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Induction of Helical Handedness and DNA Binding Properties of Peptide Nucleic Acids (PNAs) with Two Stereogenic Centres

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A systematic study on the combined effects of two lysine-derived stereogenic centres at C2 and C5 of a PNA monomer inserted within an achiral PNA strand was performed by circular dichroism, UV spectroscopy and ESI mass spectrometry by taking into account all four diastereomers. The stereogenic centres induced different preferential helix handednesses in the PNA strands, which in turn affected their ability to bind to the complementary DNA. The helical induction, studied on the PNA–PNA duplexes, evidenced that in the case of "chiral conflict" (induction of opposite helices), the stereogenic centre at C5 was clearly prevalent in determining the preferential helix handedness. The PNA–DNA duplex stability was found to be related to the number and the position of the stereogenic centres inducing a preference for a right-handed helix, as well as with the strength of the helical induction. Thermodynamic parameters evaluated by the Van't Hoff model clearly outlined the different enthalpic and entropic contributions of the substitutions at C2 and C5. These findings expand the knowledge of the role of punctual stereogenic centres in inducing supramolecular chirality in PNAs, demonstrate the ability of right-handed DNA to act as a chiral selector and are essential for the future design of chiral PNAs with improved DNA affinity.

Introduction

Peptide nucleic acids (PNAs) are achiral oligonucleotide analogues with a pseudopeptide backbone first introduced in 1991 by Nielsen and coworkers.[1] Given the ability of PNAs to bind to complementary DNA and RNA with outstanding affinity and specificity, a huge number of applications, both in the diagnostic and in the therapeutic field, have been reported in the literature since their first appearance.[2] Moreover, the peptidic nature of PNAs allowed the introduction of many modifications in the original structure by using well-established peptide chemistry.[3] One of the most studied modifications is the introduction of amino acid derived stereogenic centres within the PNA backbone.[4]

Because PNAs assume a right-handed helical conformation upon binding to DNA,[5] their ability to adopt the proper helical conformation that affects the actual binding is influenced by the eventual stereogenic centres present in their backbone. Extensive studies on the influence of chirality of PNA strands on the PNA–DNA duplex stability were performed by our group.[6] In particular we inserted stereogenic centres at the C2 position of the monomer by developing a simple model that allowed to rationalize the effect of PNA configurations.[7] Briefly, when using PNAs with an amino acid derived stereogenic centre at the C2 position, the intrastrand steric hindrance of the amino acid side chain influences the PNA helical structure, which thus imparts a preference for a right-handed (when D-amino acids are used as synthons) or a left-handed (for L-amino acids) helicity. The PNA–DNA duplex stabilities were found to be linked to the helical preferences of the PNA molecules: as DNA is a right-handed helix (in the common B-form), it preferentially bound to D-PNAs, which showed the same preferred handedness. L-PNAs, which favour the "wrong" left-handed helix, were found to bind to DNA more weakly as were forced to assume an unfavourable conformation. This model was consistent with the first-published crystal structure of a PNA–DNA duplex,[5] where the PNA had three chiral D-lysine-based monomers in the middle of an achiral strand ("chiral box"). From the crystal structure, it clearly appeared that the PNA strand in the PNA–DNA duplex had almost the same conformation (P-helix) as in a PNA–PNA duplex: in particular, the D configuration of the stereogenic centre at the C2 carbons was best suited to fit into the P-helix, whereas the pro-L protons were more hindered. Ly et al. demonstrated that this chiral selection might have striking consequences also in vivo: PNAs bearing arginine side chains at the C2 positions were used in order to increase the cellular uptake,[8] but quite interestingly PNAs based on L-arginines showed less sequence

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specificity on the target DNA than PNAs based on D-arginines, although the cellular uptake was similar in the two cases.[9]

Another potential point in the PNA backbone in which a stereogenic centre can be inserted is the C5 position. Several authors inserted L-amino acid derived residues in this position. Seitz et al. synthesized a PNA bearing at the N-terminus a 5-substituted monomer based on L-cysteine: in combination with another PNA strand derivatized at the C terminus as a thioester, PNA synthesis by chemical ligation was performed.[10] Appella et al. synthesized PNAs with 5-substituted backbones derived from L-lysine and found that the lysine side chains could be used as linking groups for different moieties without affecting the PNA binding ability to DNA.[11,12] Ly et al. confirmed that 5-substituted PNAs bearing side chains derived from L-alanine and L-serine actually prefer a right-handed helical conformation.[13] By using NMR and circular dichroism (CD) spectroscopic studies, they showed that a single-stranded PNA dimer derived from L-alanine possessed a right-handed helical conformation and that PNAs bearing an L-serine-derived monomer substituted at C5 showed a very high degree of helical preorganization and very high DNA binding affinity. However, the effect of a 5-substituted PNA monomer based on a D-amino acid has never been studied so far.

