Bioorganic & Medicinal Chemistry Letters 19 (2009) 6585–6587

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Aptamers as a model for functional evaluation of LNA and 2′-amino LNA

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ARTICLE INFO

Article history:
Received 11 August 2009
Revised 6 October 2009
Accepted 7 October 2009
Available online 13 October 2009

Keywords:
LNA
2′-Amino-LNA
Aptamer
Surface plasmon resonance
Affinity

ABSTRACT

The affinity change upon incorporation of LNA and 2′-amino-LNA monomers into an avidin binding DNA aptamer is described. The kinetic profile of selected modified-aptamer was obtained by surface plasmon resonance experiments and compared with the profile of the parent unmodified DNA aptamer. We report significant improvement of avidin binding affinity by the incorporation of single LNA modifications into the aptamer, and successful incorporation of 2′-amino LNA as a novel monomer in aptamers with potential function as carrier unit for additional molecular entities.

Aptamers are synthetic nucleic acids that can recognize a wide variety of targets, from a simple ion to complex structures or even whole cells.[1] They bind to their targets with a dissociation constant (K_D value) typically in the low nanomolar range. Furthermore, they have high specificity with a capacity to distinguish target molecules with minimal structural differences, such as the existence of either a methyl or a hydroxyl group on the target molecule.[2] In many medical applications, high affinity and specific molecular recognition are achieved by using antibodies. However, there are some limitations and disadvantages with antibodies, especially in their production which requires animals or cell lines.[3] In contrast, in vitro selected aptamers can be synthesized in a reproducible manner in short time. Moreover they can easily be modified using chemical methods to improve their thermodynamic stability, decrease their nuclease sensitivity or allow addition of reporter groups to broaden their range of applicability. Aptamers have been widely used in different research areas and they are involved in applications based on molecular recognition, including diagnostics,[4,5] therapeutics,[6,7] and biosensing.[8,9]

Aptamers are composed of RNA or DNA nucleotides but sequences with non-native nucleotides or combination of nucleic acids including LNA (Locked Nucleic Acid), 2′-*O*-methyl and 2′-fluoro nucleotides amongst others have been exploited as well. LNA is one of the most promising modified nucleotides (Fig. 1) for applications within siRNA,[10] antisense oligonucleotides,[11] and aptamers,[12–15] wherein its unprecedented properties regarding stability against nucleases,[16] low-toxicity,[17] binding affinity and nuclease resistance[18–21] are being widely exploited. In the study presented below, a new avidin-aptamer has been modified with LNA and an improvement in affinity of 8.5-fold was observed. In addition, we present the first example of incorporation of 2′-amino-LNA into an aptamer. 2′-Amino-LNA nucleotides (Fig. 1) offer a new alternative as a linker molecule to which a functional group can be attached to the N2′-position.

An avidin-aptamer (61*n* DNA oligomer) with high selectivity and affinity (K_D = 1.30 nM) has previously been reported.[9] In this study, the avidin-aptamer has been truncated and three derivate sequences (49 oligomer, 21 oligomer, and 15 oligomer) were synthesized and their affinity profiles measured by SPR (Surface Plasmon Resonance). The sequences and the obtained K_D values are shown in Table 1.

As the K_D value for the truncated 15*n* oligomer (13.11 nM) is very similar to the K_D value obtained for the truncated 21*n* oligomer (9.79 nM) we believe that positions 16 to 21 in the 21*n* oligomer that constitute the difference between the 21*n* oligomer and the 15*n* oligomer are not very important for affinity, and might

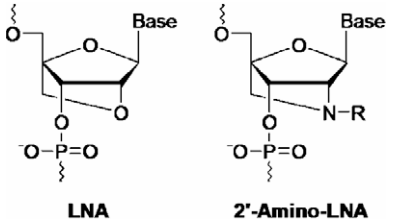

Figure 1. Structures of LNA and 2′-amino-LNA nucleotide monomers.

