Tetrahedron Letters 52 (2011) 300–304

Contents lists available at ScienceDirect

Tetrahedron Letters

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

DNA and RNA binding properties of an arginine-based 'Extended Chiral Box' Peptide Nucleic Acid

Alessandro Calabretta, Tullia Tedeschi, Roberto Corradini, Rosangela Marchelli, Stefano Sforza *

Department of Organic and Industrial Chemistry, University of Parma, Viale G.P. Usberti 17a, University Campus, I-43124 Parma, Italy

ARTICLE INFO

Article history:
Received 3 September 2010
Revised 4 November 2010
Accepted 8 November 2010

Keywords:
Chiral PNA
Binding selectivity
DNA recognition
RNA recognition
Backbone modification

ABSTRACT

Lys-based 'chiral box' Peptide Nucleic Acids (PNAs with three adjacent 2D-Lys-based chiral monomers) have shown unsurpassed specificity in DNA recognition. In this Letter, the binding performances of arginine-based chiral PNAs were evaluated for PNAs containing in the middle part of the strand either a 2D,5L-Arg monomer or three adjacent 2D-; 2D,5L-; 5L-Arg monomers ('Extended Chiral Box'), a combination never studied before. The binding performances of the PNAs were studied by evaluating the melting temperatures of fullmatch and mismatch PNA–DNA and PNA–RNA hybrids and by studying their structure by circular dichroism (CD). The data indicated that the arginine side chains inserted in the PNA structure are perfectly equivalent to lysine side chains as far as oligonucleotide recognition is concerned. The insertion of an 'Extended Chiral Box' into PNA differently influences the binding properties to DNA and RNA: the additional side chains had no observable effect on binding affinity and selectivity toward DNA, whereas, seemed to slightly disturb the binding affinity to RNA but at the same time highly enhancing the recognition selectivity.

1. Introduction

The development of new molecules able to efficiently bind specific nucleic acid sequences is one of the hot topics in the field of nucleic acid chemistry. Improved DNA and/or RNA recognition is actually mandatory for many diagnostic and therapeutic applications. Oligonucleotide-like molecules, either modified in the backbone[1] or in the nucleobases,[2] allowed to obtain, in some cases, promising probes able to bind complementary sequences with enhanced affinity and specificity. Peptide Nucleic Acids (PNAs, Fig. 1A) are among the most promising oligonucleotide analogs.[3] PNAs are chemically different from natural nucleic acids, due to their backbone structure based on a pseudopeptide motif and for the carboxymethylene linker between the backbone and the nucleobases. Anyway, they are able to bind complementary DNA or RNA sequences via Watson–Crick interactions. Due to the neutral backbone and the different structure, PNA–DNA and PNA–RNA complexes show enhanced affinity and specificity, when compared to DNA–DNA or DNA–RNA counterparts.[3] Moreover, PNAs have a very high enzymatic and chemical stability.[4] Many modifications of the basic PNA structure have been studied in order to further improve their properties. The introduction of different type of modifications within the backbone, based on cyclic or acyclic structures,[5] led to significant improvements in terms of affinity for complementary sequences, selectivity, solubility, or bioavaibility.[6]

In particular, chiral amino acid-based PNAs seem to be particularly interesting:[5,7] these molecules are characterized by the presence of one or more chiral monomers substituted in position 2 or 5

