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Induced Chirality in PNA-PNA Duplexes†

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Abstract: Complementary peptide nucleic acids (PNA) form Watson–Crick base-paired helical duplexes. The preferred helicity of such a duplex is determined by a chiral amino acid attached to the C-terminus. We here show that the induced helicity, as measured by circular dichroism (CD), is drastically dependent on the nucleobase sequence proximal to the chiral center. Chemically linked PNA tetramer duplexes of all 16 combinations of the two bases proximal to a carboxy terminal lysine residue were studied by CD. We conclude that the base next to the chiral center must be either a guanine or a cytosine for efficient stabilization of one helical sense. In case of cytosine, the subsequent base should preferably be a purine. We also show that the side chain properties of the C-terminal amino acid influence the resulting sense of helicity. The propagation length of induced chirality in PNA duplexes is found to be around 10 base pairs. Theoretical calculations of the circular dichroism for B-DNA, using the quantum mechanical matrix method of Schellman, give spectra in reasonable agreement with those found experimentally for PNA duplexes. The rate of helix conversion of the duplexes shows first-order kinetics with a rate constant in the range of minutes. Shorter duplexes are found to have lower activation energy and larger negative activation entropy for helix conversion, in agreement with a conversion mechanism in which a perfect helix is switched to the opposite handedness.

Introduction

DNA (or in a few cases RNA) is the fundamental genetic material of all living organisms. The flow of genetic information both from one generation to the next and from DNA to messenger RNA within an organism is rooted in the nucleobase complementarity within the structural contexts of helical DNA duplexes (or DNA-RNA heteroduplexes). Thus, it is of considerable chemical and conceptual interest that PNA (peptide nucleic acid), a structural homologue of DNA in which the deoxyribose phosphate backbone has been replaced by a pseudopeptide backbone composed of N-(2-aminoethyl)glycine units (Chart 1), acts as an excellent mimic of DNA.[1] We have previously found that complementary PNA oligomers form DNA-like helical duplexes, which generally exhibit high thermal stability.[2]

Since the PNA backbone is inherently achiral, PNA duplexes can be expected to form as a racemic mixture of right and left-handed helices.[2] Most interestingly, the attachment of a chiral lysine to the C-terminal base of the PNA strand has been shown to result in a thermodynamically preferred handedness of the helix[2]. The seeding of preferred helicity is believed to occur relatively slowly as a conversion process within the initially

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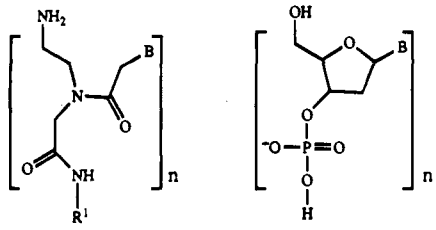
Chart 1



PNA DNA

formed racemic mixture of PNA-PNA duplexes. As expected, helices induced by D- and L-lysine were found to be of opposite helical sense.[2] Substitution of L-lysine with L-phenylalanine or L-isoleucine was found to give essentially the same results,[2] indicating that the amino acid side chain is not involved in any specific interactions stabilizing the preferred helical form. Preferred sense of helicity is of interest from a fundamental physicochemical point of view, and it may also be relevant for the discussion of the evolution of homochirality in living organisms.[2,3]

In order to better understand the induced chirality in PNA duplexes containing a terminal chiral amino acid, we now report a systematic study with respect to variations of nucleobase sequence and duplex length, as well as amino acid functionality and position.

Experimental Section

The PNA oligomers were synthesised as described previously.[4] The sequences are given in the N to the C direction. Lysine (lysinyl amide) or other amino acids are attached to the carboxy terminus, unless stated otherwise. Hairpin forming PNAs were of the sequence H-Y*X*AG-$(eg)_3$-CTXY-(L-Lys)-NH_2, where X and X*, as well as Y and Y*, refer to Watson–Crick complementary bases, and "eg" denotes an ethylene glycol linker (8-amino-2,6-dioxaoctanoyl).[5] The PNA H-AGTGATC-TA(Cβ-Ala)-(L-Lys)-NH_2, with a β-alanine (β-Ala) replacing the glycine in the linker to the C-terminal lysine, was synthesized as described previously.[6] All PNAs were characterized by mass spectrometry.

PNA concentrations were determined spectrophotometrically using ϵ_{260} as for DNA of the corresponding sequence. Hybridization of PNA strands was performed by heating the sample solution to 80 °C for 30 min followed by slow cooling to room temperature. The melting temperatures were determined as previously described[1] in 10 mM sodium phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.0. Circular dichroism measurements were performed in 5 mM sodium-phosphate buffer, pH 7.0. The kinetics was detected by mixing equimolar amounts of complementary PNA strands and following the appearance of induced circular dichroism at 220 nm with time. Circular dichroism was measured on a Jasco 720 spectropolarimeter in a 1-cm strain-free quartz cell. All spectra were obtained as averages of 8 scans. Calculations of theoretical circular dichroism spectra for B-DNA were performed as earlier described.[7] Structural modeling was done with the programs Insight and Discover (Biosym Inc.).

