Crystallization and preliminary X-ray analysis of a PNA–DNA complex

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Received 6 March 1995

Abstract Peptide nucleic acids (PNAs) are DNA mimics with a peptide backbone. PNAs are being intensely investigated owing to a potential as gene-targeted drugs. A PNA (H-GTAGAT-CACT-NH_2)–DNA (5′-AGTGATCTAC-3′) complex has been crystallized in a tetragonal space group $P4_122$ with cell dimensions $a = b = 79.8$, $c = 99.9$ Å. The crystals diffract to about 5 Å resolution.

Key words: PNA; DNA; Crystallization; Complex

1. Introduction

PNA (peptide nucleic acid) is a recently developed oligonucleotide analogue in which the entire backbone has been replaced by a pseudopeptide backbone composed of *N*-(2-aminoethyl)glycine units [1–3]. Although the new backbone chemically does not even remotely resemble the deoxyribose phosphodiester backbone of DNA, it is structurally homomorphous to this. Experiments revealed that PNA is close to a perfect structural mimic of DNA in terms of its ability to form double helical complexes with complementary oligonucleotides [4] and complementary PNAs [5], and the complex formation obeys the Watson–Crick base pairing rules (adenine-thymine, and guanine-cytosine recognition) (Fig. 1).

PNA can also bind to double-stranded DNA by strand displacement [1,6]. Two PNA strands are bound to the one DNA strand, by Watson–Crick or Hoogsteen base pairing [7].

Upon binding to mRNA, PNA interferes with ribosome function and thus acts as an antisense inhibitor of translation [8]. PNA bound at a protein (restriction enzyme) recognition site efficiently inhibits the cleavage at this site by the enzyme [9]. When a PNA binding site is positioned downstream from a promoter, transcription elongation by RNA polymerase is affected when PNA is bound to the template strand, but is only little affected when PNA is bound to the non-template strand [8,10]. Thus, PNA has many of the properties desired of an antisense/antigene drug candidate [8,11], including stability towards nucleases, proteases, peptidases and human serum [12].

Circular dichroism results have shown that PNA–DNA complexes adopt a B-like helical structure while PNA–RNA complexes resemble more the A-form [4]. These conclusions were recently confirmed by two-dimensional NMR spectroscopy [13,14]. However, a high resolution molecular structure is not yet available and would be indispensable for further rational design of new DNA mimics and for a molecular understanding of the DNA binding properties of PNA.

We now report the crystallization of a PNA–DNA complex as a first step towards this goal.

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Abbreviations: PNA, peptide nucleic acid; PEG, polyethylene glycol.

2. Materials and methods

The PNA H-GTAGATCACT-NH_2 (I) was synthesized as described [2] and purified by HPLC. The complementary, antiparallel oligonucleotide 5′-AGTGATCTAC-3′ (II) was synthesized by the standard phosphoramidite procedure. The ratio of PNA to oligonucleotide to be used for crystallization experiments was determined experimentally by titration using a gel shift assay and a trace amount of ^{32}P-labeled oligonucleotide. The oligonucleotide was labeled with ^{32}P at the 5′-end using [γ^{32}P]ATP (5000 Ci/mmol; Amersham) and T_4 polynucleotide kinase [15].

PNA (I) and DNA (II) were mixed in an approximately 1:1 ratio, yielding a concentration of 3.5 mg/ml for the PNA–DNA complex in water. Crystals were obtained by the hanging drop vapor diffusion method [16]. 3–5 μl of PNA–DNA were mixed on a siliconized coverslip with equal volumes of well solution, typically 14% PEG 6000, 200–300 mM zinc acetate and 100 mM sodium cacodylate buffer, pH 6.5, and equilibrated at room temperature over 0.5 ml of well solution using a Lindbro tissue culture plate (Flow Lab Inc.; McLean, VA, USA). Within 4 days crystals grow to a size of 0.2 × 0.2 × 0.2 mm. By repeated seeding experiments crystal size was improved to 0.5 × 0.5 × 0.5 mm within an additional 4 days. The crystal was moved into a new drop containing 2 mg/ml PNA–DNA, 5% PEG 6000, 130 mM zinc acetate, and 40 mM cacodylate buffer, pH 6.5, and equilibrated over 0.5 ml of well solution containing 12% PEG 6000, 300 mM zinc acetate, and 100 mM sodium cacodylate buffer, pH 6.5.

A full X-ray data set was collected on one crystal to 5 Å resolution on an R-AXIS II imaging plate detector system using a Rigaku RU200 rotating anode generator (λ = 1.542 Å, 50 kV, 180 mA). Data were processed using the program DENZO [17] and the CCP4 suite of programs [18] and visualized with the program PRECESS [19].

