ}\text{C}$ for mismatched duplexes, respectively.

2.3 Real-time PCR experiment

Eight pairs of primers were designed to amplify a designated fragment of plasmid pcDNA3.1-survivin. All the reverse primers were identical and the forward primer in each pair was different from each other only at the 3'-end (supplementary table 2). The ultimate nucleotide at the 3'-end was either LNA or DNA. Real-time PCR was performed on 7500 Real-Time System (Applied Biosystems, USA). The total volume of PCR system was 25 μL containing 12.5 μL SYBR Premix Ex Taq (TaKaRa), 0.2 ng template, 0.32 μM primers, 0.5 μL Rox Reference Dye, and 10 μL ddH₂O. Thermal cycling conditions were as follows: pre-denaturation at 95°C for 10 min, followed by 40 cycles of a denaturation at 95°C for 5 s and an annealing/extension at 60°C for 34 s. A thermal melting profile was used to confirm the identity and purity of the amplicons.

3. Results and discussion

Rapid progress in genomics has had a profound impact on life sciences in the post-genomics era: single nucleotide polymorphism (SNP) identification, epigenetic analysis, microRNA targeting and genetic diagnosis have become routine approaches in fundamental research and medical utilizations (Braasch and Corey 2001; Wilson and Keefe 2006; Kaur *et al.* 2007). The success of these analyses and diagnoses rely on the specificity of oligonucleotide probes. Based on the principle of complementary hybridization, oligonucleotide probes should hybridize with their DNA or RNA targets through perfect matches (PM), and at the same time, these probes are expected to exclude those non-targeted DNA or RNA fragments that contain the mismatched (MM) base pairs in the hybridized duplexes. It presents a great challenge to improve the ability of oligonucleotide probes to have a high binding affinity towards complementary targets as well as a high discrimination power to effectively repel those nucleotide interferants in the same sample. A proposed solution for this problem is to utilize different nucleotide analogues developed specifically (Wilson and Keefe 2006).

Figure 2a shows melting temperatures of oligonucleotide duplexes containing a complementary DNA:DNA, LNA:

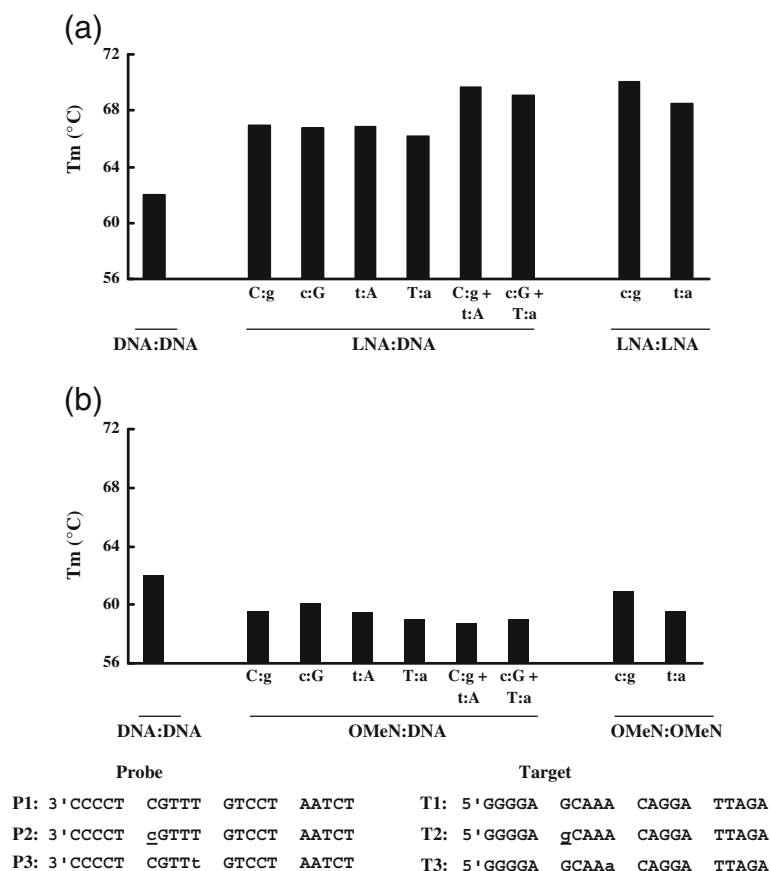


Figure 2. Melting temperatures of duplexes containing a complementary DNA:DNA, LNA:DNA and LNA:LNA base pair (a), and duplexes containing a complementary DNA:DNA, OMeN:DNA and OMeN:OMeN base pair (b). LNA and OMeN nucleotides are labeled in lowercase and underlined. For convenience, the probe strands are written from the 3'-end and the target strands are written from the 5'-end.

