BIOORGANIC CHEMISTRY **25,** 321–329 (1997)
ARTICLE NO. BH971078

Hybridization Studies with Chiral Peptide Nucleic Acids

Gordon Lowe,[1] Tirayut Vilaivan, and Martin S. Westwell

Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, U.K.

Received October 16, 1997

A novel class of chiral peptide nucleic acids has been synthesized in which the sugar-phosphate backbone of DNA has been replaced with the glycyl-proline backbone of both the L- and the D-configurations, nucleobases being attached through the 4-position of proline with *cis*- and *trans*-stereochemistry. The T_{10} homopolymers with *cis*-stereochemistry in the L- and D-series bind strongly to poly(dA) with T_m values of 69 and 70°C, respectively. They bind more strongly to poly(rA) with T_m values of 73 and 72°C, respectively, and with apparent 1 : 1 stoichiometry. Using a mixed sequence decamer it was found that the thermal stability of the chiral peptide nucleic acid/oligonucleotide complex was comparable to that formed by Nielsen's polyamide nucleic acid.
Key Words: glycyl-proline backbone; stereoisomers; homo- and heterodecamers; melting temperature; hybrids; oligo- and polynucleotides; peptide nucleic acids; polyamide nucleic acids.

INTRODUCTION

The search for analogs of oligonucleotides which are stable intracellularly and which bind more effectively than natural oligonucleotides to complementary DNA or RNA sequences has been the objective of much endeavor since Zamecnik and Stephenson showed that oligonucleotides could control genetic expression (*1*, *2*). Their observation adumbrated the possibility that oligonucleotide analogs could be used to selectively arrest cellular processing at the translational or transcriptional level, such molecules now being known as antisense or antigene agents, respectively (*3*, *4*). These agents have immense potential, for example, in the identification of gene function from sequence, in diagnosis of genetic diseases, and in therapy. Oligonucleotide analogues reported include mono- (*5*) and dithiophosphates (*6*, *7*), methylphosphonates (*8*, *9*), boranophosphates (*10*), formacetals (*11*), carbamates (*12*, *13*), siloxanes (*14*), dimethylenethio-, sulfoxido-, and sulfono-linked species (*15–18*), etc. (*19*). The discovery by Nielsen and his colleagues that the *N*-(2-aminoethyl)-glycine backbone to which nucleobases are attached through a methylene carbonyl linker binds to complementary oligonucleotides with higher T_ms than oligonucleotides themselves provided great encouragement (*20*). We reasoned that if the sugar-phosphate moiety of nucleotides could be replaced with a dipeptide (both contain six bonds) with appropriate stereochemistry, a novel class of chiral

[1] To whom correspondence should be addressed. Fax: +44(0)1865275674. E-mail: gordon.lowe@chem.ox.ac.uk.

0045-2068/97 $25.00

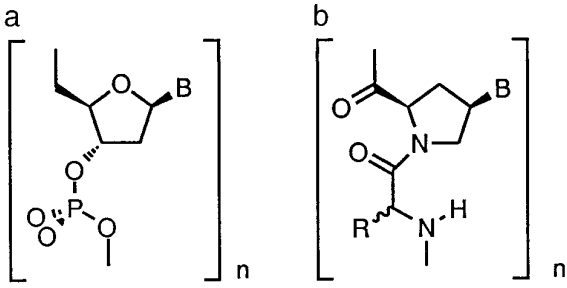


FIG. 1. Structural comparison of (a) DNA with (b) the target chiral peptide nucleic acid.

peptide nucleic acids (cPNAs)[2] could be synthesized by the solid phase method using standard peptide chemistry.

The conformational constraints and stereochemical relationship between substituents on the 2′-deoxyribose ring of nucleotides in DNA appear to be well matched by D-proline with nucleobases incorporated at the 4-position in a *cis*-relationship to the carboxyl group (Fig. 1). Moreover, peptides containing proline derivatives at alternating sites have the added attraction that they are unlikely to be susceptible to proteolysis especially if they have the D-configuration. Numerous amino acids could be used in the "spacer site" of the dipeptide synthons, allowing a whole family of cPNAs to be made with varied physical and biological properties. Thus by using lysine or arginine as a "spacer," a positively charged cPNA could be made which would be expected to bind exceptionally strongly to DNA (*21*). Initially, however, glycine was selected as the spacer primarily for synthetic expediency. In any event such uncharged cPNAs are expected to bind more strongly to nucleic acids than complementary oligonucleotides, and independently of ionic strength, because they do not introduce the electrostatic effect which offsets the binding free energy arising from hydrogen bonding between Watson–Crick base pairs, van der Waals forces, and π-stacking between adjacent base pairs in DNA duplexes (*22*).

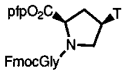
EXPERIMENTAL

The synthesis of the three chiral synthons **1, 3,** and **5** has been reported (*23*) as has the synthesis of T_{10} chiral peptide nucleic acids **2, 4,** and **6** (*24*) (Scheme 1).

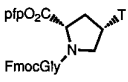
Sterile deionized water was used for all experiments involving oligonucleotides and cPNAs.

Poly(2′-deoxyadenylic acid) [poly(dA)] (sodium salt, average M_r 8.9×10^4) was obtained from Pharmacia Biotech. Polyadenylic acid [poly(rA)] (potassium salt,

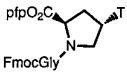
[2] Abbreviations used: cPNA, chiral peptide nucleic acid; PNA, polyamide nucleic acid; Fmoc, 9-fluorenylmethoxycarbonyl; pfp, pentafluorophenyl; A^{Bz}, N^6-benzoyladenin-9-yl; C^{Bz}, N^4-benzoylcytosin-1-yl; G^{Ibu}, N^2-isobutyrylguanin-9-yl; HPLC, high performance liquid chromatography.