3. Results and discussion

3.1. Crystallization of the PNA (I)–DNA (II) complex

Bipyrimidal crystals of PNA–DNA were obtained by the hanging drop method using PEG as precipitant and zinc acetate as additive (Fig. 2). The addition of zinc acetate was essential to crystal growth, and substitution with other divalent cations (magnesium, calcium, cobalt, and copper) did not result in any regular crystals. In addition, the acetate anion was clearly to be preferred over chloride and sulphate as anions. Zinc ions have not been used for crystallization of any DNA–DNA or DNA–RNA complexes deposited in the Biological Macromolecule Crystallization Database [20].

Crystals were grown to a maximum size of 0.2 × 0.2 × 0.2 mm

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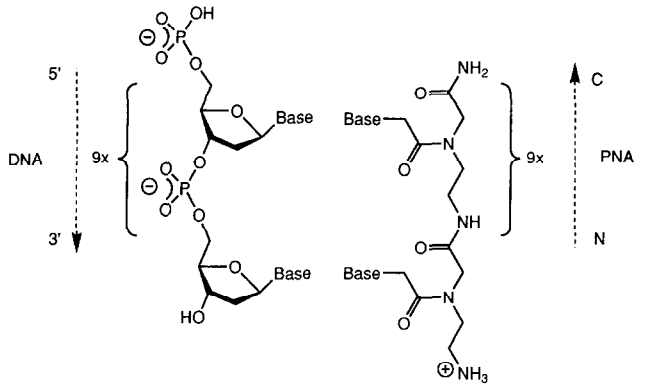

Fig. 1. Schematic representation of a PNA–DNA complex. The repeating PNA and DNA units are marked by brackets.

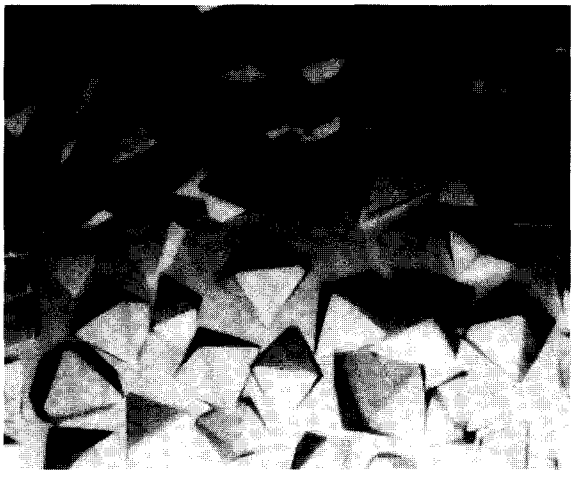

Fig. 2. Crystals of the PNA–DNA complex (bar indicates 0.1 mm).

within 4 days at room temperature. These crystals show diffraction to about 13 Å resolution. After repeated seeding crystal size was increased to 0.5 × 0.5 × 0.5 mm; hereby the diffraction was improved to 5 Å resolution. Within this resolution shell the diffraction is very strong, while outside this shell the crystals show no diffraction at all (Fig. 3). The crystals are stable for at least 4 days in the beam showing no decrease in diffraction.

A complete (98.8%) data set was collected to 5.0 Å resolution (R_{sym} = 12.1%, $I/\sigma(I)$ = 3.8). PNA–DNA crystallizes in the tetragonal space group $P4_122$ with cell dimensions of $a = b = 79.8$, $c = 99.9$ Å. Estimating the solvent content as for protein crystals [21], the crystals of PNA–DNA contain 64% solvent for 32 PNA–DNA complexes in the unit cell, 46% for 48, and 28% for 64. Comparing the volume occupied by different DNA–DNA decamers from structures deposited in the Protein Data Bank, January release 1995 [22], an average volume of 13.460 Å^3 per DNA–DNA complex is found, ranging from 10.070 Å^3 to 17.492 Å^3. The decamer structures deposited crystallize in either a monoclinic, orthorhombic or hexagonal space group. The average volume indicates 48 complexes of