DNA or LNA:LNA base pair. As shown, one complementary LNA:DNA base pair (c:G, g:C t:A or a:T_i) can increase the duplex stability by ~5.0°C in comparison with DNA:DNA counterparts. (The lowercases and uppercases represent LNA and DNA nucleotides, respectively.) When oligonucleotide duplexes contain two complementary LNA:DNA base pairs (c:G + a:T or g:C + t:A), the duplex stability is enhanced by ~7.3°C. Furthermore, when two LNA nucleotide forms a complementary LNA:LNA base pair, this

duplex is further stabilized by 6.5°C (t:a) and 8.0°C (c:g), respectively. These data demonstrate a consistency with previously reported results (Vester and Wengel 2004; Kaur *et al.* 2006). Different hypotheses have been proposed to interpret the duplex stability enhancement, including a combination of high amount of counter-ions and low amount of water molecules (Kaur *et al.* 2008), the favourable enthalpy and entropy from the thermodynamic studies (Petersen *et al.* 2000), and a stronger stacking interaction of the local

Table 1. Melting temperatures (°C) of duplexes containing DNA:DNA or LNA:DNA base pairs

| | A/a | G/g | C/c | T/t |
|----|----------------------|----------------------|-----------------------|-----------------------|
| A: | 56.0/60.1 (9.7/10.2) | 58.0/58.8 (6.5/12.0) | 55.2/60.8 (11.0/9.3) | 62.0/66.2 |
| G: | 60.8/62.9 (1.9/6.0) | 59.4/59.3 (4.2/11.2) | 62.0/67.0 | 58.8/63.2 (5.2/4.5) |
| C: | 57.0/59.5 (8.1/11.1) | 62.0/66.8 | 51.0/55.7 (17.7/16.8) | 55.1/59.0 (11.1/10.9) |
| T: | 62.0/66.9 | 57.1/59.2 (7.9/11.5) | 53.4/56.1 (13.9/16.3) | 55.1/58.5 (11.1/11.6) |

*The top numbers in each cell are melting temperatures in °C and the bottom numbers in parentheses are the temperature differences in percentage [$\Delta T_m/T_m(\text{PM}) = (T_m(\text{PM}) - T_m(\text{MM}))/T_m(\text{PM})$]. The numbers before and after the backslash are the melting temperatures of duplexes containing a DNA:DNA base pair and a LNA:DNA base pair, respectively.

phosphate backbone geometry (Nielsen *et al.* 2000; Bondensgarrrd *et al.* 2000; Kaur *et al.* 2006).

As LNA-modified oligonucleotide probes are increasingly being used as a common strategy in bioanalytical applications, LNA:DNA mismatches become noticed. However, the effect of LNA:DNA mismatches on the duplex stability has not been studied systematically. In the current study, we prepared a group of duplexes containing LNA:DNA mismatches to address this question. Table 1 compares melting temperatures of duplexes containing a complementary LNA:DNA base pair, a LNA:DNA mismatch or a DNA:DNA mismatch, respectively. When a canonical T:A base pair was modified to T:a base pair, the duplex stability was elevated from 62.0°C to 66.9°C. When this complementary T:A base pair becomes a mismatched G:A, or A:A, or C:A base pair, the duplex stability is reduced to 60.8, 56.0 and 57.0°C, respectively (equivalent to by -1.9, -9.7 and -8.1%). However, when the complementary LNA-modified T:a base pair becomes a mismatched G:a, or A:a, or C:a base pair, the duplex stability is reduced from 66.9°C to 62.9, 60.1 and 59.5°C, respectively (equivalent to by -6.0, -10.2 and -11.1%). These data indicate that the decreased duplex stabilities caused by mismatched LNA adenine are more than that of the mismatched DNA adenine. We also observed similar phenomenon for LNA guanine residue. The canonical A:G, G:G and T:G mismatches reduce the duplex stabilities by -6.5, -4.2 and -7.9%, whereas the A:g, G:g and T:g mismatches decrease the duplex stabilities by -12.0, -11.2 and -11.5%, respectively. This large temperature drop between the LNA-purine matches and mismatches provides a possibility for the LNA-modified oligonucleotide probes to