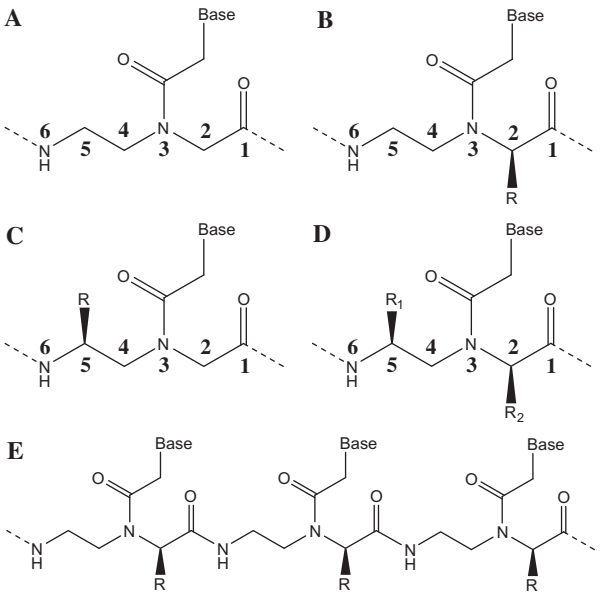

Figure 1. Structure of standard PNA (A) and of different generation of chiral PNAs: 2D (B); 5L (C); 2D,5L (D) and 2D-Chiral Box (E).

* Corresponding author. Fax: +39 0521 905472.
E-mail address: stefano.sforza@unipr.it (S. Sforza).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.11.034

(or both) with amino acid-derived side chains. Chiral PNAs modified with lysine side chains[8] (Lys-PNAs) have been mostly investigated,[8,9] and the results demonstrated that 2D;[10] 5L,[11] and 2D,5L[12] Lys-PNAs (Fig. 1B, C, and D) have enhanced affinity for the complementary nucleic acid sequences compared to the unmodified molecules.[13] As far as selectivity is concerned, all these modifications showed improved specificity in mismatch recognition, and the most selective modified PNAs were the so called 'chiral box' PNAs,[14,15] obtained by inserting three 2D lysine-based modified monomers in adjacent positions in the middle part of the PNA strand (Fig. 1E). 'Chiral box' Lys-PNAs showed, together with a good affinity for perfectly complementary sequences, an extremely high selectivity, which usually results in a total absence of binding when a single mismatch is introduced in the complementary DNA sequence.[14] This property has been exploited in several diagnostic applications.[16,17] Recently, a new class of chiral PNAs has been introduced: arginine-based PNAs (Arg-PNAs), obtained by inserting in position 2 or 5 or both of the backbone, an arginine rather than a lysine side chain. Such molecules demonstrated interesting properties in terms of cellular and nuclear uptake,[18,19] and good performances in the realization of diagnostic devices on the surface,[20] when high selectivity is required.

In order to further improve affinity and selectivity of chiral PNAs, in this work the synthesis and the study of a new class of Arg-PNA will be presented, obtained with three consecutive chiral Arg-based monomers, respectively, substituted in position 2(D-Arg), 2(D-Arg), and 5(L-Arg) and 5(L-Arg). The synthesis of this new PNA, never reported before, will be described and its binding performances toward DNA and RNA will be studied and compared with those of a 2D,5L-Arg PNA having the same sequence, in order to further understand the structural determinants affecting PNA performances. We called this new PNA an 'Extended Chiral Box' PNA, given the presence of four adjacent stereogenic centers.

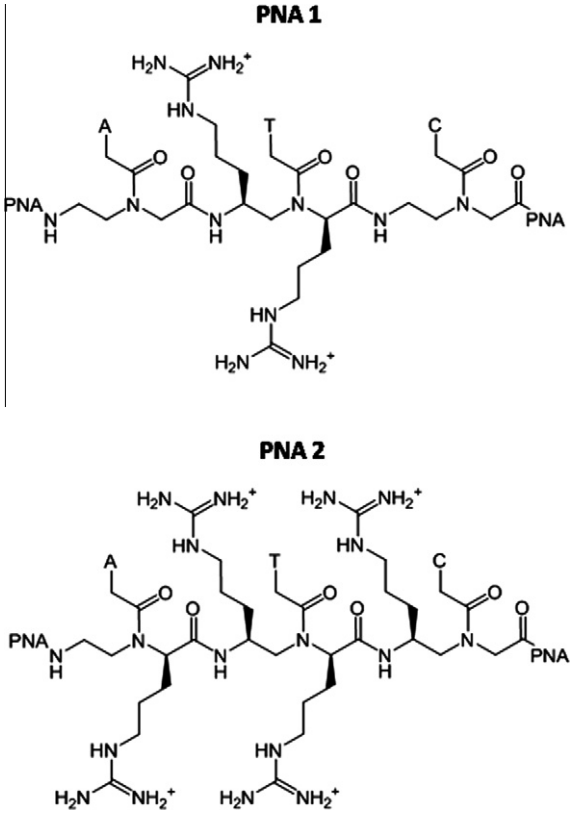

Figure 2. Chemical structure of PNA **1** (one 2D,5L-Arg monomer) and PNA **2** (adjacent 2D-Arg, 2D,5L-Arg, 5L-Arg monomers).

