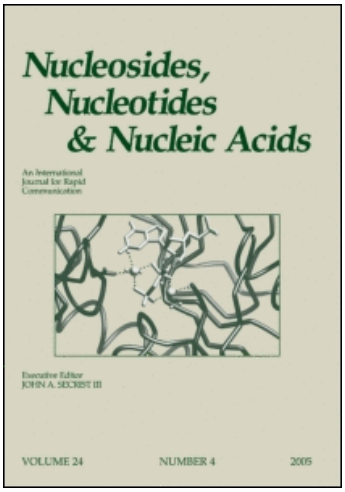

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BENT OLIGONUCLEOTIDE DUPLEXES AS HMGB1 INHIBITORS: A COMPARATIVE STUDY

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□ *In this work we explore the ability of a chimeric LNA/DNA bent duplex, in which the kink is induced by 2 unpaired adenines in the middle of one strand, to bind HMGB1, a protein involved in many inflammatory processes. The LNA/DNA duplex was compared with the corresponding full DNA and PNA/DNA chimera duplexes from a thermodynamic and spectroscopic point of view.*

Keywords LNA; bent duplexes; HMGB1 inhibition

INTRODUCTION

In a recent work[1] we proposed a novel approach to identify new therapeutic targets, based on extracellular proteins which contain nucleic acid binding domains and which are also involved in some pathologies, to be targeted by ODN molecules chosen on the basis of the informations relative to the DNA-protein binding. By selecting extracellular targets one can avoid delivery problems; however the ODN drug has to be resistant to the enzymatic degradation. One of the proteins that fits the aforementioned requirements and that has been object of our research for more than two years is HMGB1. This nuclear protein contains the HMG box, a classical DNA binding domain, and, as it has been recently shown, is involved in a number of different inflammatory diseases when released extracellularly, acting as a cytokine.[2]

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Our idea was to use the HMGB1 nuclear ligand (ODN duplexes) outside its natural location to antagonise RAGE receptor, the protein extracellular ligand involved in the inflammation process (ligand exchange). Since HMGB1 in the nucleus binds DNA through its DNA-binding domains in a manner that does not depend on the nucleobase sequence, playing an architectural role in DNA bending, looping, folding, etc., it should be possible to target HMGB1 by using pre-bent DNA duplexes.

In order to verify our idea we initially designed and synthesized 3 short DNA bent duplexes with different curvature angles, obtained varying the number of unpaired bases (adenines) in the middle of one of the strands (6A, 4A, and 2A DNA duplexes).[3] We demonstrated, by CD spectroscopy (Tandem mix cell), that the protein binds the three bent duplexes.[3] The most stable HMGB1/DNA complex was obtained with the 2A, which was also the most stable duplex. The affinity of the protein for the 2A duplex was studied by CD titration experiments, and a K_d of 25 nM was calculated.[3] We also showed that this kind of duplex is able to inhibit HMGB1 activity in vitro (migration and proliferation assays, IC_{50} ~10 nM).[3]

RESULTS AND DISCUSSION

In order to improve nuclease resistance of the 2A full DNA double helix and to increase its thermal stability, we realized the corresponding PNA/DNA chimera duplex, composed by two chimeric strands carrying three PNA units at 3′ ends with the following sequences: GAACGTAACAAA-GAATCC (**a**) and GGATTCTTTAAGTTACGTTC (**b**). The resulting duplex has both ends capped by a PNA-DNA double helix.

From our studies, PNA/DNA chimeric duplex can bind HMGB1, efficiently inhibits its activity in vitro (IC_{50} ~10 nM) comparably with the full DNA duplex, and is enough resistant to serum nucleases having an half-life of 9 hours in 100% fresh human serum.[3]

Another kind of examined chimeric duplexes contained LNA monomers instead of the PNA ones in the **a** and **b** sequences (duplex **3**).

By UV melting experiments the LNA/DNA chimera duplex **3** resulted the most stable one (T_m 54.6°C, Table 1) in comparison to duplexes **1** and **2**. In agreement with Erdmann et al.,[4] we found that the T_m was raised by

TABLE 1 UV T_m obtained in 100 mM NaCl, 10 mM phosphate buffer, pH 7.5.

Duplex	$T_m \pm 0.5$ (°C)
Full DNA **a–b (1)**	44.7
PNA/DNA chimera **a–b (2)**	43.5
LNA/DNA chimera **a–b (3)**	54.6

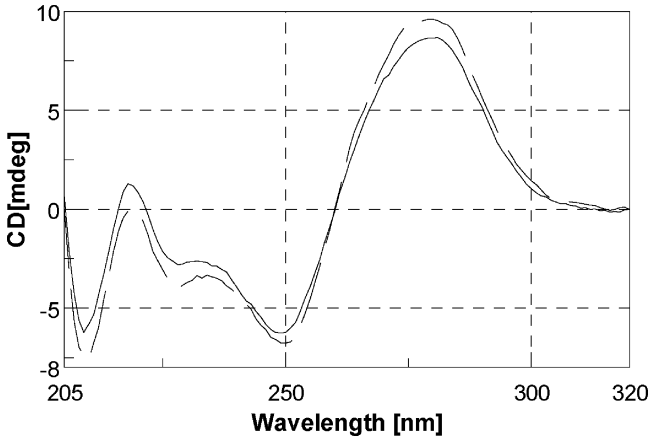

FIGURE 1 Sum (dashed line) and Mix (solid line) CD spectra of HMGB1/duplex **3** in 4:1 ratio.

1.75°C per LNA unit for the LNA/DNA chimera duplex **3** with respect to the full DNA duplex **1**.

To derive structural informations on the bent duplexes **1**, **2**, and **3** we compared their structures by their CD spectra evidencing that the 3 duplexes contain mainly B-form structure, even if, in the case of **3**, some CD characteristics revealed also an A-like contribution.

Successively, we found that also duplex **3** was able to interact with HMGB1, as evidenced by CD spectroscopy using a Tandem Mix cell (Figure 1).

The stability of HMGB1/duplex **3** complex was also estimated by CD ($T_m = 56.2$°C).

The duplex **3** is promising as HMGB1 inhibitor because presents a T_m of 54°C, binds HMGB1 and thus it is interesting to be tested in in vitro studies (data not yet ready).

In order to explore other bent duplexes with high stability, we realized a duplex containing one strand entirely composed of PNA (**a** sequence) and its complementary full DNA strand with the protruding 2A bulge (**b** strand). This duplex showed the highest melting temperature (65.1°C) but failed to bind HMGB1 (CD experiments) probably because its structure (P-like) is different from that of the full DNA duplex **1**, bound by HMGB1, as we revealed comparing their CD spectra. Thus we conclude that an important requirement of ODN duplexes to be used as ligands for HMGB1 is to adopt a B-like structure.

Our future research will focus on: 1) evaluation of the HMGB1 binding affinity to duplex **3**; 2) in vitro tests on the bent LNA/DNA duplex **3**; 3) in vivo tests on both duplexes **2** and **3**; 4) exploration of other classes of bent ODN-like duplexes (*e.g.* 2′-OMeDNA/DNA chimeras); 5) studies of bent duplexes containing bulges with bases different from adenines.

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