distinguish N:a and N:g mismatches more effectively than N:A and N:G counterparts. In contrast, LNA cytosine and LNA thymine do not show such enhanced destabilization effect since the duplex stability was decreased by almost the same degree as that of DNA cytosine and DNA thymine (last two columns in table 1). These results indicate that LNA purines can discriminate the LNA:DNA mismatches more effectively than DNA nucleotides as well as than LNA pyrimidines.

To assess the observed discrimination capability of LNA-modified probes, real-time PCR experiments were conducted. Eight pairs of primers were prepared, where eight reverse primers were identical whereas eight forward primers varied from each other just by the ultimate nucleotide at the 3'-end. The ultimate nucleotide at the 3'-end was either LNA nucleotide or DNA nucleotide as well as either perfect match or mismatch, respectively. When hybridizing with the template, they formed eight duplexes with a LNA:DNA perfect match, a DNA:DNA perfect match, 3 LNA:DNA mismatches and 3 DNA:DNA mismatches at the 3'-end (supplementary table 2). The shape and the cycle threshold number (Ct) of each PCR fluorescence curve were compared.

The forward primers PM-C and PM-c formed complementary matches at the 3'-end, and generated PCR products with Ct numbers of 19.4 and 21.6, respectively (figure 3). When the forward primers MM-T and MM-t created mismatched T:G and t:G at the 3'-end, their Ct numbers of their PCR experiment became 23.1 and 27.8, indicating an increased discrimination capability of LNA modification against mismatches ($\Delta C_t = 3.7$ and 6.2). Similarly, the forward primers MM-A and MM-a had Ct numbers of 28.9 and 37.0, respectively ($\Delta C_t = 7.5$ and 15.4), and the forward

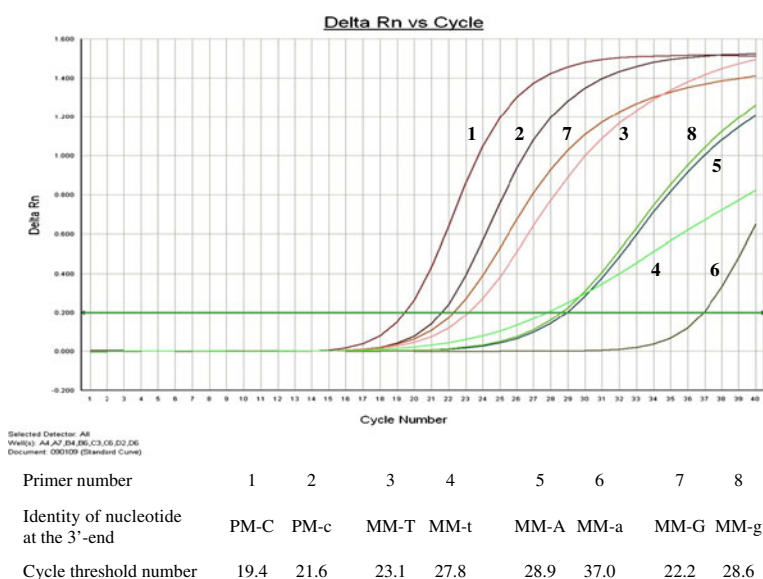


Figure 3. Real-time PCR fluorescence curves of plasmid pcDNA3.1-survivin using eight different forward primers. DNA and LNA nucleotides are labeled in lowercase and uppercase, respectively.

primers MM-G and MM-g had Ct numbers of 22.2 and 28.6 ($\Delta C_t=2.8$ and 7.0). All these data suggest that the mismatched LNA:DNA base pairs could be discriminated more effectively than DNA:DNA mismatches.