Results

When extending our studies of induced chirality in PNA duplexes,[2] we observed that only one of the carboxy terminal lysines in our originally studied PNA duplex, the pair of sequences H-AGTGATCTAC-(L-Lys)-NH_2 and H-GTAGAT-CACT-(L-Lys)-NH_2, contributes to the induction of CD, namely the lysine of the former PNA strand (Figure 1). This suggested that the base sequence proximal to the lysine may be critical for the "chiral communication" between the amino acid and the PNA helix. We therefore synthesized several PNA decamers in which the terminal base pairs were varied (Table 1). The CD signals of these duplexes were dramatically different in amplitude (Figure 1), although all duplexes were found to have similar high thermal stability (Table 1). Sequences terminating with CT-(L-Lys)-NH_2 and CA-(L-Lys)-NH_2 exhibit no significant CD. The duplex with AC-(L-Lys)-NH_2 shows a distinctly

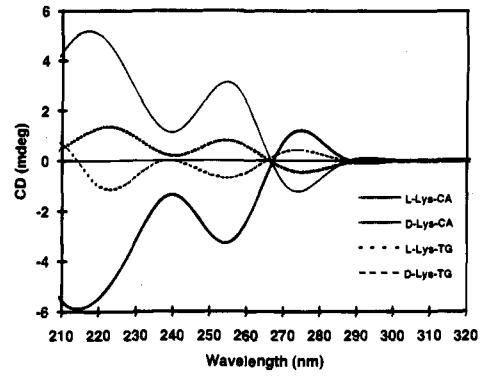


Figure 1. CD spectra for two 10mer PNA duplexes with D- or L-lysine at the carboxy terminus: H-AGTGATCTAC-(L/D-Lys)-NH_2 and H-GTAGATCAGT-(L/D-Lys)-NH_2, each hybridized to the complementary sequence without lysine. (Attachment, or not, of L- or D-lysine to the complementary strand of the first listed sequence (with L-lysine) gave identical spectra.) The spectra are labeled with the sequence of the two bases closest to the terminal lysine. Note that the (L-lys)-CA duplex gives a substantially stronger CD signal than (L-lys)-TG, and a helix with apparently opposite helicity. Base pair concentration 50 μM. The sequences H-AGTGATCTCA-(L-Lys)-NH_2, H-GTAGATCACT-(L-Lys)-NH_2, and H-AGTGATCTA(C-βAla)-(L-Lys)-NH_2, each hybridized to its complementary sequence without terminal lysine showed no CD signal.

Table 1. Melting Temperatures of Decamer PNA-PNA Duplexes with Terminal Lysine Residues (L or D Form) As Indicated

PNA-PNA duplex	T_m (°C)
H-GTAGATCACT-(L-Lys)-NH_2 NH_2-(L-Lys)-CATCTAGTGA-H	67
H-GTAGATCACT-NH_2 NH_2-(L-Lys)-CATCTAGTGA-H	67
H-GTAGATCACT-NH_2 NH_2-(D-Lys)-CATCTAGTGA-H	69
H-GTAGATCACT-(L-Lys)-NH_2 NH_2-CATCTAGTGA-H	67
H-GTAGATCACT-NH_2 NH_2-(L-Lys)-(βAla-C)ATCTAGTGA-H	69
H-GTAGATCAGT-(L-Lys)-NH_2 NH_2-CATCTAGTCA-H	66
H-GTAGATCAGT-(D-Lys)-NH_2 NH_2-CATCTAGTCA-H	66
H-TGAGATCACT-NH_2 NH_2-(L-Lys)-ACTCTAGTGA-H	72

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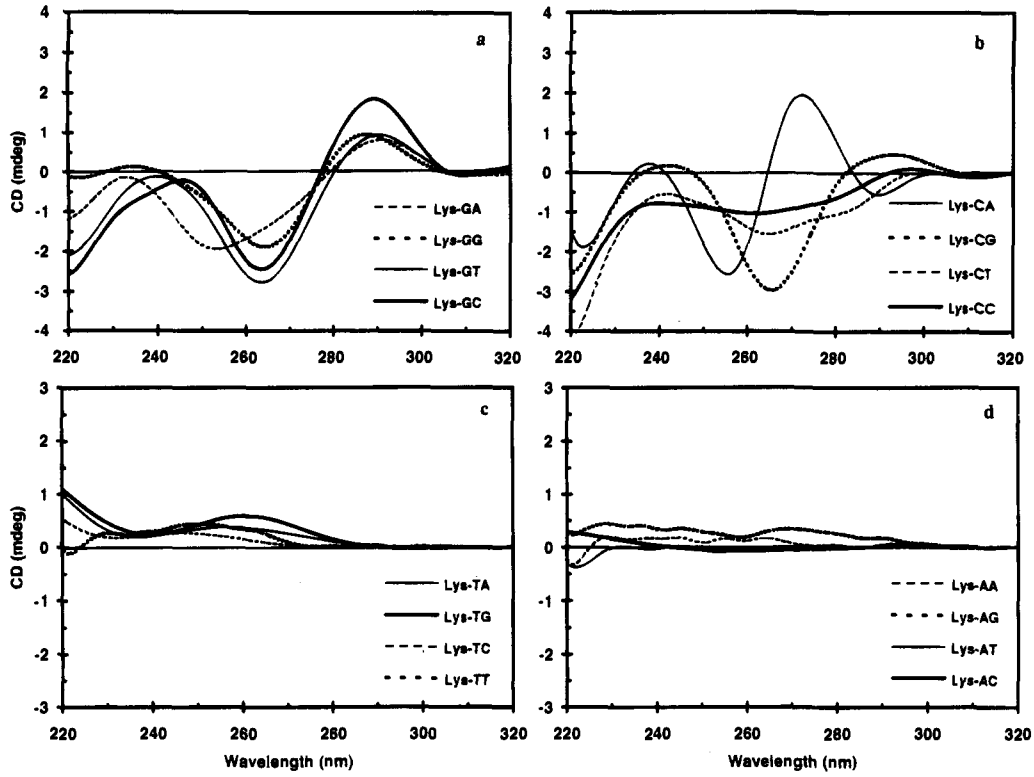