In order to improve the PNA binding abilities by inducing the appropriate helical handedness, it is important to determine the effect of multiple stereogenic centres on PNA conformation and DNA binding, particularly if present in the same monomer. Moreover, PNAs may be endowed with new properties derived from several amino acidic side chains simultaneously present in the backbone. By looking at the PNA–DNA crystal structure, it is evident that the steric constraint in the PNA backbone involves the configurations at both C2 and C5, but the simultaneous influence of both stereogenic centres in the same backbone and their mutual interference is difficult to predict and it has never been experimentally studied so far. In a preliminary report we showed how the affinity for DNA can be strongly affected by the simultaneous presence of two stereogenic centres,[14] although no explanations for the observed effects has yet been provided. In order to fully clarify the stereochemical effects at both the C2 and C5 positions, in this work we describe a systematic study of all the possible combinations of configurations. CD spectroscopy was used in order to establish which configuration at which position has the stronger ability to induce right-handed helices and how this in turn can be correlated to DNA binding ability. These studies were carried out by CD and UV spectroscopy and ESI mass spectrometry. Analysis of the UV profiles with the Van't Hoff model was also performed in order to identify the enthalpic and entropic contributions to DNA binding.

Results and Discussion

A preliminary report concerning the synthesis of PNAs bearing in the middle of the sequence 2- and 5-substituted lysine-based monomers with all the possible diastereomeric combinations (2D,5D; 2L,5L; 2D,5L; 2L,5D) was recently published by our group.[14] The mixed purine/pyrimidine sequence chosen was widely used in previous studies[6] on chiral PNAs (H–GTAGATCACT–NH_2; the chiral monomer is underlined). In order to have a complete model system, PNAs bearing one chiral monomer in the middle of the sequence with only one lysine-derived side chain either at the 2- or 5-position were also synthesized. The structures of the PNA monomers and oligomers are reported in Figure 1.

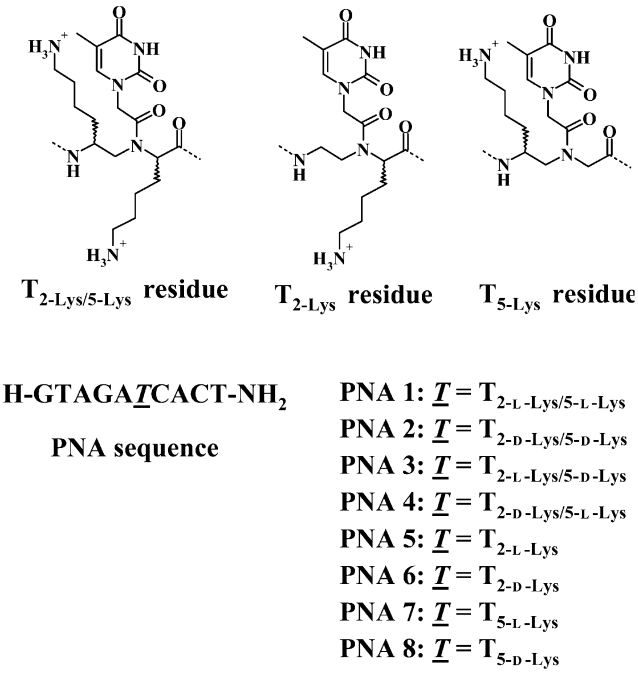

Figure 1. Structures of the modified chiral lysine-based PNA residues and PNA sequences studied in this work.

Induced Preferential Helicity in PNA–PNA Duplexes

The helical preference induced in chiral PNAs is most apparent in PNA–PNA duplexes given their "natural" helical structures (P-helix[15]): if a chiral PNA is hybridized with an achiral one, the resulting duplex generally shows a preferential helix handedness induced by the stereogenic centre(s). Therefore, PNAs **1**–**8** were first hybridized with their complementary antiparallel achiral PNA (H–AGTGATCTAC–NH_2, apPNA) and the preferential helicity was verified by CD spectroscopy. Spectra were recorded for solutions containing the PNA–PNA duplexes (Figure 2).

