Observation of a PNA−PNA−PNA Triplex

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Peptide nucleic acid (PNA) is a DNA mimic in which the deoxyribose phosphate backbone of DNA has been replaced by an achiral pseudopeptide backbone.[1,2] Mixed-sequence PNA oligomers have been found to form stable hybrids with DNA and RNA.[3] In addition, two PNA strands, if complementary, can form Watson−Crick base-paired helical duplexes.[4] While homopyrimidine PNA oligomers had been found to bind very strongly to homopurine DNA by PNA−DNA−PNA triplex formation,[1,5,6] no pure PNA triplexes have so far been reported. We here show that, indeed, an all PNA−PNA−PNA triplex is formed between one adenine−PNA decamer and two thymine−PNA decamers. When a chiral cyclohexyl moiety is located at a single position in the thymine−PNA backbone (Figure 1), the formation of a helically stacked complex could be sensitively monitored by circular dichroism (CD).

Two complementary mixed-sequence PNA oligomers have earlier been found to form a Watson−Crick base-paired helical duplex.[4,7] A fast base-pairing step resulting effectively in a 1:1 racemic mixture of left- and right-handed double helices is followed by a relatively slow inversion of the whole double helix to adopt the helical sense preferred by the terminal amino-acid residue.[4] We here demonstrate the existence of a corresponding triple-helical structure consisting of solely PNA molecules.

In order to use the CD of helically stacked nucleobases[8] to monitor the hybridization, we studied a thymine−PNA decamer having a chiral cyclohexyl moiety incorporated in the backbone at a single central position (see Table 1), interacting with an adenine−PNA decamer (lacking intrinsic chirality). As seen in Figure 2, when PNA-T_{10}-((*SS*)-cyclohexyl) is titrated into PNA-A_{10}, a strong CD signal arises, which increases linearly with PNA concentration and saturates at a 2:1 T:A stoichiometry. This shows that the PNA-T_{10} is indeed able to bind to the PNA-A_{10} as a triplex. The linearity of the titration curves as well as the appearance of isodichroic points in the CD spectra indicates that no duplex is formed but that the triplex is the only product. Also, a plot of hypochromicity as a function of PNA ratio (not shown) verified a 2:1 stoichiometry of thymine to adenine bases. The melting profile, measured by CD, showed a sharp one-step melting in agreement that no duplex intermediate is effectively formed. The melting temperatures (T_m in Table 1) for the triplexes formed between PNA-A_{10} and either PNA-T_{10}-((*SS*)-cyclohexyl) or PNA-T_{10}-((*RR*)-cyclohexyl) were found to be high and comparable with those for the $(PNA\text{-}T_{10})_2$-DNA-A_{10} triplexes. As expected, the CD spectrum of the triplex with PNA-T_{10}-((*SS*)-cyclohexyl) is the mirror image[9] of the triplex formed with PNA-T_{10}-((*RR*)-cyclohexyl) (Figure 3).[10] Furthermore, the CD spectrum of the $(PNA\text{-}T_{10}(SS))_2$-PNA-$A_{10}$ triplex strongly resembles those of the $(PNA\text{-}T_{10})_2$-DNA-A_{10} and $(PNA\text{-}T_{10}(SS))_2$-DNA-$A_{10}$ triplexes,[11,12] suggesting similar right-handed helical structures.

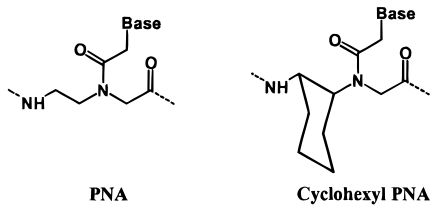

Figure 1. Chemical structure of PNA backbone with and without cyclohexyl.

Table 1. PNA Complexes

complex	T_m (°C)[a]
$(PNA\text{-}TTTTT_{SS}TTTTT\text{-}L\text{-}LysNH_2)_2$ + PNA-A_{10}-$GlyNH_2$	62 ± 2[b]
$(PNA\text{-}TTTTT_{RR}TTTTT\text{-}L\text{-}LysNH_2)_2$ + PNA-A_{10}-$GlyNH_2$	61 ± 2[b]
$(PNA\text{-}TTTTTTTTTT\text{-}L\text{-}LysNH_2)_2$ + PNA-A_{10}-$GlyNH_2$	76.0[c]
$(PNA\text{-}TTTTT_{SS}TTTTT\text{-}L\text{-}LysNH_2)_2$ + DNA-A_{10}	70[c]
$(PNA\text{-}TTTTT_{RR}TTTTT\text{-}L\text{-}LysNH_2)_2$ + DNA-A_{10}	52[c]
$(PNA\text{-}TTTTTTTTTT\text{-}L\text{-}LysNH_2)_2$ + DNA-A_{10}	71.5[c]

[a] Buffer: 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7.0. [b] CD measurements. [c] Absorption (hypochromicity) measurements.

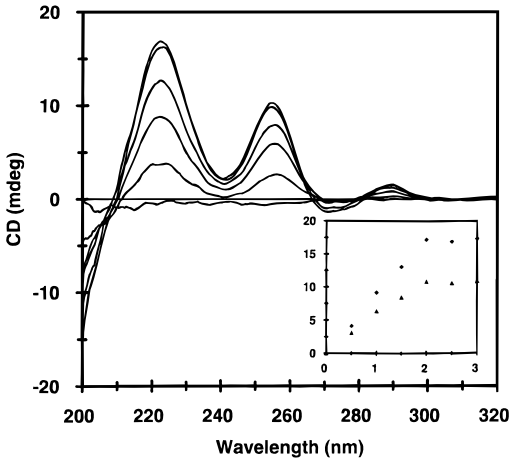

Figure 2. CD titration of PNA-T_{10}(SS) into PNA-A_{10} in 5 mM sodium phosphate (pH 7.0, 20 °C), insert showing the CD at 223 and 255 nm for various thymine−PNA to adenine−PNA ratios. Concentration at the saturation point: 25 mM adenine bases, 50 mM thymine bases. CD spectra recorded on a JASCO 720 spectropolarimeter.