Figure 2. CD spectra of chemically linked PNA tetramer duplexes, with all sequence combinations of the two bases adjacent to the terminal L-lysine. The sequence is H-X*Y*AG-(eg)$_3$-CTYX-(L-Lys)-NH_2 with X and Y varied as indicated below (X* and Y* complementary to X and Y) and eg = ethylene glycol bridge. The spectra are labeled starting from the carboxy terminus, Lys-XY, as indicated. Base concentration 100 μM in spectra a and b and 50 μM in spectra c and d: (a) **X** = G, **Y** = A, T, G, or C, (b) **X** = C, **Y** = A, T, G, or C, (c) **X** = T, **Y** = A, T, G, or C, (d) **X** = A, **Y** = A, T, G, or C.

structured CD which may be related to helical stacking apparently involving most of the base pairs (see evidence below). The duplex ending with GT-(L-Lys)-NH_2 shows a CD spectrum which is very weak and significantly different from that of AC-(L-Lys)-NH_2. Using D- instead of L-lysine, mirror image spectra show that structures of opposite helicity are formed (Figure 1). The self-complementary sequence H-CGCGCGCGCG-(L-Lys)-NH_2, designed for the purpose of searching for *Z*-like conformations, was found to exhibit a CD spectrum (not shown) very similar to that of the AC-(L-Lys)-NH_2 in Figure 1, showing that sequences ending with CG-(L-Lys)-NH_2 also exhibit a significant preference of helical sense. These results prompted us to examine the sequence dependence of the induced chirality of the PNA duplex more systematically.

All possible combinations of the two base pairs next to the terminal lysine were studied in chemically linked tetramer duplexes. Figure 2 shows the circular dichroism spectra of these PNAs and Table 2 gives the melting temperatures (T_m). No simple correlation between T_m and induced CD is apparent, but the T_ms are fairly well correlated with the GC content in full analogy to the behavior of DNA (Table 2). The CD curves of the PNAs show individual variations of the amplitudes and the shapes (wavelength positions of maxima/minima). Oligomers terminating with G-(L-Lys)-NH_2 and C-(L-Lys)-NH_2 (Figure 2, a and b) show distinct CD signals, whereas oligomers terminating with T-(L-Lys)-NH_2 and A-(L-Lys)-NH_2 show no structured CD (Figure 2, c and d). From the results presented in Figure 2 we conclude that efficient stabilization of a preferred helical sense is only obtained when the base next to the lysine is either a G or a C. Also the second base of the sequence from the lysine appears to be of importance. If the base closest to the lysine is a C, a more distinct CD spectrum is observed if the subsequent base is a purine (A or G) (Figure 2b). If the base closest to the lysine is a G, however, essentially the same CD spectrum is obtained whether the second base is a purine or a pyrimidine (Figure 2a).

We have previously found that the hydrophobic amino acids L-phenylalanine and L-isoleucine had the same effect as L-lysine if attached to the C terminus of one strand (H-AGTGATCTAC-(L-Lys)-NH_2) in a PNA duplex stabilizing what was tentatively assigned to be a right-handed helix.[2] In further search for effects related to the nature of the side chain of the attached amino acid, 10mer PNA duplexes (of the above sequence) with the more hydrophilic amino acids L-alanine and L-glutamic acid attached to the C terminus were prepared. At neutral pH we find that both L-Ala and, to a seemingly greater degree, L-Glu stabilize left-handed helices, as seen from their CD spectra (Figure 3). However, in acidic solution (pH 5.0), where glutamic acid is protonated, a right-handed helix is stabilized. These results suggest that the hydrophobic properties of the side chains are important for the preferred sense of helicity (see Discussion).

