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COMMUNICATION

Solid-Phase Synthesis of Peptide-Derived Enantiospecific Nucleic Acid Analogs[1]

The first solid-phase synthesis of novel peptide-derived enantiospecific nucleic acid analogs (PDNAs), employing all three stereoisomers of 4-(N^9-adeninyl)-2-amino(*t*-boc)-butyric acid as ADNA (amino acid-derived nucleoside analogs) monomers has been described. The preliminary melting temperature (T_m) data obtained by hybridization experiments demonstrated that these PDNAs can anneal to their complementary nucleic acid strand dT_{10}.

In a previous paper we reported the enantiospecific synthesis of α-amino-acid-derived nucleoside analogs (ADNAs) in which the purine and pyrimidine nucleic acid bases are substituted on the side chain at the γ-carbon. We also stated that these ADNAs could be employed either alone or in association with any other natural amino acid to prepare enantiomerically pure novel oligopeptides, called peptide-derived nucleic acid analogs (PDNAs). We also indicated that to recognize and to hybridize with the complementary nucleic acid (or oligonucleotide) these oligopeptides optimally should be composed of both (D)- and (L)-monomers [i.e., (*R*)- and (*S*)-monomers].

To test this hypothesis we decided to synthesize the following three model homo-PDNAs, employing all three stereoisomers of 4-(N^9-adeninyl)-2-amino(*t*-boc)-butyric acid [AhA, (1 DL, 1L, 1D)] as ADNA monomers. Initially, in order to keep the peptide synthesis simple and to avoid aggregation and/or nonproductive folding of the growing peptide chain during the later stages of synthesis on the solid support, we decided to make 10-mers of AhA (**I**–**III**). The peptide synthesis was carried out semiautomatically on an Applied Biosystems peptide synthesizer (Model 431A) using standard Boc-chemistry protocols (1–3). In order to obtain the end product as an amide at the C-terminus after HF cleavage, *p*-methylbenzhydrylamine resin (0.2 mM scale) was used as the polymeric support to anchor the growing peptide chain. In each case the peptide synthesis was carried out on a 0.2 m*M* scale. Several coupling reagents such as DCC-HOBT, BOP-HOBT, or HBTU-HOAT in the presence of DIEA as a base were used as needed (4–7). Incorporation of each AhA residue was attempted by dissolving a two-equivalent excess of corresponding stereoisomers of Boc-AhA-OH in DMSO/DMF[2] as a

[1] An abstract of this work was submitted to the Division of Medicinal Chemistry on December 9, 1994 and was later presented in part as a poster (MEDI 228) at the 209th American Chemical Society National Meeting held in Anaheim, CA, April 2–6, 1995. A U.S. patent for this work also has been applied for and is pending approval.

[2] Abbreviations used: ODN, oligodeoxynucleotide; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DIEA, diisopropylethylamine; DCC, *N*, *N*-dicyclohexylcarbo-diimide; HOBT, *N*-hydroxybenzotriazole; BOP, benzotriazole-*l*-yl-oxy-tris(dimethylamino)-phosphoniumhexafluoro-phosphate; HOAT, *N*-hydroxy-7-azabenzotriazole; HBTU, 2-(1H-benzotriazole-1-yl)-1,1,3-tetramethyluronium hexafluorophosphate; HF, hydrofluoric acid; HPLC, high-pressure liquid chromatography.

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solvent mixture at elevated temperature (above 80°C) for 5 min, followed by 2 h coupling. Each coupling was monitored for completion by following the Kaiser ninhydrin test (3) and each monomer residue was coupled until the Kaiser ninhydrin test turned negative (two to four times). Additionally, in order to increase the solubility of the resulting PDNAs in water, (L)-lysine was incorporated as the flanking residue both at the N-terminus as well as at the C-terminus in all three model homo-PDNAs using DCC and HOBT as the coupling reagents.

Synthesis of Model I: [*H-Lys-*{(DL)*-AhA*}$_{10}$*-Lys-NH*$_2$]

The synthesis of the racemic Model **I** was accomplished from lysine and the corresponding racemic monomer, (*R*S)-4-(*N*^{9}-adeninyl)-2-amino(*t*-boc)-butyric acid [(DL)-Boc-AhA-OH, 1DL] as the only ADNA monomer unit using BOP and HOBT as the coupling reagents.

Synthesis of Model II: [*H-Lys-*{(D)*-AhA-*(L)*-AhA*}$_5$*-Lys-NH*$_2$]

The new reagent HOAT in combination with HBTU was used as the coupling reagent to synthesize the enantiospecific Model **II** from the corresponding enantiomers (*R*)-4-(*N*^{9}-adeninyl)-2-amino(t-boc)-butyric acid [(*D*)-Boc-AhA-OH, **1D**] and (*S*)-4-(*N*^{9}-adeninyl)-2-amino(*t*-boc)-butyric acid [(L)-Boc-AhA,OH, **1L**] as the adjacent monomeric units.

Synthesis of Model III: [*H-Lys-*{(L)*-AhA*}$_{10}$*-Lys-NH*$_2$]

Similarly, the enantiospecific Model **III** was synthesized from the corresponding enantiomer (*S*)-4-(*N*^{9}-adeninyl)-2-amino(*t*-boc)-butyric acid [(L)-Boc-AhA-OH, **1L**] as the only ADNA monomer unit.