In genetic information transfer processes, mutations on DNA or RNA can occur all the time spontaneously or under the influence of certain stimulating factors. Identification of these genetic mutations could uncover the

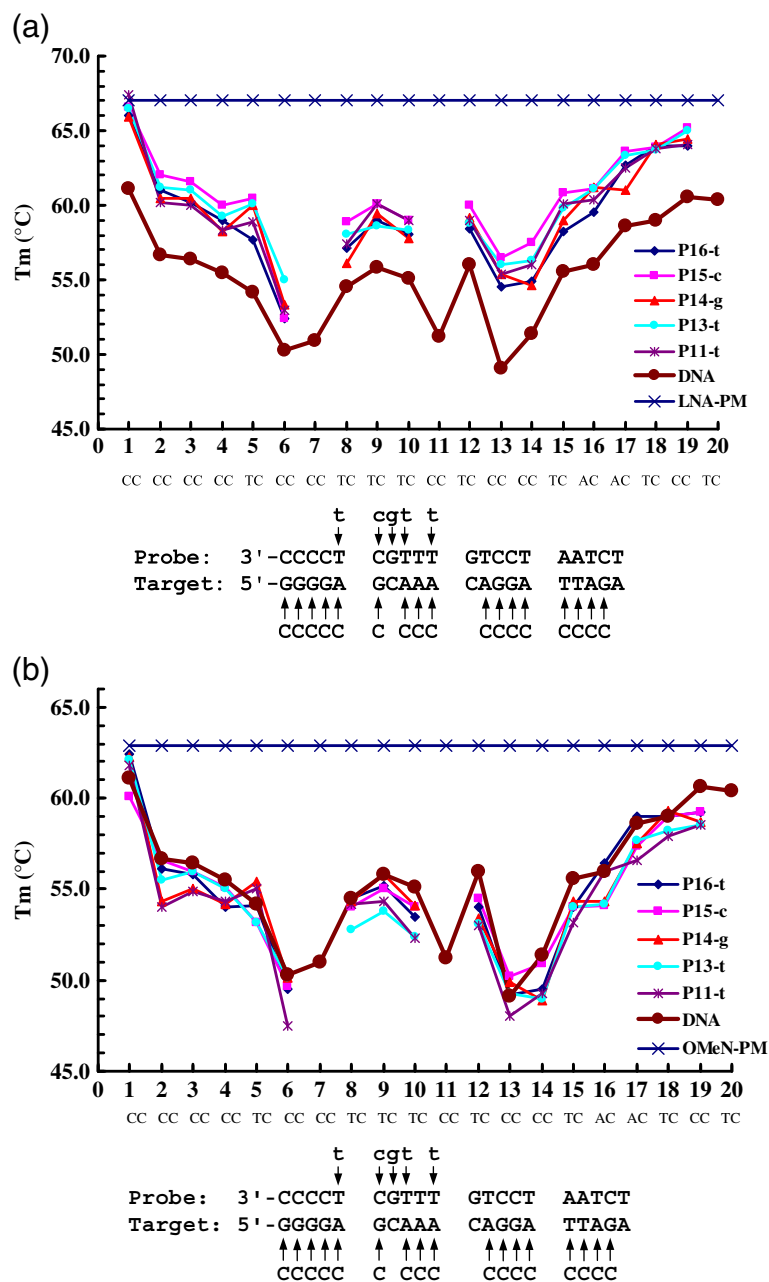


Figure 4. (a) Graphic illustration of collective effect of a complementary DNA:LNA base pair and a DNA:DNA mismatch on the duplex stability. The numbers indicate the position from the 5'-end on the target strand. Letters and arrows above the probe sequence are the positions and types of LNA modification, and C and arrows below the target sequence represent the position and type of mismatched nucleotides intentionally created. (b) Graphic illustration of collective effect of a complementary DNA:OMeN base pair and a DNA:DNA mismatch on the duplex stability. The numbers indicate the position from the 5'-end on the target strand. Letters and arrows above the probe sequence are the positions and types of LNA modification, and C and arrows below the target sequence represent the position and type of mismatched nucleotides intentionally created.