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doi:10.1016/j.bmcl.2009.10.039

Table 1
K_D value and sequences of truncated H18-avidin-aptamers

H18-avidin-aptamer	Sequence 5′ to 3′	K_D (nM)
61mer[a]	CCAACCGCAATTGTAGTTGACTCAACATAGTACCGGACTCGGCTAATAGACCTGGGGTTGG	1.30
49mer	GCAATTGTAGTTGACTCAACATAGTACCGGACTCGGCTAATAGACCTGG	3.62
21mer	GGCTAATAGACCTGGGGTTGG	9.78
15mer	GGCTAATAGACCTGG	13.11

[a] Original aptamer.[9]

Table 2
List of oligonucleotides synthesized to investigate the effect of incorporation of LNA nucleosides

Aptamer	Sequence 5′ to 3′
Unmodified	GGCTAATAGACCTGGGGTTGG
LNA-G1	G_LGCTAATAGACCTGGGGTTGG
LNA-G2	GG_LCTAATAGACCTGGGGTTGG
LNA-A5	GGCTA$_L$ATAGACCTGGGGTTGG
LNA-A6	GGCTAA$_L$TAGACCTGGGGTTGG
LNA-A8	GGCTAATA$_L$GACCTGGGGTTGG
LNA-G9	GGCTAATAG$_L$ACCTGGGGTTGG
LNA-A10	GGCTAATAGA$_L$CCTGGGGTTGG
LNA-G14	GGCTAATAGACCTG$_L$GGGTTGG
LNA-G15	GGCTAATAGACCTGG$_L$GGTTGG
LNA-G16	GGCTAATAGACCTGGG$_L$GTTGG
LNA-G17	GGCTAATAGACCTGGGG$_L$TTGG
LNA-G20	GGCTAATAGACCTGGGGTTG$_L$G
LNA-G21	GGCTAATAGACCTGGGGTTGG$_L$
LNA-A5A6	GGCTA$_L$A$_L$TAGACCTGGGGTTGG
LNA-A5A8	GGCTA$_L$ATA$_L$GACCTGGGGTTGG
LNA-A5A10	GGCTA$_L$ATAGA$_L$CCTGGGGTTGG
LNA-A6A8	GGCTAA$_L$TA$_L$GACCTGGGGTTGG
LNA-A6A10	GGCTAA$_L$TAGA$_L$CCTGGGGTTGG
LNA-A8A10	GGCTAATA$_L$GA$_L$CCTGGGGTTGG
O-Methyl-G2	GG$_m$CTAATAGACCTGGGGTTGG

LNA nucleotides are shown as A_L and G_L.

be used as a linker region. The 21*n* oligomer was thus selected for incorporation studies of LNA and 2′-amino-LNA nucleotides. We wanted to evaluate both the effect in general from incorporation of one or more LNA nucleoside(s) in the aptamer and the influence from incorporation of a 2′-amino LNA in the carrier region.

To investigate the effect of LNA incorporation all A and G nucleotides in the 21*n* oligomer sequence were systematically replaced by LNA nucleotides. All nine single G-LNA, four single A-LNA substitutions and all six dual A-LNA substitutions were investigated. These 19 sequences (Table 2) were synthesized on an automated nucleic acid synthesizer using commercially available DNA and LNA phosphoramidite building blocks and published procedures.[22]

To assay binding of the LNA containing 21*n* oligomer aptamers to avidin we used SPR. First, avidin was immobilized on a CM5 sensor chip (BIACORE). Next, each aptamer was injected at a flow rate of 15 μL/min, 500 nM final concentration for 3 min at 25 °C. The SPR signals recorded for each sample are shown in Figure 2. The aptamers **LNA-G2** and **LNA-G20** displayed significantly increased target binding compared to the parent 21*n* oligomer. In addition it can be seen that LNA is allowed at many positions without interference with binding.

To further analyze the effect of LNA incorporation, the parent 21*n* oligomer and **LNA-G2** were chosen for synthesis with biotin at the 3′-end. The biotinylated aptamers were bound to the surface of a streptavidin-coated sensor chip SA (BIACORE) in order to perform affinity studies.

