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Crystallization and preliminary X-ray diffraction studies of a D-lysine-based chiral PNA–DNA duplex

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A 10-mer duplex formed between a PNA containing a 'chiral box' of three adjacent D-Lys-based monomers and its complementary DNA strand has been crystallized for the first time. Crystals have been obtained using PEG 8000 as precipitant and cacodylate at pH 6.3 as buffer. The crystals belong to the space group $P3_1$ or to its enantiomorph $P3_2$, with unit-cell parameters $a = b = 35.00$, $c = 35.91$ Å. A complete data set has been collected at the synchrotron source Elettra in Trieste to 1.85 Å resolution, using a single frozen crystal.

1. Introduction

Peptide nucleic acids (PNAs) are nucleic acid analogues in which the sugar–phosphate backbone has been replaced by a pseudopeptide skeleton made up of *N*-(2-aminoethyl)glycine units (Nielsen *et al.*, 1991). Nucleobases are linked to this skeleton through a carboxymethyl moiety, which allows a two-atom spacer between the backbone and the bases.

PNA oligomers can be synthesized in relatively large amounts. PNAs recognize DNA and RNA sequences with high specificity and selectivity by forming Watson–Crick base pairs leading to RNA–PNA and DNA–PNA hybrids more stable than the corresponding regular nucleic acid complexes (Egholm *et al.*, 1993). Their high thermal stability and high resistance to proteases and nucleases make PNAs ideal candidates for antisense or antigene therapeutic agents (Ulhmann *et al.*, 1998; Nielsen, 2000; Ray & Norden, 2000) and they are currently used as very powerful tools in molecular biology and in diagnostics. However, the development of PNA-based therapeutics is prevented somewhat by the poor solubility of PNAs under physiological conditions and by their limited uptake into cells (Gildea *et al.*, 1998; Soomets *et al.*, 1999). Furthermore, PNAs can bind to complemetary DNA strands in both parallel and antiparallel orientations. Thus, several variants of the basic PNA structure were proposed in order to improve the binding specificity, solubility and uptake into cells (reviewed in Ganesh & Nielsen, 2000). Duheolm *et al.* (1994) modified the PNA backbone by introducing chiral aminoethylamino acids. Circular dichroism experiments demonstrated that the configuration of stereogenic centres induces preferential PNA helicity: namely, D-monomers induce a preferred right-handedness, while L-monomers induce left-handness in the PNA strands (Sforza *et al.*, 1999; Smith *et al.*, 1999). As a consequence of the observed enantioselectivity, right-handed DNA was found to bind with higher affinity to PNAs containing D-monomers (Sforza *et al.*, 1999). Moreover, it has been demonstrated that with the introduction of positively charged lysine-based monomers more stable PNA–DNA duplexes can be obtained owing to stabilizing electrostatic interactions with the negatively charged phosphate groups (Haaima *et al.*, 1996). On the basis of these results, Sforza *et al.* (2000) recently reported the synthesis of a chiral PNA decamer containing three adjacent chiral monomers ('chiral box') based on D-lysine in the middle of the PNA sequence (Fig.1). Binding studies demonstrated that this 10-mer PNA hybridizes with the complementary DNA sequence only in the antiparallel mode; furthermore, the difference between the melting temperatures of its full-matched and single-mismatched hybrids with DNA strands was greatly enhanced compared with those observed for other achiral or chiral PNA. Thus, it possesses many of the properties required for the detection of point mutations in diagnostics and for the development of gene therapeutics.

Three-dimensional structures have been determined for the major families of PNA complexes. A PNA–RNA duplex (Brown *et al.*, 1994) and a PNA–DNA duplex (Eriksson & Nielsen, 1996) were solved by NMR and a PNA_2–DNA triplex (Betts *et al.*, 1995) and two PNA–PNA duplexes (Rasmussen *et al.*, 1997; Haaima *et al.*, 1999) were solved by X-ray crystallography. Analysis of the structures of the DNA–PNA (Eriksson & Nielsen, 1996) and RNA–PNA (Brown *et al.*, 1994) duplexes have shown that PNA, when hybridized to a nucleic acid, seems to adopt the conformation of its partner. In fact, the PNA strand adopts

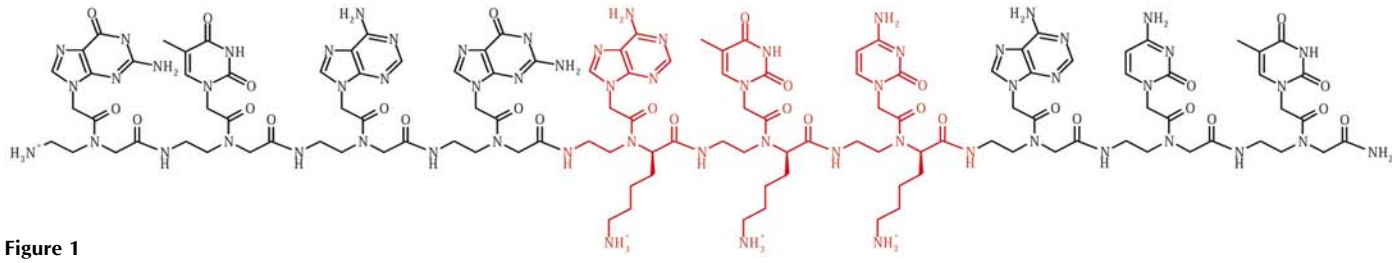

Figure 1
The three D-lysine-based PNA monomers (chiral box) introduced in the middle of the PNA sequence used in the present study are shown in red.