Structural differences between the PDNAs Model **II** and Model **III** can be best viewed in extended β-sheet-like conformations as illustrated by the molecular models shown in Fig. 1. Our hypothesis is that Model **III** should hybridize and form a more stable complex with the complementary thymidine oligonucleotide dT_{10} than Model **II**. Although there have been a few examples in the literature incorporating either (*RS*)- or (*S*)- or (*R*)-stereoisomers of monomers (8–11), there is no precedence for such an enantiospecific oligopeptide incorporating both (*R*)- and (*S*)-stereoisomers in the same molecule.

All three peptides were cleaved from the resin by HF using standard protocols. The crude peptides were extracted from the resin and lyophilized. All three stereospecific peptides were purified by HPLC on a C_{18} reverse-phase column, using water–acetonitrile–0.1% TFA as eluant, and all of them have shown similar chromatographic behavior. The chromatographic separation was somewhat more difficult than anticipated; however, detection of a molecular ion peak of mass (MH^+ = 2456.7) confirmed the presence of the racemic PDNA (Model **I**) in the crude peptide sample by electrospray mass spectrometry. Although the biophysical evaluation in terms of their interaction with the complementary oligonucleotide ideally should require pure samples of these peptides, we decided to test these partially purified peptides as a preliminary evaluation of their effectiveness as the

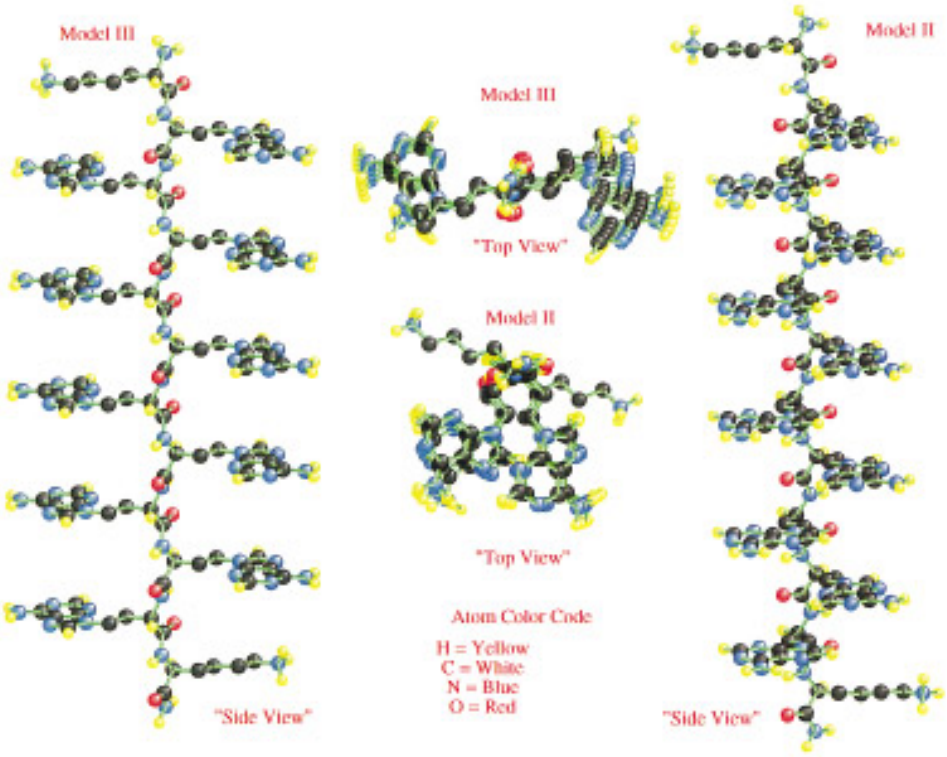

FIG. 1. Modeled structures of PDNAs (Model II and Model III) in β-sheet-like conformation.

nucleic acid binding agents. We have isolated and collected several major peaks with similar retention times for all the PDNA models (**I**–**III**). All these fractions were lyophilized and tested individually against their intended target nucleic acid strand, thymidine deoxyoligonucleotide 10-mer (dT_{10}).

The thermal stability of a nucleic acid strand bound to its complementary strand, a measure of their binding affinity (i.e., hybridization, base-pairing, and base-stacking), is defined by its melting temperature, T_m, at which 50% of each oligonucleotide exits in the single-stranded form. The T_m can be inferred from the hyperchromicity, which is the increase in uv absorbance due to duplex dissociation. Thus the measurement of uv absorbance as a function of temperature can provide qualitative and quantitative structural information about the nucleic acid bound to its complementary strand.

We carried out the binding experiments by hybridizing the isolated HPLC fractions of each of the enantiospecific PDNAs (Models **I**–**III**) to its complementary oligodeoxynucleotide dT_{10}, followed by thermal denaturation and measurement of uv absorbance at 260 nm as a function of temperature. The synthetic oligonucleotides dT_{10} and dA_{10} were used as reference complementary nucleic acid strands. The concentrations of dA_{10}, dT_{10}, and PDNAs were determined spectrophotometri-