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(8) CD in the nucleobase absorption region of the DNA bases arises from helical stacking of the bases as a result of exciton interactions between the transitions in neighboring bases and from their interactions with transitions of the chiral deoxyribose moiety. By contrast, the PNA backbone is achiral and the electronic interaction between the majority of the bases with the chiral terminal lysine is small. Thus, the CD of PNA is due practically only to a chiral orientation of the base pairs relative to each other.
(9) A slight deviation from perfect symmetry can be ascribed to the presence of the terminal L-lysine in both the (*RR*)- and (*SS*)-cyclohexyl PNA-T_{10} (see Table 1).
(10) CD titrations were also performed in a buffer containing 100 mM NaCl. Identical results as obtained without NaCl were found, in agreement with the earlier verified lack of salt-dependence for PNA−PNA duplexes.
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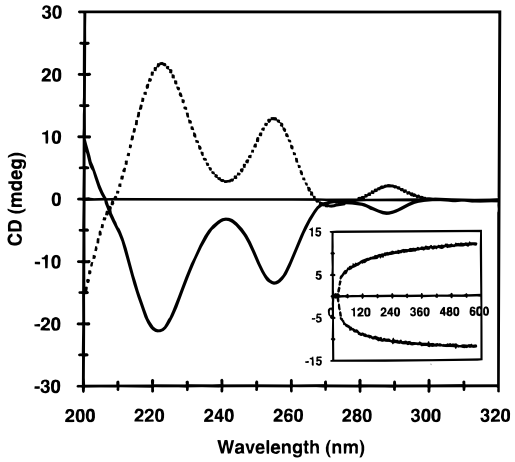

Figure 3. CD spectra of (PNA-T_{10}(*SS*))$_2$-PNA-A_{10} and (PNA-T_{10}-(*RR*))$_2$-PNA-A_{10} triplexes. Base-triplet concentration 25 mM. The insert shows the CD at 255 nm as a function of time (in seconds) upon mixing the PNAs in a 2:1 base ratio (20 °C).

We also studied the complex formed between PNA-A_{10} and a PNA-T_{10} having a terminal L-lysine residue as the only chiral moiety. Absorbance measurements (hypochromicity) indicated that also here a complex with a 2:1 thymine to adenine stoichiometry is formed. The melting temperature for this triplex was also high (Table 1), similar to those of the cyclohexyl-containing PNA_3 triplexes. The T−A−T triplet with the PNA-T_{10}−lysine did not exhibit any significant CD (not shown) which is not surprising in view of the previous finding that an A:T base-pair is a poor transmitter of chirality from a terminal lysine in PNA−PNA duplexes.[7] For the cyclohexyl as well as the lysine PNA-T_{10} oligomers in mixtures with PNA-A_{10}, electrospray mass spectroscopy verified that triplexes but not duplexes were formed.

In order to study the kinetics of triplex formation, we followed the evolvement of ellipticity at 255 nm as a function of time upon mixing the PNA-T_{10}(*SS*) or PNA-T_{10}(*RR*) with PNA-A_{10} (examples of kinetic traces shown in insert of Figure 3). Within experimental errors, these "mirror-image" reactions proceeded with identical rates showing that the chirality of the end-terminal lysine does not significantly affect the reaction.[13] The time-dependence could be fitted to a first order kinetics, and the rate constant was found essentially independent of the PNA concentration (not shown). In contrast to the slow CD change, the hypochromicity upon mixing occurs within seconds, showing that the association of the PNA strands is fast. We thus conclude that the CD kinetics reflects a monomolecular internal reorganization (inversion) process in an already formed triplex, in analogy with what was previously found for the reorganization within PNA−PNA duplexes.[7] From the CD kinetics as a function of temperature, an activation energy of approximately 73 kJ/mol and an activation entropy of about −31 J/(mol K) were estimated. These values could be compared to those observed for helix-propagation in PNA−PNA duplexes induced by a terminal lysine residue (activation energy 34 kJ/mol and activation entropy −70 J/(mol K)),[4] although one should recall that the chiral centers are different. The helix propagation in the PNA triplex, hence, seems to require a larger activation energy, in accordance with a larger energy cost for rearranging three strands compared to only two strands in a duplex.

The possibilities of formation of corresponding CGC or CGC^+ triplex structures were tested also for a mixed purinic motif. UV and CD titrations of the PNA H-GAGAGGAAAA-L-$LysNH_2$ with PNA H-TTTTCCTCTC-L-$LysNH_2$ gave a duplex complex only (both at pH 5 and 7), indicating that formation of PNA pyrimidine−purine−pyrimidine structures may not be a general property. For these mixed-base sequences, both strand polarity and pH may play crucial roles.

In conclusion, here we demonstrate the existence of pure peptide nucleic acid triplexes. These are formed as extremely cooperative Watson−Crick−Hoogsteen T-A-T triplets which virtually form and dissociate (melt) in one step. As concluded from CD they have helical stuctures, right-handed with PNA-T_{10}(*SS*) (left-handed with *RR*), and display an inversion rearrangement like that previously reported for PNA duplexes. Together with the latter, PNA triplexes may be regarded as a novel type of robust supramolecular structures that may find exploitation as structural elements for the design of various molecular devices in nanotechnology.

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(13) CD kinetics in presence or absence of 100 mM NaCl were identical.