To evaluate the length by which chirality propagates along a PNA duplex, three PNAs of different lengths, each forming an intramolecular duplex (4, 6, and 8 base pairs) and terminating with the sequence AC-(L-Lys)-NH_2, were analyzed. As shown in Figure 4a, there is a clear correlation between the length of the PNA duplex and the amplitude of the signal. For the main peaks at 220 and 270 nm the CD amplitude increases approximately linearly with the number of base pairs. Thus, all base pairs seem to contribute to the rotational strength and, hence, the helix appears to propagate throughout the duplex. We also studied intermolecular PNA-PNA duplexes of 8, 10, and 12 base pair lengths. The CD amplitude per duplex (Figure 4b) was found not to increase above 10 base pairs, indicating convergence of induced helicity at this length of the helix. Thus, the effective correlation length is estimated to be 8 to 10 base pairs.

Table 2. Melting Temperatures (and GC Content) of Chemically Linked PNA Tetramer Duplexes, Carrying an L-Lysine Residue at the Carboxy Terminus

PNAs	% GC	T_m (°C)*
H-CCAG-$(eg)_3$-CTGG-(L-Lys)-NH_2	75	~64
H-CGAG-$(eg)_3$-CTCG-(L-Lys)-NH_2	75	69
H-CAAG-$(eg)_3$-CTTG-(L-Lys)-NH_2	50	48
H-CTAG-$(eg)_3$-CTAG-(L-Lys)-NH_2	50	47
H-GCAG-$(eg)_3$-CTGC-(L-Lys)-NH_2	75	~66
H-GGAG-$(eg)_3$-CTCC-(L-Lys)-NH_2	75	~70
H-GAAG-$(eg)_3$-CTTC-(L-Lys)-NH_2	50	~54
H-GTAG-$(eg)_3$-CTAC-(L-Lys)-NH_2	50	51
H-TCAG-$(eg)_3$-CTGA-(L-Lys)-NH_2	50	~50
H-TGAG-$(eg)_3$-CTCA-(L-Lys)-NH_2	50	63
H-TAAG-$(eg)_3$-CTTA-(L-Lys)-NH_2	25	38
H-TTAG-$(eg)_3$-CTAA-(L-Lys)-NH_2	25	33
H-ACAG-$(eg)_3$-CTGT-(L-Lys)-NH_2	50	52
H-AGAG-$(eg)_3$-CTCT-(L-Lys)-NH_2	50	58
H-AAAG-$(eg)_3$-CTTT-(L-Lys)-NH_2	25	42
H-ATAG-$(eg)_3$-CTAT-(L-Lys)-NH_2	25	37

* The melting profiles marked by ~ were broad.

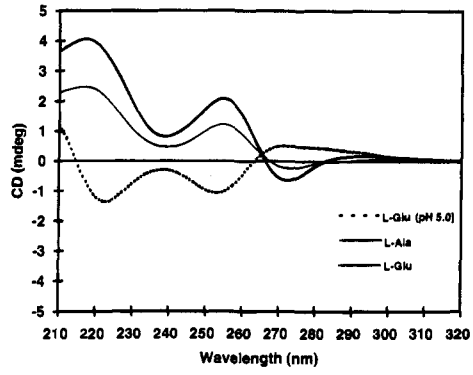

Figure 3. CD spectra of the PNA duplexes consisting of either H-AGTGATCTAC-(L-Ala)-NH_2 or H-AGTGATCTAC-(L-Glu)-NH_2 hybridized to H-GTAGATCACT-NH_2, at pH 7.0. Also shown is the CD spectrum of the L-Glu duplex at pH 5.0. Base pair concentration 50 μM.

According to our model, the PNA duplexes initially form as an equimolar mixture of left- and right-handed helices,[2] which then convert into one preferred handedness determined by the attached amino acid. The kinetics of the helix reorganization process was investigated by monitoring the appearance of the induced CD signal at 220 nm upon mixing PNA oligomers of