SPR analysis was used to determine the kinetic constants at concentrations ranging from 0.2 to 25.6 nM. The K_D for both aptamers were calculated by global fitting of four concentrations of avidin over a constant density aptamer surface (Fig. 3). A 1:1 binding with mass transfer fitting was used to obtain kinetic data and showed χ^2 values below five. It is assumed that a χ^2 value less than 10 provides reliable data for determination of K_D.[23] The obtained K_D values were 9.78 nM for the parent 21*n* oligomer and 1.15 nM for **LNA-G2**. These results are in good agreement with the binding profile presented in Figure 2. The data show an enhancement in affinity of 8.5-fold by the incorporation of one single LNA nucleotide at position 2. Corresponding control interaction studies were performed by the addition of BSA (Bovine Serum Albumin) at 10 nM concentration and showed very low signals for both the parent truncated-21mer and **LNA-G2**.

To explore whether the 8.5-fold enhancement in affinity by incorporation of one single LNA nucleotide at position 2 was due to site specificity or modification specificity we synthesized an aptamer with 2′-*O*-methyl G at position 2 (Table 1) and measured its binding to avidin. As seen in Figure 2 this oligo showed a very weak binding signal that is much lower than the signal from the unmodified-aptamer, indicating that the **LNA-G2** enhancement is due to the LNA modification.

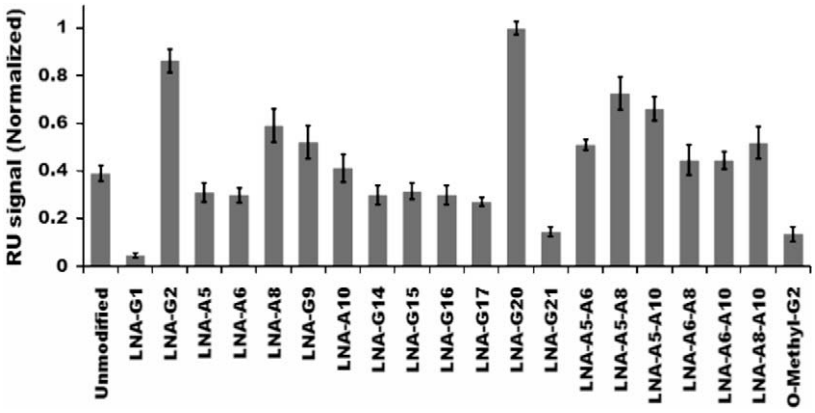

Figure 2. Binding signals of the 21*n* oligomer and the LNA-modified aptamers measured by SPR.

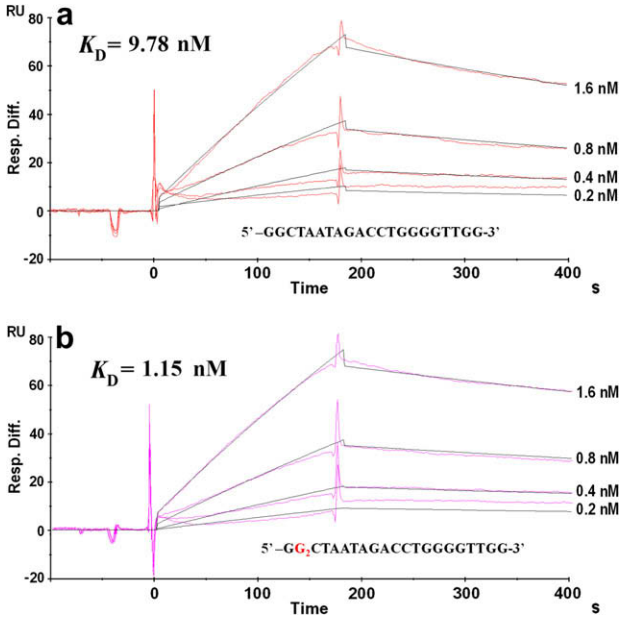

Figure 3. Kinetic analysis of the high-affinity interaction between immobilized aptamers and avidin. Four different avidin concentrations were analyzed and fitted using 1:1 binding with mass transfer. (a) Truncated-21mer, and (b) **LNA2789**.