According to the literature data,[6] CD curves showing positive Cotton effects around 260 nm could be attributed to right-handed helices, whereas curves showing negative Cotton effects around 260 nm could be attributed to left-handed helices, which thus allows a clear-cut attribution of the preferential handedness. A further confirmation of the duplex handedness came by adding 3,3′-diethylthiadicar-

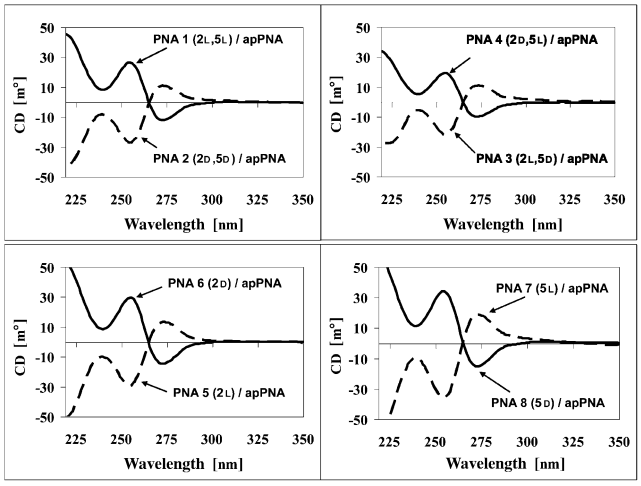

Figure 2. CD spectra of antiparallel PNA–PNA duplexes recorded in phosphate buffer solution at pH = 7 (PNA concentration: 5 μM each strand). Spectra corresponding to right-handed helices are indicated by full lines, spectra corresponding to left-handed helices are indicated by dotted lines.

bocyanine dye to the solutions containing the PNA–PNA duplexes in accordance with a method proposed by Armitage and coworkers.[16] The aggregation of the dye onto the duplexes gave rise to the appearance of a diagnostic band in the visible spectrum at 540 nm: the same band in the CD spectrum was characterized by a typical exciton coupling (due to the oligomerization of the dye) and thus allowed the determination of the handedness of the dye aggregate, which in turn is the same handedness of the PNA–PNA duplex acting as template. The results of these experiments (Supporting Information, Figure S1) fully confirmed the handedness attribution.

The effect of the stereogenic centres on the preferential handedness of the antiparallel PNA–PNA duplexes was quite straightforward when only one stereogenic centre was present. In the 2-position, the D configuration favours right-handed helices (PNA **6**), whereas the L configuration favours left-handed helices (PNA **5**), which is consistent with our previous reports.[6] Quite interestingly, the opposite is true if the stereogenic centre is placed in the 5-position: the D enantiomer favours left-handed helices (PNA **8**), whereas the L enantiomer favours right-handed helices (PNA **7**).

When two stereogenic centres were simultaneously present, two different situations could arise: the stereogenic centres could impart the same preferential handedness ("chiral accordance"), or alternatively, they could give rise to a "chiral conflict" by inducing opposite helical handedness. Cases of "chiral accordance" were represented by PNAs **3** (2L,5D) and **4** (2D,5L). Because the D configuration in the 2-position induced the same handedness as the L configuration in the 5-position (right-handed), PNA **4** was expected to give, as it did, a right-handed helix in the PNA–PNA duplex. Analogously, the L configuration in the 2-position induced the same handedness as the D configuration in the 5-position (left-handed), and therefore, PNA **3** hybridized with its complementary antiparallel PNA giving a left-handed helix. In contrast, in case of "chiral conflict" the outcome was very interesting: for PNA **1** (2L,5L) (the 2L stereogenic centre favours a left-handed helix; the 5L stereogenic centre favours a right-handed helix) a well-defined right-handed helical conformation was found for the PNA–PNA duplex. Thus the 5-position appeared to be prevalent in inducing the preferred helical conformation and completely overwhelmed the effect of the chiral induction exerted by the stereogenic centre at the 2-position. Analogously, in PNA **2** (2D,5D) the two stereogenic centres induce opposite helical preferences, but again the effect of the D stereogenic centre in the 5-position was found to be predominating over that of the D stereogenic centre in the 2-position and the resulting PNA–PNA duplex had a well-defined left-handed helical conformation.