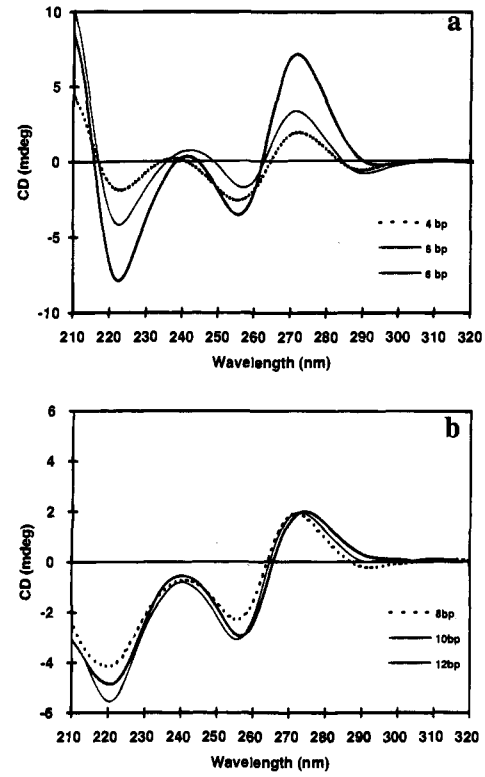

Figure 4. (a) CD spectra of PNA hairpins, forming 4, 6, and 8 base pair duplexes: H-GTAG-$(eg)_3$-CTAC-(L-Lys)-NH_2, H-GTAGAT-$(eg)_3$-ATCTAC-(L-Lys)-NH_2, and H-GTAGATCA-$(eg)_3$-TGATCTAC-(L-Lys)-NH_2. The spectra refer to identical hairpin concentrations, 12.5 μM (i.e., 100, 150, and 200 μM bases, respectively). The longest duplex exhibits the largest CD amplitudes, in agreement with contribution from more base pairs. (*b*) CD spectra of PNA duplexes 8, 10, and 12 base pairs long: H-TGATCTAC-(L-Lys)-NH_2, H-AGTGATCTAC-(L-Lys)-NH_2, and H-ACAGTGATCTAC-(L-Lys)-NH_2, each hybridized to the complementary sequence without lysine. The spectra refer to identical duplex concentrations, 5 μM (i. e., 80, 100, and 120 μM bases, respectively).

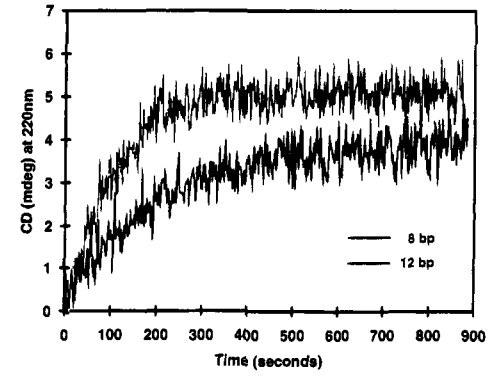

Figure 5. CD kinetics at 220 nm monitoring helical rearrangement of the PNA duplexes: H-TGATCTAC-(L-Lys)-NH_2 + H-GTAGATCA-NH_2 and H-ACAGTGATCTAC-(L-Lys)-NH_2 + H-GTAGATCACTGT-NH_2 at 30 °C. Base-pair concentration 50 μM.

complementary, antiparallel sequences. PNA duplexes of three different lengths (8, 10, and 12 base pairs) were used. In all cases first-order kinetics could describe the observed changes (Figure 5 gives an example, see also ref 2). Table 3 reports activation parameters obtained from Arrhenius plots of the temperature dependence of the rate constants. The reaction rates decrease, and the activation energies increase, with increasing length of PNAs. The entropy of activation ($\Delta S^{\ddagger}$) is negative and relatively large, suggesting an ordered transition state. The magnitude of $\Delta S^{\ddagger}$ decreases with increasing length of the PNA.

Table 3. Kinetic Data for Helical Seeding in PNA–PNA Duplexes

PNA–PNA duplex	k_{inv}(30 °C)/s^{-1}	E_a/kJ mol^{-1}	$\Delta S^{\ddagger}$/J mol^{-1} K^{-1}	$\Delta G^{\ddagger}$/kJ mol^{-1}
8 base pairs[a]	$22 \times 10^{-3} \pm 3 \times 10^{-3}$	28 ± 3	-190 ± 15	86 ± 2
10 base pairs[b]	$9 \times 10^{-3} \pm 2 \times 10^{-3}$	34 ± 3	-173 ± 15	87 ± 2
12 base pairs[c]	$6 \times 10^{-3} \pm 2 \times 10^{-3}$	42 ± 3	-150 ± 15	88 ± 2

[a] H-TGATCTAC-(L-Lys)-NH_2 and the complementary sequence without lysine. [b] H-AGTGATCTAC-(L-Lys)-NH_2 and the complementary sequence without lysine. [c] H-ACAGTGATCTAC-(L-Lys)-NH_2 and the complementary sequence without lysine.

In an attempt to correlate the measured CD spectrum with the structure of a helical PNA-PNA duplex, we calculated the CD spectrum predicted for a helical stack of base pairs, using the Schellman matrix quantum mechanical method.[7] The experimental CD spectrum of the 10mer PNA, H-AGTGATC-TAC-(L-Lys)-NH_2, hybridized to the complementary PNA sequence, is quite similar to the theoretical CD spectrum calculated for a canonical B-form DNA of the same sequence, as shown in Figure 6a, supporting a right-handed structure for the PNA-PNA duplex. In fact, the agreement of the calculated CD spectrum with the experimental one for the PNA-PNA duplex is better than with that of the corresponding DNA-DNA duplex.

CD calculations, assuming a B-form DNA conformation, were also carried out for the linked tetramer PNA duplexes that had been found to exhibit distinct CD spectra (Table 2). The calculated CD spectra show variations in shape depending on the sequence (Figure 6, b and c). Previous calculations on dimers and tetramers[7] have shown the CD to be sensitively dependent on sequence, while relatively insensitive to structural variations. Therefore, the observed differences are more likely to be the result of sequence dependence (variations in couplings between the nucleobases) than of marked structural variations. The experimental CD spectra of these PNA duplexes (Figure 2, a and b) also show considerable sequence variations which, for several of them, are similar to those predicted by the calculations. Cases in which calculated and experimental spectra differ may reflect deviations from a B-form structure. However, there is still controversy regarding the directions of some of the transition moments of the nucleobases,[8] and it is therefore difficult to interpret these spectral variations in structural terms.