Based on the results from the affinity analysis, aptamer **LNA-G2** was selected as a template for the incorporation of 2′-amino-LNA nucleotides in the second region encompassing positions 16 to 21. The thymine derivative of 2′-amino-LNA was selected as the modified nucleotide to be incorporated at positions 18 and 19 thus generating **LNA-G2T18$_2$** (G-LNA at position 2 and T-2′-amino-LNA at position 18) and **LNA-G2T19$_2$** (G-LNA at position 2 and T-2′-amino-LNA at position 19). Both these chimeric oligonucleotides were synthesized with a biotin label at the 3′-end to facilitate binding to chips for affinity analysis by SPR as described above. The kinetic profiles showed a K_D value of 4.20 nM for **LNA-G2T18$_2$** and a similar K_D value of 4.97 nM for **LNA-G2T19$_2$**. The profiles with the four avidin concentrations are presented in Figure 4.

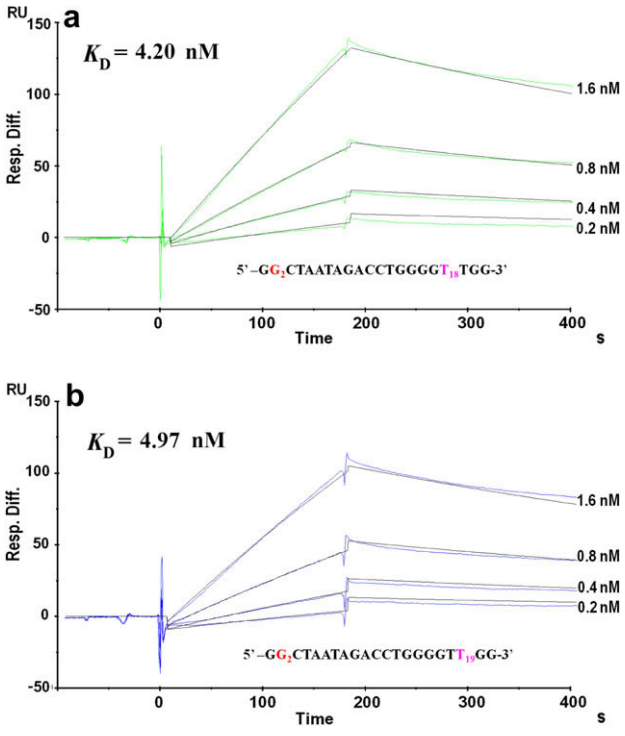

Figure 4. Kinetic analysis of the high-affinity interaction between avidin and (a) immobilized **LNA-G2T18$_2$** and (b) immobilized **LNA-G2T19$_2$**.

The obtained K_D value shows that 2′-amino-LNA nucleotides can be incorporated into a functional nucleic acid such as an aptamer without impeding the molecular recognition. The two chimeric aptamers show similar target affinity in accordance with our view that this region of the aptamer is not critical for recognition and binding. A minor influence is indicated as the insertion of one 2′-amino-LNA nucleotide induce a decrease in affinity compared to **LNA-G2**, but still an increased affinity compared to the parent 21*n* oligomer. These changes might be associated with subtle allosteric or structural changes of the molecule.

In summary, a significant enhancement of avidin-aptamer affinity have been accomplished by the incorporation of single LNA nucleosides and also by combined incorporation of one LNA and one 2′-amino-LNA nucleotide. The latter has been introduced as a novel constituent in aptamers and offers N2′-derivatization which could be useful for future aptamer functionalization.

Acknowledgments

The Nucleic Acid Center is a research center of excellence funded by the Danish National Research Foundation for studies on nucleic acid chemical biology. We thank the Danish National Research Foundation, the Danish Agency for Science Technology and Innovation and the Graduate School for Nucleic Acid Based Drug Design (NAC-DRUG) for financial support.

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