This behaviour may be rationalized according to the following considerations. The preferential helicities are supposed to arise from the attempt to minimize the intrastrand steric clashes due to the amino acid side chains.[7] When a PNA with a "chiral conflict" adopts a P-helix conformation, either right-handed or left-handed, this helical arrangement should be sterically unfavourable for one of its two "conflicting" amino acid side chains; therefore, one or more bonds must rotate in order to minimize the clashes, eventually inducing a modification in the preferred PNA conformation. According to the published crystal structure of a PNA–PNA duplex,[15] the torsion angle between C4 and C5 is the most conserved all along the PNA strands, whereas the C1/C2 and C2/N3 torsion angles show some variability. Therefore, it appears reasonable to assume that, for a PNA in its "natural" P-helix conformation, steric disturbances are less tolerated in the 5-position than in the 2-position, and consequently, the stereogenic centre in the 5-position is "dominant" in helical induction.

PNA–DNA Duplexes: The Stability is Correlated to the Helical Preferences of the PNAs

PNAs **1**–**8** were then hybridized to their complementary antiparallel DNA (5′-AGTGATCTAC-3′, apDNA). Because all diastereomeric PNAs experience the same hydrogen bond pattern, the affinity for DNA should depend only on the preferential helix handedness according to the model previously discussed: PNAs showing a preference for the right-handed helix were expected to give well-defined and stable PNA–DNA duplexes, whereas PNAs showing a preference for the left-handed helical arrangement were expected to bind more weakly to DNA. In order to test this hypothesis, CD spectra were recorded of the hybrid PNA–DNA solutions (Figure 3).

As expected, the CD spectra for the solutions containing the DNA and the PNAs preferring the right-handed helical conformation (PNAs **1**, **4**, **6**, **7**) were typical of antiparallel PNA–DNA duplexes,[5] with a positive band characterized by a maximum around 260 nm and a shoulder around 280 nm. In contrast, the solutions containing the DNA strand and the PNAs preferring left-handed helices showed

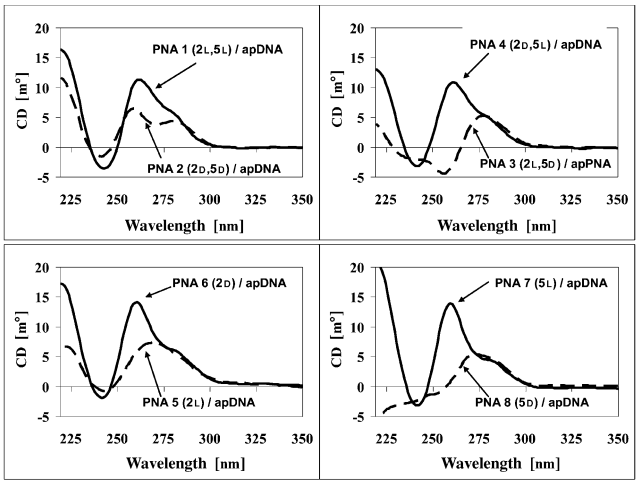

Figure 3. CD spectra of the solutions containing the PNAs and their antiparallel DNA strand recorded in phosphate buffer solution at pH = 7 (PNA and DNA concentrations: 5 μM each strand). Spectra of the solutions containing the PNAs preferring right-handed helical conformations are indicated by full lines and are typical for antiparallel PNA–DNA duplexes, whereas spectra of the solutions containing the PNAs preferring left-handed helical conformations are indicated by dotted lines.

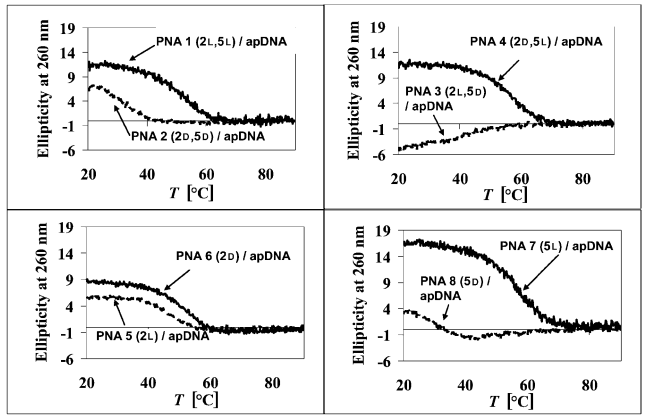

Figure 4. CD melting curves of the hybrid solutions containing the PNAs and the complementary antiparallel DNA strand recorded in phosphate buffer solution at pH = 7 (PNA and DNA concentration 5 μM each strand). The positive CD signal at 260 nm disappears when the PNA–DNA duplex melts; therefore, the CD melting curves of PNA–DNA duplexes are always characterized by a transition from positive ellipticities to zero. Melting temperatures were estimated as the minimum of the first derivatives of the melting curves.

anomalous CD spectra. Only in the case of PNA **2** (2D,5D) and **5** (2L) could the presence of partially formed (about 30–40%, as determined by the CD spectra) PNA–DNA duplexes be hypothesized. A very small amount of duplex (not more than 15%) could be present also in the case of PNA **8** (5D), whereas for PNA **3** (2L,5D) the CD spectra was very similar to the canonical conservative spectrum of a free DNA strand, which indicates that the PNA–DNA duplex is probably not formed.