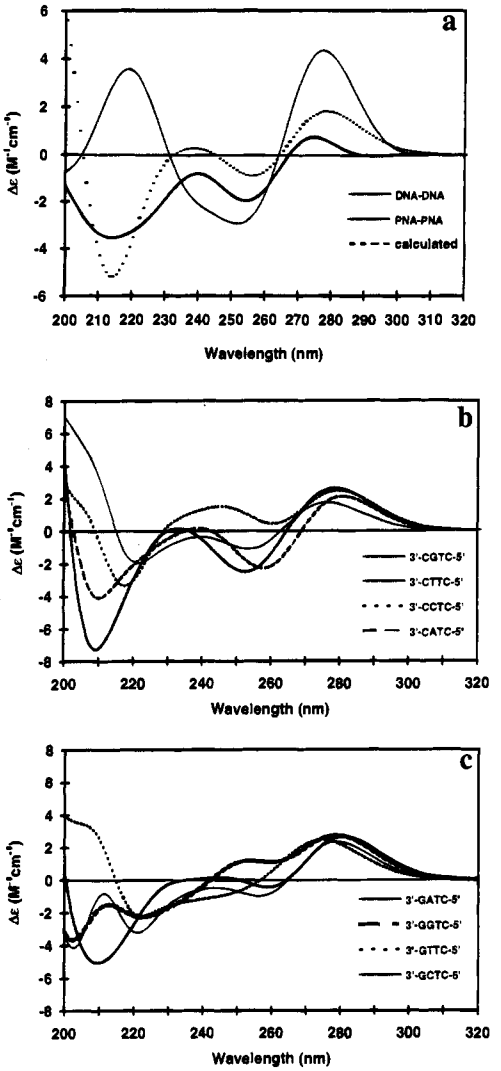

Figure 6. (a) Calculated CD spectrum for a right-handed stack of nucleobases corresponding to a duplex in B conformation, with the strands 5′-GTAGATCACT-3′ + 5′-AGTGATCTAC-3′ (dotted line). Also shown are the experimental CD spectra for the same sequences of DNA-DNA (solid line) and PNA-PNA (thick solid line) duplexes ($\Delta\epsilon$ in M^{-1} cm^{-1} per base pair). (b and c) Calculated CD spectra for B-form duplexes corresponding to PNA duplex sequences in Figures 2a and 2b ($\Delta\epsilon$ in M^{-1} cm^{-1} per base pair): (b) 3′-CCTC-5′, 3′-CATC-5′, 3′-CGTC-5′, and 3′-CTTC-5′ (c) 3′-GCTC-5′, 3′-GATC-5′, 3′-GGTC-5′, and 3′-GTTC-5′.

Discussion

Induced Chirality. Evidently the choice of base sequence is important for the helicity of a PNA-PNA duplex, stabilized by a chiral amino acid attached to the carboxy terminus. We find that the base closest to the chiral amino acid must be either a guanine or a cytosine in order to give a well-defined circular dichroism, indicative of a preferred helicity of the PNA duplex (in the cytosine case the subsequent base should preferably be a purine). We have mainly used L-lysine (lysinyl amide) as a chiral center in the sequence H-AGTGATCTAC-(L-Lys)-NH_2. Replacement of the L-lysine by L-isoleucine or L-phenylalanine gives essentially the same result for this sequence,[2] showing that the intrinsic optical activity of the amino acid is of no major importance for the resulting circular dichroism. The circular dichroism is thus predominantly an effect of coupling between the transition moments of the nucleobases as a result of their helical stacking.

Two important issues in the discussion of the induced preferred helicity of a nucleic acid double helix devoid of local chiral centers are (1) the origin of helicity induced by the amino acid attached at the end of the helix (local chiral bias) and (2) the propagation of preferred helicity along the duplex, i.e. the length in which the induced helicity extends.

Local Chiral Bias. We find that helicity can be introduced effectively in a PNA duplex with either a guanine or a cytosine on the C-terminus to which the lysine is attached. Since G and C residues have no structural features in common that may be involved in a specific type of interaction with the lysine, we believe that the base probably does not interact specifically with the amino acid. The higher thermal stability of a G-C base pair, as compared to an A-T base-pair, may provide the structural stability required for the amino acid to induce a preferred handedness. The importance of hydrogen bonds in hindering free rotation of amino acids was recently illustrated in a study of induction of α-helices in short peptides by end capping. A correlation between helix stability and the amount of hydrogen

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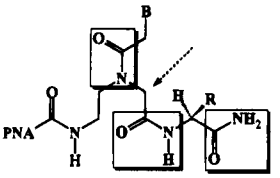

Figure 7. Schematic model of C-terminal nucleobase (B) on a PNA strand with attached L-lysinyl. The three planar amides proposed to transmit the chiral communication are boxed. The arrow points to the position in the backbone for the CH_2 extension in the H-AGTGATCTA-(C-βAla)-(L-Lys)-NH_2 PNA.

bonds formed was found.[9] From induced rotatory power in amino acid complexes with transition metals[10] we tentatively infer that effective mechanisms of inducing chirality will involve some immobilization of rotation around the bonds of the α carbon of the amino acid.