mechanisms of human diseases as well as to pinpoint potential targets for clinical treatment. However, mutation identification is a time-consuming and laborious process, although many robotic nucleotide sequencing instruments of high-throughput and parallel processing capability have been developed. Our finding in the current study presents a feasibility to differentiate different mutations in a more convenient manner, but even this cannot solve these problems completely. For example, oligonucleotide probes modified with LNA adenine can identify the pyrimidine transition mutation (T→C or C→T) more accurately than the purine transition mutation since this transition mutation could create a larger drop of duplex stability (complementary a:T→mismatched a:C). Similarly, LNA guanine can facilitate the detection of the g:C→g:T mutation. By the same token, LNA modifications can advance the epigenetic study as well. DNA methylation occurs naturally where cytosine residues on DNA are methylated, consequently creating different phenotypes without changing the original genetic sequences. To identify the methylated cytosine residues, bisulphite sequencing approach is used to convert the non-methylated cytosines to uracils while remaining the methylated cytosines unchanged (Kanai 2010). In the LNA-modified microarray mini-sequencing, once a cytosine is methylated, the probe will constitute a g:U mismatch on the methylated sites, while the non-methylated cytosines are still perfectly g:C matched. Based on the current study, these two situations could be identified more efficiently than the normal DNA microarray.

When the LNA-modified oligonucleotide probes are used for bioanalytical applications, the detection specificity and accuracy are still facing another challenge. The LNA-modified oligonucleotide probes could have a chance to form duplexes that might contain a complementary LNA:DNA base pair and DNA:DNA mismatches at other positions, mixing the enhanced stability of the LNA:DNA perfect match and the destabilization effect of DNA:DNA mismatches. To examine the mixed effect on the hybridization stability, we designed oligonucleotide probe strands containing single LNA substitution at a selected position. Seventeen oligonucleotide strands were also synthesized, each of which differed from the target strand by one nucleotide at different positions. Thus, when the LNA-modified probe was paired with them, a set of 17 duplexes were constructed which have a complementary LNA:DNA

base pair at the designated position and a DNA:DNA mismatch at another position. LNA-modifications were made at the positions of 11, 13, 14, 15 or 16 on the probe strand, namely P11-t, P13-t, P14-g, P15-c and P16-t, where the letters are the identity of LNA nucleotides and the numbers are the positions from the 5'-end of the probe strand. DNA cytosine was chosen to make mismatches intentionally since it has shown to have the most profound destabilization effect on the duplex stability (Piao *et al.* 2008). The position of the C:N mismatch was designed to move stepwise from the 5'-end to the 3'-end on the target strands.

The stability curves of five LNA-modified oligonucleotide probes are shown in figure 4a. For comparison, the stability curve of the native DNA:DNA duplex (thicker line) is also included. Five LNA-modified probes present the same stability trend as that of the C:N mismatch-containing DNA:DNA duplex. In comparison with the native DNA probe (thicker line), these five curves were elevated by a few degrees. The stability curves of LNA-modified probes display a strong dependence on the positions of the DNA:DNA mismatches regardless of the chemical identities and positions of the LNA modifications. Mismatches at each end of the duplexes experience the least destabilization effect, and the duplexes become destabilized gradually as the mismatch is moving toward the center. These results are compatible with previous observations (Naiser *et al.* 2008a, b; Zhen *et al.* 1997). The most unstable positions take place at the position #6 ($T_m = -13^\circ\text{C}$) and #13 ($T_m = -11^\circ\text{C}$), and this behaviour is still waiting for rationalization.

In contrast, although both LNA and OMeN have a functional group attached to the O2' atom and both share a similar C3'-endo sugar pucker conformation, they do display different types of hybridization behaviour. Figure 2b shows that duplexes containing a complementary OMeN:DNA base pair become destabilize by a few degrees. Duplexes containing double complementary OMeN:DNA base pairs as well as one complementary OMeN:OMeN base pair are also destabilized differently, but there is no additive effect. Previous studies have reported a stability trend of hybrids: OMeN:RNA>DNA:RNA>DNA:DNA>OMeN:DNA, indicating that the OMeN-modified probes preferred to hybridize with RNA targets in a higher binding affinity than with