Further confirmation came from the measurements of the CD melting curves at 260 nm (Figure 4). All PNAs with a preference for the right-handed helix (PNAs **1**, **4**, **6**, **7**) gave sharp melting transitions of the corresponding PNA–DNA duplexes. PNAs **2** (2D,5D), **5** (2L) and **8** (5D) also gave positive-to-zero transitions, which could be attributed to the melting of PNA–DNA duplexes, although with lower melting temperatures. For PNA **3** (2L,5D) the curve was totally inconsistent with the presence of a PNA–DNA duplex. CD melting temperatures are reported in Table 1.

By comparing the preferential PNA handedness with the PNA–DNA duplex stabilities (Table 1), interesting correlations emerge. First, as expected, all PNAs with a preference for the right-handed helix gave PNA–DNA duplexes more stable than the analogous achiral PNA, whereas PNAs with a preference for left-handed helix consistently gave PNA–DNA duplexes less stable than the achiral PNA. Moreover, the effect exerted by the substituent in the 5-position, either favourable or unfavourable on DNA binding, was always stronger than the effect exerted by the substituent in the 2-position, which significantly matches the stronger helical induction given by 5-substituted chiral PNA monomers. Actually, PNA **7** (5L, right-handedness preferred) gave a PNA–DNA duplex more stable than the corresponding duplex formed by PNA **6** (2D, right-handedness preferred); analogously, PNA **8** (5D, left-handedness preferred) gave a PNA–DNA duplex less stable than the corresponding duplex formed by PNA **5** (2L, left-handedness preferred). Finally, the PNAs containing two stereogenic centres clearly showed how "chiral accordance" and

Table 1. Helical induction exerted by the single stereogenic centres in chiral PNAs, preferential handedness through chiral accordance or chiral conflict in PNA–PNA duplexes and thermal stabilities of PNA–DNA duplexes.

PNA	Helical induction C2	Helical induction C5	Overall helical preference	Chiral accordance/conflict	PNA–DNA Thermal stability [°C][a]
4 (2D,5L)	right-handed	right-handed	right-handed	accordance	57
7 (5L)	–	right-handed	right-handed	–	56
1 (2L,5L)	left-handed	right-handed	right-handed	conflict	52
6 (2D)	right-handed	–	right-handed	–	52
Achiral	–	–	–	–	50[b]
5 (2L)	left-handed	–	left-handed	–	47
2 (2D,5D)	right-handed	left-handed	left-handed	conflict	33
8 (5D)	–	left-handed	left-handed	–	32
3 (2L,5D)	left-handed	left-handed	left-handed	accordance	<20

[a] Thermal stabilities were determined by CD melting curves registered at 260 nm. [b] From ref.[6]

"chiral conflict" finely tuned the PNA–DNA duplex stabilities. PNA **4** (2D,5L) (favourable preference for right-handedness given by "chiral accordance") gave a PNA–DNA duplex more stable than PNA **1** (2L,5L) (favourable preference for right-handedness given through a "chiral conflict" between the two stereogenic centres). Analogously, PNA **2** (2D,5D), (unfavourable preference for left-handedness as result of a "chiral conflict") was found to bind DNA, although very weakly, whereas PNA **3** (2L,5D), in which both stereogenic centres induce a wrong left-handed helix, was not able to form a stable PNA–DNA duplex even at room temperature.

In order to gain further evidence about the PNA–DNA duplex formation, MS spectra (ESI) were recorded on the hybrid solutions by following a procedure developed by our group.[17] Reconstructed MS spectra for the hybrid solutions of PNAs **1**–**4** are reported in Figure 5.

From the relative mass peak intensities of the PNA–DNA duplexes and of the free DNA, we could detect the presence (or the absence) of the duplexes, and even a correlation with their relative stabilities appeared to emerge. Actually, the intensities of the PNA–DNA peaks, if compared to the intensities of the free DNA strand, finely match with the stability order found by CD. PNA **4** (2D,5L) gave the highest intensity of the mass peaks of the PNA–DNA duplex, followed by PNAs **1** (2L,5L) and **2** (2D,5D), whereas in the case of PNA **3** (2L,5D) the duplex was totally absent and only free DNA was detectable. The same experiment was done with PNAs **5**–**8** with analogous results (Supporting Information, Figure S2).