As in any conformational equilibrium, the predominance of one isomer over another can be described by both stabilizing (hydrogen bonding, hydrophobic interactions, and electrostatic attractions) and destabilizing forces (steric interference and electrostatic repulsion). Although we do not have a detailed model for the molecular interactions responsible for the amino acid induced chirality in the PNA helix, several conclusions can be drawn from our results.

It is obvious that the nature of the amino acid side chain is of predominant importance in determining the helical sense. Since we observe virtually identical effects with Phe and Ile as with Lys, hydrogen bonding donation of the amino acid side chain cannot be a crucial factor. Similarly, the helical induction by Glu at acidic pH is less efficient than Phe, Ile, and Lys. Therefore, hydrogen bonding acceptor properties of the amino acid side chain cannot be essential either. Furthermore, steric interference can be ruled out since the helicity of the Glu-PNA duplex is inverted simply by deprotonating the carboxyl group. It is also difficult to reconcile the similar effect of the cationic lysine side chain with those of the charge neutral phenylalanine and isoleucine side chains with the importance of any electrostatic attraction/repulsion forces.

Thus we are left with hydrophobic interactions. We notice that the amino acids Phe, Ile, and Lys all have long, hydrophobic hydrocarbon side chains. Similarly, the side chain of Glu is relatively hydrophobic at acidic pH, while it is hydrophilic (charged) at neutral pH. The methyl group of Ala is also relatively hydrophilic. It thus appears that hydrophobic side chains stabilize one helical sense, while hydrophilic side chains stabilize the other. Computer model building shows that hydrophobic interactions with the amino acid side chain may occur in the minor groove of the PNA helix as well as by "stacking" over the last base pair. More elaborate computations might distinguish between the relative importance of these two modes of interaction.

Finally, we note that the three terminal amide groups of the PNA (Figure 7) are at "α-carbon distance" from each other and will therefore, due to dipole–dipole interactions of the planar amide groups and steric hindrance between these, tend to assume a preferred orientation as also observed for natural peptides.[11] The importance of such an interaction is supported by the finding that the chiral communication between the amino acid and the PNA helix is disrupted by insertion of one methylene group between the last base and the chiral amino acid (Figure 7, Table 1). Therefore we propose that the preferred chiral conformer is stabilized primarily by a combination of dipole interactions of the amide groups and hydrophobic interactions between the amino acid side chain and the PNA helix. The more precise features of the stabilization/destabilization forces may be revealed by choosing other amino acids, nucleobases, and PNA backbones and eventually by solving three-dimensional structures by NMR or X-ray crystallographic techniques.

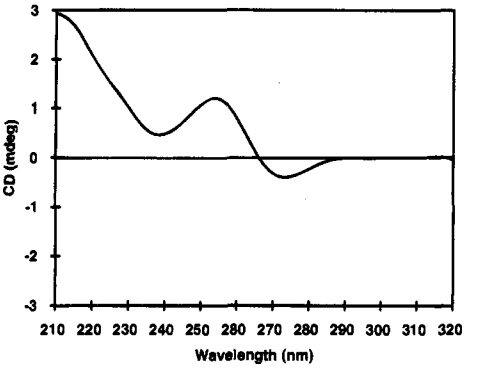

Figure 8. CD spectrum of a 10mer PNA duplex with L-lysine attached to the amino terminus: H-(L-Lys)-GTAGATCACT-NH_2 hybridized to H-AGTGATCTAC-NH_2. Base pair concentration 50 μM.

We also studied a PNA-PNA duplex in which the lysine residue was attached to the N-terminus of one of the PNA strands (H-(L-Lys)-GTAGATCACT-NH_2) (Figure 8). This duplex showed a significantly different CD spectrum, corresponding to opposite helicity (i.e. putatively left-handed), with a somewhat lower amplitude compared to that with the L-lysine in the carboxy terminus. This again supports that the chemical nature of the environment of the chiral center on the atomic level is critical.

Propagation of Helicity. As concluded from the results of Figure 4, spectra a and b, the correlation length of the induced helicity is around ten base pairs. In principle there are two mechanisms for helix propagation. First, due to dipole–dipole interactions between two stacked base pairs, asymmetric conformations may be preferred. Within a stack of equivalent bases, each step will then correspond to a certain twist angle defining the pitch of a helix. Second, the backbones of a duplex structure will provide a means by which the chirality of a preceding pair of base pairs could be transduced, topologically, to the following base pair. Furthermore, the helicity of a base pair step may be effectively transferred through the backbone, which does not exclude the dipole mechanism (possibly accounting for structural variations depending on base sequence).