Table 2. Melting temperatures ($^\circ\text{C}$) of duplexes containing DNA:DNA or OMeN:DNA base pairs

| | A/a | G/g | C/c | T/t |
|---|---------------------|----------------------|-----------------------|-----------------------|
| A | 56.7/58.0 (9.7/1.7) | 58.0/53.7 (6.5/13.1) | 55.2/50.0 (11.0/16.4) | 62.0/59.5 |
| G | 60.8/54.5 (1.9/7.6) | 59.4/61.2 (4.2/1.0) | 62.0/60.1 | 58.8/53.1 (5.2/10.8) |
| C | 57.0/54.0 (8.1/8.5) | 62.0/61.8 | 51.0/49.7 (17.7/17.3) | 55.1/52.3 (11.1/12.1) |
| T | 62.0/59.0 | 57.1/56.2 (7.9/7.4) | 53.4/53.8 (13.9/6.3) | 55.1/50.8 (11.1/14.6) |

normal DNA targets (Tsourkas *et al.* 2003; Kumar and Ganesh 2007). This high binding affinity of OMeN towards RNA could even allow the OMeN probes to bind double-stranded regions of structured RNA, and shorter OMeN-containing probes could replace the longer DNA probes to assay the RNA materials (Majlessi *et al.* 1998).

Table 2 summarizes the melting temperatures of duplexes containing a complementary OMeN:DNA base pair, a OMeN:DNA mismatch or a DNA:DNA mismatch, respectively. As shown, OMeN:DNA mismatches have reduced the duplex stabilities in comparison with that of complementary OMeN:DNA base pairs. Table 2 also shows that the c:C base pair is still the least stable mismatch among the 12 pairs of mismatches (Piao *et al.* 2008). When evaluating the duplex stability in terms of the percentage of the melting temperature decrease, OMeN:DNA mismatches showed similar trends as DNA:DNA mismatches, not offering any advantage.

Using the same procedure, we also assessed the utility of the OMeN-modified oligonucleotide probes by measuring the collective effect of an OMeN:DNA perfect match and a DNA:DNA mismatch on the duplex stability. Five OMeN-modified oligonucleotide probes have been prepared and were used to form 17 duplexes containing an OMeN:DNA perfect match and a DNA:DNA mismatch. As shown in figure 4b, the stability curves also displayed a similar trend to that of the native DNA probes except that the formers were less stable by a few degrees. These curves were strong dependence on the positions of the DNA:DNA mismatch, and, in contrast, the chemical identity and position of OMeN modifications did not influence the duplex stability.

Acknowledgements

The authors are grateful for the financial support to YG from the National Natural Science Foundation of China (No. 31070705) and to YY from the Postdoctoral Research Grant of Ministry of Education (20081042).