Because PNA conformations were found to be essentially the same in PNA–PNA[15] and PNA–DNA[5] duplexes, it is not surprising that the stronger influence on DNA binding of the stereogenic centre in the 5-position is directly linked to its stronger ability to induce a preferential helix handedness in the PNA strand. Because the C4–C5 torsion angle is the most conserved in the PNA–DNA duplexes, as it was in the PNA–PNA duplexes, a right-handedness-compatible L configuration in the 5-position is a strong prerequisite for efficient DNA binding, whereas steric disturbances induced by the "wrong" left-handedness-compatible D configuration are highly detrimental. This is also consistent with the published structure of the PNA–DNA duplex,[5] where it is clearly evident that the pro-*S* hydrogen in the 5-position is much less hindered than the pro-*R* hydrogen in the same position (Figure 6).

However, beside the enthalpic effects, an entropic advantage due to the helical preorganization could contribute to the PNA–DNA duplex stability.[13] In order to better define enthalpic and entropic contributions to the improved DNA affinity, UV melting curves of the PNA–DNA duplexes were also measured and fitted by Van't Hoff plots[18] in the cases of PNA **4** (2D,5L), **7** (5L), **1** (2L,5L), **6** (2D), **5** (2L) and of the achiral homologous PNA sequence. For the other PNA sequences the melting temperatures were too low to allow a correct Van't Hoff fitting of the corresponding curves. The original and the fitted curves are reported in the Supporting Information (Figure S3). The data obtained by the plots are reported in Table 2. The measured UV melting temperatures were nearly the same (within the ex-

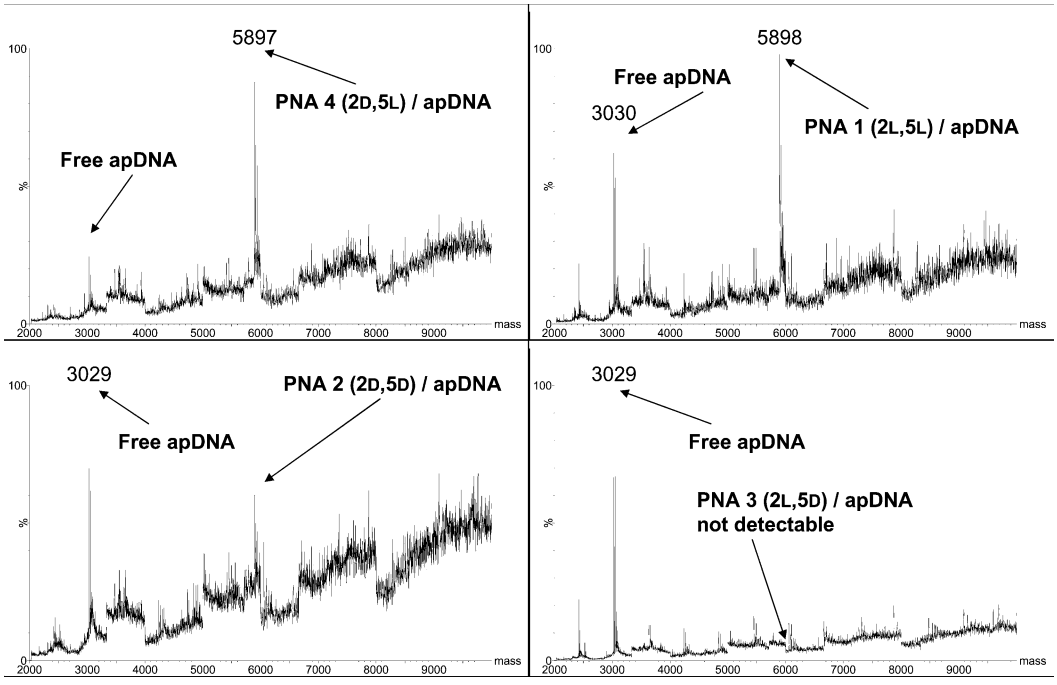

Figure 5. Reconstructed MS (ESI) spectra of the solutions containing the PNAs with two lysine-derived stereogenic centres and their antiparallel DNA strand recorded in ammonium formate buffer solution at pH = 7 (PNA and DNA concentrations: 20 μM each strand). All spectra are scaled to the most intense peak. Calculated MW for the free DNA strand: 3027 Da, calculated MW for the PNA–DNA duplexes 5897 Da.

