Solid-phase synthesis and properties of chiral peptide nucleic acids bearing cationic side chains

Hirotake Harada, Sho Funayama, Yoshiaki Shoji, and Takeshi Wada

Department of Medical Genome Science, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan

ABSTRACT

A novel chiral peptide nucleic acid, ornithine-based nucleobase-linked polyamide (ONA), aimed to hybrid with a DNA duplex to form triplexes, was designed and synthesized. The chiral monomers composed of the ornithine-based backbone and the serine-derived side chains were synthesized in stereocontrolled manners. The diastereopure oligomers were synthesized by a solid-phase method using the diastereopure monomers. Highly cross-linked polystyrene (HCP) resin was employed as a solid support to minimize the aggregation of the oligomer which was suspected to occur during the chain elongation. By the use of new solid-phase method, homo-thymine ONA oligomers were synthesized in good yields. The ONA hexamer was found to form a triplex with the dsDNA in a sequence specific manner.

INTRODUCTION

One of the most effective antigen reagents is peptide nucleic acid (PNA), in which the negatively charged sugar-phosphate backbone is replaced with neutral 2-aminoethylglycine amides. Due to its non-charged backbone property, PNA forms exceptionally stable complexes with the complementary DNA and RNA. It is also resistant to cellular enzymes such as nucleases and proteases, which will bring about a longer inhibition effect for the target gene expression.

The required properties for ideal antigen reagents are high affinity toward dsDNA, sequence-selective recognition of dsDNA, and resistance to cellular enzymes. The low sequence-selective binding properties of PNA due to its anomalously high affinity toward dsDNA limits its application as an ideal antigen reagent. Other drawbacks of PNA are its ambiguous orientation of the binding and poor solubility in aqueous media.

We, therefore, designed a novel peptide nucleic acid termed ornithine-based nucleobase-linked polyamide (ONA), bearing a chiral backbone and cationic sidechains. In ONA, alanine-based cationic chiral side chains linked with nucleobases is bound to the chiral ornithine-based backbone (Fig. 1). The amino groups incorporated at the side chains is aimed at improving its solubility to water, and induces high binding affinity to dsDNA by strong electrostatic interactions between the cationic side chains and phosphates under physiological pH. Chirality incorporated at the main and side chains would provide opportunities for ONA to induce a helical structure, and therefore would recognize dsDNA in a stereospecific manner. Furthermore, the ornithine-based backbone structure is also intended to realize the sequence-selective recognition of dsDNA. Ornithine-based backbone is destined to be flexible and is presumed to dissociate smoothly when a mismatched base pair exists in the triplex.

RESULTS AND DISCUSSION

Four diastereomers of thymine-ONA monomers were synthesized in solution. Starting from L- or D- ornithine, and L- or D- serine, four kinds of monomers with two chiral centers were synthesized in optically pure forms.
Scheme 1. Solid-phase synthesis of ONA oligomer.

Next, we tried to synthesize ONA oligomers by the solid-phase method on an MBHA Rink amide resin. As the chain length of the oligomer increased, the significant decrease of the coupling yield was observed.

It seemed that the chain elongated ONA oligomers would interact with the oligomers located nearby on the solid support, resulting in the aggregation of oligomers and diminishing its reactivity. To overcome this obstacle, we employed a highly cross-linked polystyrene (HCP) resin. The HCP resin has its 50% of polystyrene cross-linked with divinylbenzene, and therefore having a non-swelling firm structure. In addition, fewer amino groups are loaded to the HCP resin compare to those for the MBHA Rink amide resin. It was expected that its non-swelling property and lower loading amount would prevent oligomers from aggregation and improve the coupling efficiency.

Under the reaction conditions optimized for suppressing racemization during the coupling, the ONA oligomers were successfully synthesized with average coupling yield of over 94%.

The binding affinity of the ONA oligomers toward the complementary dsDNA, ssDNA, and ssRNA was also examined. The thermal denaturation studies revealed that the homo-thymine ONA hexamer formed a stable triplex with the complementary dsDNA. It is noteworthy that the ONA hexamer did not form duplexes with either the complementary ssDNA or ssRNA. These results strongly indicate that ONA would be potentially useful as a new class of antigen reagent.

CONCLUSION

A novel peptide nucleic acid, ornithine-based nucleobase-linked polyamide (ONA), was designed and synthesized. The highly cross-linked polystyrene (HCP) resin was found to be effective for the efficient coupling reaction in the solid-phase synthesis of ONA oligomers.

Its properties as antigen reagent were also explored and the data demonstrated that the ONA oligomer would bind selectively with dsDNAs and not with ssDNAs or ssRNAs.

REFERENCES


*Corresponding Author. E-mail: wada@k.u-tokyo.ac.jp