2. Sequence design

Although 2D,5L-Arg PNAs have been already introduced in a previous work,[20] their performances have never been compared with those of Lys-PNAs having the same sequence. For this reason a 2D,5L-Arg PNA, homologous to the Lys-PNA studied in previous works[12,21] was first devised (sequence H-GTAGA**T**CACT-NH_2, with the bold and underlined monomer bearing two 2D,5L stereogenic centers with Arg-based side chains, PNA **1**, Fig. 2). Then, starting from PNA **1**, two Arg-based side chains were designed on both sides of the chiral monomer, on the account of the fact that close proximity of the side chains seemed to help the recognition specificity on the 'chiral box' Lys-PNAs. In all cases, the chosen configurations were those already demonstrated to give the best performances in nucleic acid recognition. For this reason a 2D-Arg monomer was to be included at the N-terminal side of the 2D,5L-Arg-monomer and a 5L-Arg monomer was to be included at the C-terminal side. In this way, a chiral Arg-based PNA bearing four stereogenic centers in close proximity in the middle of the strand was designed (sequence H-GTAG**ATC**ACT-NH_2, with the bold and underlined monomers bearing respectively one 2D, two 2D,5L, and one 5L stereogenic centers with Arg-based side chains, 'Extended Chiral Box' PNA **2**, Fig. 2). Such a design has never been reported before for chiral PNAs.

3. PNA synthesis

Chiral submonomers and chiral monomers were synthesized as described in previous Letters.[20,22] PNAs were synthesized by using the common SPPS protocol based on the Boc strategy for the standard monomers and for the monomer bearing the modification on the carbon atom in position 5, whereas the Boc submonomer strategy was used for the monomers bearing the substituents in position 2 or 2 and 5, in order to preserve the optical purity at the 2 position.[23] According to previously reported procedures,[20,23] the chiral submonomers were inserted by manual coupling with the HATU/DIEA protocol and, after Fmoc deprotection, the nucleobase residues were introduced by a double coupling with DIC/DhBTOH. All the PNA oligomers were purified by preparative RP-HPLC and characterized by LC/ESI-MS and LC/UV.[24]

4. PNA–PNA duplexes

The helical preference of PNA **1** and **2** was studied in order to demonstrate that the modifications inserted within the probe actually induced, as expected,[13,21] a preference for right-handed helical conformation, an essential pre-requisite for efficient DNA binding.

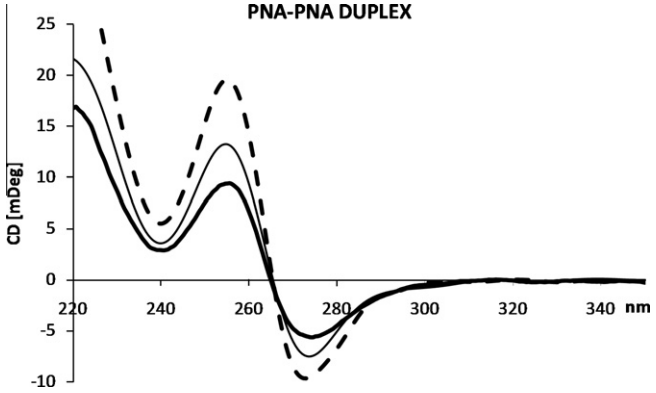

Figure 3. CD spectrum of PNA **1**–PNA duplex (thin line), PNA **2**–PNA duplex (thick line) and 2D,5L-Lys-PNA–PNA duplex (dotted thick line) obtained at 15 °C.