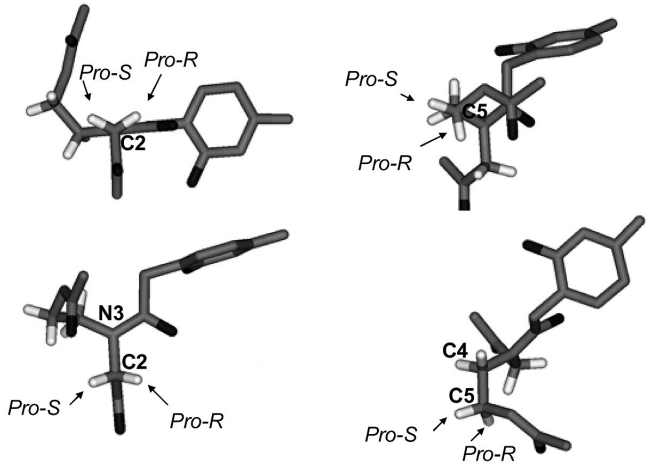

Figure 6. Different projections of a typical PNA monomer conformation in a PNA–DNA duplex, as determined by the crystal structure reported in ref.[5] The less-hindered protons that can be substituted by amino acidic side chains with minimal steric disturbances are the H2 pro-*R* (D in case of Lys side chain) and the H5 pro-*S* (L in case of Lys side chain).

Table 2. Enthalpic and entropic contributions to PNA–DNA duplex formation determined by Van't Hoff plots of the UV melting curves,[a] free energies at 25 °C and UV melting temperatures.

PNA	$\Delta H°$ [kJ mol^{-1}]	$\Delta S°$ [J K^{-1} mol^{-1}]	$\Delta G°$ (at 25 °C) [kJ mol^{-1}]	Thermal stability [°C][b]
4 (2D,5L)	–262	–682	–59	59
7 (5L)	–259	–688	–54	55
1 (2L,5L)	–279	–750	–55	54
6 (2D)	–303	–827	–56	53
Achiral	–300	–827	–53	50
5 (2L)	–241	–646	–48	48

[a] Average relative standard deviation for the given values is 5%. [b] Thermal stabilities were determined by UV melting curves registered at 260 nm.

perimental standard deviations) as those obtained by CD melting curves.

2D-Substituted PNA **6** appears to form a more stable PNA–DNA duplex than the corresponding achiral PNA due to the more favourable enthalpic term, whereas 2L-substituted PNA **5** experiments a loss of stability in the PNA–DNA duplex mostly related to the less-favourable enthalpy. Although direct electrostatic interactions of lysine side chains with the phosphate groups can be ruled out, according to the structure of the PNA–DNA duplex,[5] positive contributions from long-range interactions are expected to stabilize the lysine–PNA–DNA duplexes. In the case of the 2L-substituted PNA, this factor is overwhelmed by the enthalpy loss due to the steric hindrance of the lysine side chain. A recent paper accordingly confirmed by quantum chemical calculations that 2D-substituted PNAs better fit into the P-helix formed in PNA–DNA duplexes.[19]

In contrast, the stability gained by the PNA–DNA duplex formed by 5L-substituted PNA **7** is mainly ascribable to the lower entropy loss upon duplex formation, which is consistent with the preorganization of the PNA strand in a right-handed helical arrangement.[13] If both stereogenic centres are simultaneously present and both configurations are suited for DNA binding (2D,5L, PNA **4**) the PNA–DNA duplex is even more stable. In contrast, when the correct right-handed preorganization induced by the 5L configuration is disturbed by the sterically hindered 2L side chain (PNA **1**), the PNA–DNA duplex is clearly less stable due to the less-favourable entropy.

The clear different contributions to the PNA–DNA duplex stabilities exerted by the substituent in the different positions (mainly based on enthalpy in the 2-position and on entropy in the 5-position) might have striking consequences on the PNA–DNA duplex stabilities at different temperatures. Because entropic contributions are most important at high temperatures, the thermal stabilities of the PNA–DNA duplexes might overestimate the contribution of the 5-substituted PNAs. Accordingly, PNA **6** (2D) appear to give a PNA–DNA duplex slightly more stable, at 25 °C, of the duplex formed by PNA **7** (5L), although the melting temperature of the latter is slightly higher. However, the insertion of both substitutions with suitable stereochemistry is clearly the best way to improve PNA–DNA duplex stability.