1



3



5

H-[Gly-D-Pro(*cis*-4-T)]$_{10}$-Lys-NH$_2$

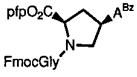
2

H-[Gly-L-Pro(*cis*-4-T)]$_{10}$-Lys-NH$_2$

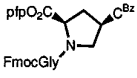
4

H-[Gly-D-Pro(*trans*-4-T)]$_{10}$-Lys-NH$_2$

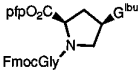
6



7



8



9

H-[Gly-D-Pro(*cis*-4-G)-Gly-D-Pro(*cis*-4-T)-Gly-D-Pro(*cis*-4-A)-Gly-D-Pro(*cis*-4-G)-Gly-D-Pro(*cis*-4-A)-Gly-D-Pro(*cis*-4-T)-Gly-D-Pro(cis-4-C)-Gly-D-Pro(cis-4-A)-Gly-D-Pro(*cis*-4-C)-Gly-D-Pro(*cis*-4-T)-Lys-NH$_2$

10

SCHEME 1

average M_r 7×10^6) was obtained from Fluka Chemicals Ltd. Triethylammonium acetate buffer was prepared by carefully mixing equimolar amounts of triethylamine (redistilled) and glacial acetic acid (redistilled) and then diluting with an appropriate volume of water to give a final concentration of the stock solution of 2.0 M. The pH was then adjusted to 7.4 by addition of acetic acid or triethylamine. Sodium phosphate buffer was prepared by dissolving equimolar amounts of $NaH_2PO_4 \cdot 2H_2O$ (BDH) and Na_2HPO_4 (BDH) in water, the pH was adjusted to 7.0 with diluted HCl or diluted NaOH, and the solution was diluted with water to give a final concentration of 0.50 M.

Oligonucleotides were synthesized by Mrs V. Cooper by the phosphoramidite method on an Applied Biosystems DNA synthesizer (Model 380B). The exocyclic amino protecting groups were removed by heating with concentrated aqueous ammonia solution at 55°C overnight and the solvent was evaporated under vacuum at 40°C on a Savant SpeedVac vacuum concentrator (Savant Instruments). The oligonucleotides were purified by ethanol precipitation in the presence of ammonium acetate, reverse phase HPLC (0.1 M triethylammonium acetate buffer–acetonitrile gradient system), or by an oligonucleotide purification cartridge (OPC column, Applied Biosystems Inc.) as appropriate and were stored as a concentrated aqueous solution at neutral pH at −20°C.

The concentration of oligonucleotide, nucleic acid, and cPNA solutions was determined from the absorbance at 260 nm (OD_{260}). The following molar extinction coefficients (ε) were used without compensation for the hypochromic effect due to the formation of ordered secondary structure of single-stranded nucleic acids [*A*,

15.4 μL/mmol·cm; *T*, 8.8 μL/mmol·cm]. The same values were also used for the chiral peptide nucleic acids.

T_m Measurements

All the T_m measurements were carried out on a Varian CARY 13 UV spectrophotometer equipped with a temperature control system. The instrument was controlled by CARY 13 software running on an IBM PS/2 system, Model 30/286. The sample for T_m measurement was prepared by mixing calculated amounts of stock oligonucleotide and cPNA solutions together and the calculated amounts of NaCl and sodium phosphate buffer (pH 7.0) were then added as stock solutions and the final volumes were adjusted to 3.0 mL by addition of water. The samples were transferred to a 10-mm quartz cell with a Teflon stopper and equilibrated at the starting temperature for at least 30 min. The OD_{260} was recorded in steps from 20 to 95°C (heater temperature) with a temperature increment of 0.25 to 0.5°C/min. The results were normalized by dividing the absorbance at each temperature by the initial absorbance. Analysis of the data was performed with KaleidaGraph software 2.1.3 (Abelbeck Software) running on a MacIntosh LC III computer. The melting temperatures were determined from the maxima of the first derivative plots of the normalized OD_{260} against temperature. Percentage hypochromicity was calculated from the ratio of the OD_{260} at the end of experiment to the initial OD_{260}.

UV-Titration

The UV titration experiment was performed on a Pye Unicam SP8-100 UV spectrophotometer at room temperature. To a solution containing the cPNA **2** (OD_{260} = 0.145; 16.5 μM dT nucleotide) and 10 mM sodium phosphate buffer, pH 7.0 (2.0 mL), was added a 10-mL aliquot of a concentrated stock solution of poly(rA) (OD_{260} = 4.34; 0.28 mM dA nucleotide) in 10 mM sodium phosphate buffer, pH 7.0. The absorbance was read against a blank (10 mM sodium phosphate) and more poly(rA) aliquots were added until a total volume of 500 μL has been added. The ratio of the observed OD_{260} and the calculated OD_{260} (Eq. [1]) were plotted against the mole ratio of T : A nucleotide (Eq. [2]) and the stoichiometry was determined from the inflection point (*25*):

$$\text{calcd. } OD_{260} = \frac{OD_{260}(T) \times V_T + OD_{260}(A) \times V_A}{V_T + V_A} = \frac{0.145 \times 2 + 4.34 \times V_A(\text{mL})}{2 + V_A(\text{mL})} \quad [1]$$

$$\text{ratio of } T:A = \frac{\varepsilon_A \times OD_{260}(T) \times V_T}{\varepsilon_T \times OD_{260}(A) \times V_A} = \frac{15.4 \times 0.145 \times 2}{8.8 \times 4.34 \times V_A(\text{mL})}. \quad [2]$$