an A-like helix in both cases, even though in the DNA–PNA duplex the DNA sugars are more B-like. By contrast, the PNA_2–DNA triplex (Betts *et al.*, 1995) was found to adopt a novel helix, called a P-helix, different from both the A- and B-forms. Finally, the structural features of the PNA–PNA double helix (Rasmussen *et al.*, 1997; Haaima *et al.*, 1999) are different from both the canonical A- and B-form and rather resemble the P-helix described for the PNA_2–DNA triplex. These results indicate that canonical PNAs are not the optimal mimic of DNA and that the structural features of new modified PNAs need to be investigated further.

To the best of our knowledge, no structure of a complex between chiral PNA and DNA has been reported so far. In a general project aimed at understanding at the molecular level the role of chirality in DNA recognition and in order to investigate the structure–activity relationships of chiral PNAs, we have undertaken a crystallographic study on a PNA decamer ($GTAGGA_{D\text{-}Lys}T_{D\text{-}Lys}C_{D\text{-}Lys}ACT$) containing a chiral box (Fig. 1), hybridized with its complementary antiparallel DNA strand (5′-AGTGATCTAC-3′). Here, we report the crystallization and preliminary crystallographic studies of this duplex.

2. Materials and methods

Synthesis and purification of the the D-lysine-based PNA decamer has previously been reported (Sforza *et al.*, 2000), while the complementary DNA single strand was purchased from PRIMM Labs and used without further purification. The 10-mer duplex was formed by mixing equal amounts of PNA and DNA single strand. The double-strand PNA–DNA was annealed by warming to 353 K and cooling to 277 K. The sample was lyophilized and then redissolved in water for the crystallization experiments.

The Hampton Natrix screen, which is based on the nucleic acid sparse-matrix sampling of Scott *et al.* (1995), was used to test 48 crystallization conditions. The screen was carried out at 298 K with Linbro tissue-culture plates in which 2 µl of duplex solution was added to 2 µl of reservoir solution in a hanging drop suspended over 1 ml reservoir.

Very tiny crystals were grown using a reservoir solution of 200 m*M* ammonium acetate, 10 m*M* magnesium acetate, 30% PEG 8000, 100 m*M* sodium cacodylate pH 6.5. Optimal conditions for crystallization were achieved with reservoir solutions consisting of 100 m*M* ammonium acetate, 28% PEG 8000, 50 m*M* sodium cacodylate pH 6.3. Two subsequent macroseeding experiments using crystals thus obtained were necessary to obtain single crystals of sizes suitable for X-ray diffraction studies. Under these conditions, crystals could be reproducibly grown to dimensions of 0.05 × 0.05 × 0.3 mm in one week.

For data collection, crystals were (i) briefly washed in solutions of 100 m*M* ammonium acetate, 28% PEG 8000, 50 m*M* sodium cacodylate pH 6.3 and 10% glycerol, (ii) retrieved with a 0.1 mm nylon loop and (iii) flash-frozen in the nitrogen stream from an Oxford Cryosystems Cryostream cooler operated at a temperature of 100 K.

X-ray diffraction data were collected on a MAR CCD detector at the Elettra synchrotron source in Trieste and were processed using the *DENZO* and *SCALEPACK* crystallographic data-reduction package (Otwinowski & Minor, 1997).

3. Results

Large well formed crystals of the D-lysine-based chiral PNA–DNA duplex were grown in one week using the hanging-drop method. The crystals exhibited hexagonal morphology (Fig. 2) and belong to the $P3_1$ space group or to its enantiomorph $P3_2$. A complete data set to 1.85 Å resolution has been collected from one flash-cooled crystal at 100 K. The scaling gave a final R_{sym} of 7.3% for 37 552 reflections, of which 4257 were unique, corresponding to 100.0% completeness. The unit-cell parameters at 100 K were $a = b = 35.00$, $c = 35.91$ Å. A solvent content of 41.7% was calculated by the methods of Matthews (1968), assuming the crystals to contain one duplex per asymmetric unit (V_M = 2.11 Å^3 Da^{-1}). Table 1 reports the crystallographic data.

Table 1
Crystal and data-collection parameters.

Values in parentheses refer to the outermost resolution shell.

Space group	$P3_1$ or $P3_2$
Unit-cell parameters (Å)	
a	35.00
c	35.91
Independent molecules	1
Resolution limits (Å)	20.00–1.85
Temperature (K)	100
Total No. of reflections	37552
No. of unique reflections	4257
Completeness (%)	100.0 (100.0)
R_{merge}†	0.073 (0.111)
Mean $I/\sigma(I)$	22.5 (15.2)

† $R_{merge} = \sum|I_i - \langle I\rangle|/\sum I_i$, where the sum is over all reflections.

Figure 2
Crystals of D-lysine-based chiral PNA–DNA duplex. Actual size of the largest crystal is 0.05 × 0.05 × 0.3 mm.

Several attempts to solve the structure by molecular replacement (Navaza, 1994) have failed; the structure determination of the duplex is therefore proceeding with multiple-wavelength anomalous diffraction techniques (Hendrickson & Ogata, 1997). A brominated derivative is currently being crystallized.

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