Conclusions

A systematic study on the effect of the presence of two stereogenic centres simultaneously present at C2 and C5 of a PNA monomer inserted in an achiral PNA strand on DNA binding was performed for the first time. The stability of duplexes formed between DNA and chiral PNAs was found to be strictly dependent on the helical preferences induced by the different configurations. The induction of a right-handed helix was demonstrated to be fundamental for achieving stable DNA binding; moreover, the different propensity of the diastereomeric PNAs to adopt a right-handed helical conformation finely tunes the binding affinity for DNA. Moreover, it was demonstrated that the stereogenic centre in the aminoethyl moiety (C5 position) of the PNA backbone is more important than the stereogenic centre of the C2 carbon for inducing the preferential helix and the subsequent effect (positive or negative) on DNA binding. This predominant effect can be ascribed to the helical preorganization induced in chiral 5-substituted PNAs (entropy effect), whereas 2-substituted PNAs appear to exert their induction only by steric clashes (or rather by the attempt to avoid them) when the duplex is formed (enthalpy effect).

The data here presented concern only one model sequence and thus avoid confusing effects due to different sequences, and in general, they are in agreement with previous results on chiral PNAs, suggesting a general validity of the conclusions here reported. Because the use of chiral PNAs is giving promising results in diagnostic[20] and possibly in therapeutic[21] applications, it is important to realize that their preferential helicity is a fundamental factor that controls the affinity for DNA. The knowledge of the combined effects on helical induction and on DNA binding

properties of multiple stereogenic centres present in a PNA backbone will be essential in designing new chiral PNAs in which several amino acid side chains can be used for inducing new properties in PNAs without hampering, or possibly even improving, their basic DNA binding abilities.

These results have deep implications also for a better understanding of the binding abilities of DNA, which has been shown to recognize not only a complementary nucleobase pattern through Watson–Crick hydrogen bonds, but to discriminate the sequences that are not able to adopt a right-handed helical conformation. It is well known that DNA does not bind to a wrong nucleobase sequence, but here it was shown that DNA can modulate the affinity for a complementary sequence according to its helical arrangement and thus act as a chiral selector.

Experimental Section

PNA Synthesis: PNAs were synthesized as already reported.[14] All PNAs were purified by semipreparative HPLC (final purity >95% in all cases) and characterized by ESI mass spectrometry.

CD Spectra and CD Melting Curves: Hybrid solutions containing PNA (5 μM) and complementary PNA (5 μM) or complementary DNA (5 μM) were prepared in a phosphate buffer (10 mM phosphate, 100 mM NaCl, 0.1 mM EDTA, pH = 7) and incubated at 90 °C for 5 min. CD spectra were recorded with a J715 spectropolarimeter (Jasco, Tokio, Japan). CD melting curves of PNA–DNA duplexes were determined with the same instrument by measuring the ellipticity at 260 nm from 15 to 90 °C. All spectra and curves were treated with noise reduction software and corrected for the drift by subtracting a blank spectrum. Melting temperatures were taken at the minimum point (given the hyperdichroic effect upon hybridization) of the first derivatives of the melting curves.

UV Melting Curves: Hybrid solutions containing PNA (5 μM) and complementary DNA (5 μM) were prepared in a phosphate buffer (10 mM phosphate, 100 mM NaCl, 0.1 mM EDTA, pH = 7) and incubated at 90 °C for 5 min. UV melting curves were recorded with a Lambda Bio Perkin–Elmer spectrophotometer by measuring the absorbance at 260 nm from 15° to 90 °C. Melting temperatures were taken as the maximum of the first derivatives of the melting curves. Van't Hoff plots were done according to ref.[18] by using home-designed software.

ESI-MS of PNA–DNA Hybrid Solutions: The ESI-MS spectra were recorded essentially as previously reported,[17] with several small modifications. PNA (20 μM) and DNA (20 μM) were dissolved in an ammonium formate buffer (2 mM, pH = 7). The solution was directly infused in a ZMD mass spectrometer (Micromass, Manchester, UK) at 10 $\mu L\,min^{-1}$ rate. MS conditions: negative ion mode, capillary voltage 2.30 kV, cone voltage 20 V, source *T* 80 °C, desolvation *T* 120 °C, cone gas (N_2) 100 $L\,h^{-1}$, desolvation gas (N_2) 500 $L\,h^{-1}$. Acquisition range 150–2000 *m*/*z*, scan time 5 s.

Supporting Information (see footnote on the first page of this article): CD spectra of PNA–PNA duplexes added of aza dye, ESI-MS spectra of PNA–DNA duplexes, fitted UV melting curves of PNA–DNA duplexes.

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