References

- Braasch DA and Corey DR 2001 Locked nucleic acid (LNA): fine-tuning the recognition of DNA and RNA. *Chem. Biol.* **8** 1–7
- Braasch DA, Liu Y and Corey DR 2002 Antisense inhibition of gene expression in cells by oligonucleotides incorporating locked nucleic acids: effect of mRNA target sequence and chimera design. *Nucleic Acids Res.* **30** 5160–5167
- Bondensgaard K, Petersen M, Singh SK, Rajwanshi VK, Kumar R, Wengel J and Jacobsen JP 2000 Structural study of LNA:DNA duplex by NMR: conformation and implication for RNase H activity. *Chem. Eur. J.* **6** 2687–2695
- Burmeister PE, Lewis SD, Silva RF, Preiss JR, Horwitz LR, Pendergrast PS, McCauley TG, Kurz JC, Epstein DM, Wilson C and Keefe AD 2005 Direct In Vitro Selection of a 2'-O-Methyl Aptamer to VEGF. *Chem. Biol.* **12** 25–33
- Chen AK, Behlke MA and Tsourkas A 2009 Sub-cellular trafficking and functionality of 2'-O-methyl and 2'-O-methyl-phosphorothioate molecular beacons. *Nucleic Acids Res.* **37** e149
- Chou LS, Meadows C, Wittwer CT and Lyon E 2005 Unlabeled oligonucleotide probes modified with locked nucleic acids for improved mismatch discrimination in genotyping by melting analysis. *BioTechniques* **39** 644–647
- Dominick PK and Jarstfer MB 2004 A conformationally constrained nucleotide analogue controls the folding topology of a DNA g-quadruplex. *J. Am. Chem. Soc.* **126** 5050–5051
- Kanai Y 2010 Genome-wide DNA methylation profiles in precancerous conditions and cancers. *Cancer Sci.* **101** 36–45
- Kaur H, Arora A, Wengel J and Maiti S 2006 Thermodynamic, counterion, and hydration effects for the incorporation of locked nucleic acid nucleotides into DNA duplexes. *Biochemistry* **45** 7347–7355
- Kaur H, Babu BR and Maiti S 2007 Perspectives on chemistry and therapeutic applications of Locked Nucleic Acid (LNA). *Chem. Rev.* **107** 4672–4697
- Kaur H, Wengel J and Maiti S 2008 Thermodynamics of DNA-RNA Heteroduplex Formation: Effects of Locked Nucleic Acid Nucleotides Incorporated into the DNA Strand. *Biochemistry* **47** 1218–1227
- Kierzek E, Ciesielska A, Pasternak K, Mathews DH, Turner DH and Kierzek R 2005 The influence of locked nucleic acid residues on the thermodynamic properties of 2'-O-methyl RNA/RNA heteroduplexes. *Nucleic Acids Res.* **33** 5082–5093
- Kumar VA, and Ganesh KN 2007 Structure-Editing of Nucleic Acids for Selective Targeting of RNA. *Curr. Top. Med. Chem.* **7** 715–726.
- Johnson MP, Haupt LM and Griffith LR 2004 Locked nucleic acid (LNA) single nucleotide polymorphism (SNP) genotype analysis and validation using real-time PCR. *Nucleic Acids Res.* **32** e55
- Laursen MB, Pakula MM, Gao S, Fluiter K, Mook OR, Baas F, Langkjaer N, Wengel SL, Wengel J, Kjems J and Bramsen JB 2010 Utilization of unlocked nucleic acid (UNA) to enhance siRNA performance in vitro and in vivo. *Mol. Biosyst.* **6** 862–870
- Majlessi M, Nelson NC and Becker MM 1998 Advantages of 2'-O-methyl oligoribonucleotide probes for detecting RNA targets. *Nucleic Acids Res.* **26** 2224–2229
- Naiser T, Kayser J, Mai T, Michel W and Ott A 2008 Position dependent mismatch discrimination on DNA microarrays - experiments and model. *BMC Bioinformatics* **9** 509–520
- Naiser T, Ehler O, Kayser J, Mai T, Michel W and Ott A 2008 Impact of point-mutations on the hybridization affinity of surface-bound DNA/DNA and RNA/DNA oligonucleotide-duplexes: comparison of single base mismatches and base bulges. *BMC Biotechnology* **8** 48–70
- Nielsen KE, Singh SK, Wengel J and Jacobsen JP 2000 Solution Structure of an LNA Hybridized to DNA: NMR Study of the d(CTLGCTLTCTLGC) : d(GCAGAAGCAG) Duplex Containing Four Locked Nucleotides. *Bioconjugate. Chem.* **11** 228–238
- Petersen M, Nielsen CB, Nielsen KE, Jensen GA, Bondensgaard K, Singh SK, Rajwanshi VK, Koshkin AA, Dahl BM, Wengel J

- and Jacobsen JP 2000 The conformations of locked nucleic acids (LNA). *J. Mol. Recognit.* **13** 44–53
- Piao XY, Sun LC, Zhang TB, Gan YL and Guan YF 2008 Effects of mismatches and insertions on discrimination accuracy of nucleic acid probes. *Acta Biochim. Pol.* **55** 713–720
- Tsourkas A, Behlke MA and Bao G 2003 Hybridization of 2'-O-methyl and 2'-deoxy molecular beacons to RNA and DNA targets. *Nucleic Acids Res.* **31** 5168–5174
- Ugozzoli LA, Latorra D, Puckett R, Arar K and Hamby K 2004 Real-time genotyping with oligonucleotide probes containing locked nucleic acids. *Anal. Biochem.* **324** 143–152
- Vester B and Wengel B 2004 LNA (Locked Nucleic Acid): High-Affinity Targeting of Complementary RNA and DNA. *Biochemistry* **43** 13233–13241
- Wilson C and Keefe AD 2006 Building oligonucleotide therapeutics using non-natural chemistries. *Curr. Opin. Chem. Biol.* **10** 607–614
- Zhen G, Liu QH and Smith LM 1997 Enhanced discrimination of single nucleotide polymorphisms by artificial mismatch hybridization. *Nat. Biotechnol.* **15** 331–335

MS received 02 September 2011; accepted 09 February 2012

Corresponding editor: BASUTHKAR J RAO