The observation that the correlation length of helicity is finite, typically corresponding to one turn of the helical duplex (assuming a DNA-like winding), is expected since the energy cost for breaking the helical pattern in a PNA-PNA duplex is rather small. The free energy cost per rotor of ordering the phosphate backbone of duplex DNA into a helical structure has been estimated to be between 2 and 6 kJ/mol.[12] An energy diagram for an N-mer of a duplex with local helicity, having a helical–bias coupling constant of ΔE, will have as a ground state the perfect helix (denoted RRR...R). The next state (RRR...RL) is degenerate with (RRR...LL) and $N - 2$ other states (R and L refer to right- and left-handed helicity, respectively). The probability of having this second state compared to the ground state will be $P_1/P_0 = N \exp(\Delta E/RT)$, increasing linearly with the degree of degeneracy and the number of bases. Thus, the memory of the original helicity is

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effectively lost after roughly 10 base pairs if $P_i/P_0 = 1 = 10 \exp(\Delta E/RT)$, i.e. if $\Delta E = 5.7$ kJ/mol base pairs.

Kinetics of Helical Propagation. The kinetics by which the CD signal arises after hybridization has been followed for PNA-PNA duplexes of various lengths. The conversion can be described by first-order kinetics and is independent of the concentration of the PNA strands, in agreement with a base-paired duplex rearranging itself into a preferred helicity determined by the single chiral center. The observed conversion may reflect either of two possible processes: an inversion of one helical structure into its mirror image, or the conversion of a mixed-helicity structure into predominantly one helical form. In the former case the absence of any inital CD is the result of a racemic mixture of opposite PNA-PNA helices. In the latter case, equal amounts of opposite turns cancel the CD of each PNA-PNA duplex. The activation energy for the conversion is found to increase with duplex length, indicating that more energy is required to convert a longer duplex. By contrast, the negative activation entropy becomes less negative with increasing duplex length. The inversion of a perfectly helical structure would exhibit this behavior if the transition state is related to the conversion of a domain of finite length. This result could also indicate that the longer duplex has a more ordered structure initially, and thus a lower starting entropy. This is reasonable, since the relative amount of less ordered ends is higher in shorter sequences. The PNA monomers have been found by NMR[13] to display slow conversions between two conformations (amide rotamers) and this activation barrier could relate also to the slow helical seeding in duplex PNA oligomers. We also note that the amplitudes of the PNA-PNA and DNA-DNA duplexes are of similar magnitudes (see Figure 6a), indicating that the predominance of one helical sense is comparable in the two molecules.

Theoretical Circular Dichroism. The CD calculations are interesting from two points of view. First, they support the assignment of a right-handed helicity of the PNA-PNA duplex with a carboxy terminal L-lysine, previously solely based on inference from the apparent similarity to the CD spectrum of a DNA-DNA double-helix with the same sequence.[2] Second, the calculations neglect the influence of the backbone as well as included chiral moieties (such as deoxyribose), and only represent the optical activity of a helical stack of nucleobases. Therefore, comparison with experimental spectra of two stacks of nucleobases with different backbones in principle allows conclusions about the contribution of the backbone to the CD spectrum. The calculated CD for the studied B-form decamer DNA sequence is found to be in better agreement with the experimental PNA-PNA duplex spectrum than with the corresponding DNA-DNA spectrum. This might indicate that in DNA there is a contribution to the CD from transitions of the deoxyribose chromophores, while in PNA the helical arrangement of base pairs alone suffices to explain the CD spectrum.

The calculated CD spectra for the tetramer duplexes (Figure 6, b and c) show considerable variations in shape depending on the sequence, in qualitative agreement with the variations experimentally observed between several (but not all) of the spectra for the PNAs with L-lysines at the carboxy termini (Figure 2, a and b). The differences between calculated and experimental CD spectra may either be due to structural deviations from the assumed B conformation or be a result of inadequacy in the theoretical description of the electronic states (transition moments) of some of the nucleobases.

Conclusions

1. The preferred chirality adopted by a PNA-PNA duplex is found to depend sensitively on the nucleobase closest to the chiral amino acid at the C-terminal end. Well-defined helical structures of one preferred helicity are only obtained if the base next to the chiral center is a guanine or a cytosine. In case the base closest to the lysine is a cytosine, the subsequent base should be a purine.

2. The helicity induced by the terminal lysine is propagated through approximately 10 base pairs of a PNA-PNA duplex.

3. The rate of conversion into a preferred helicity decreases with increasing length of the PNA-PNA duplex and probably occurs by inversion of a preformed helical structure.

4. The preferred helical sense of a PNA-PNA duplex is not related in a simple way to the absolute configuration of the amino acid attached to the carboxy terminus. Besides, the nature of the side chain also plays an important role. Amino acids with hydrophobic side chains appear to give right-handed helices, possibly as an effect of the side chain making hydrophobic interactions with the PNA duplex. More hydrophilic side chains may be directed away from the PNA and are found to stabilize left-handed helices.

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