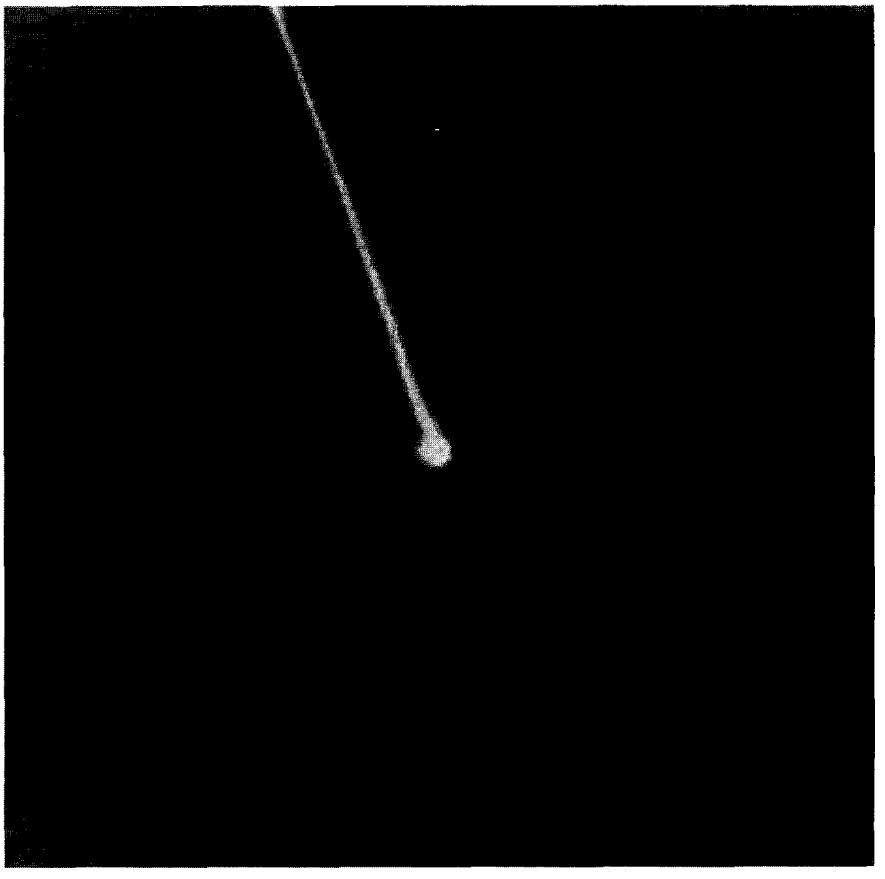

Fig. 3. Diffraction pattern obtained from a PNA–DNA crystal on an R-AXIS II imaging plate detector system using a Rigaku RU200 rotating anode generator (λ = 1.542 Å, 50 kV, 180 mA). The picture shows a 2° oscillation, crystal-imaging plate distance 90.0 mm.

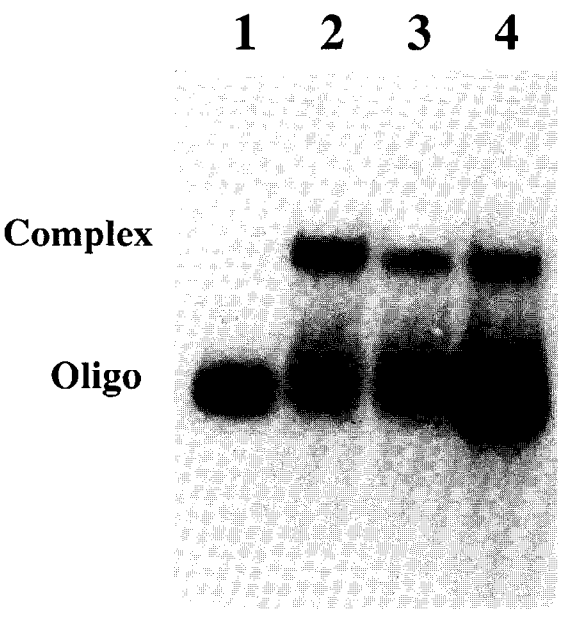

Fig. 4. Detection of oligonucleotide (II) and PNA (I) in crystals. Lane 1, 5′ ^{32}P-end labeled oligonucleotide (II); lane 2, sample as in lane 1 except that 1 μg of PNA (I) was included; lane 3, sample as in lane 1 except that crystal material corresponding to appr. 0.1 μg was added; lane 4, crystal material (appr. 0.1 μg) treated with [γ^{32}P]ATP and polynucleotide kinase. The samples were analyzed by electrophoresis in a 15% polyacrylamide gel run in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.3). The bands containing ^{32}P-labeled oligonucleotide were visualized by autoradiography using intensifying screens.

PNA–DNA per unit cell. However, the large range in observed volume might suggest numbers of 32 to 64 complexes.

3.2. Biochemical characterization of the crystals

Isolated and washed crystals were analyzed for their content of PNA (I) and oligonucleotide (II) by polyacrylamide gel electrophoresis.

In one experiment it was shown that the crystals contain material that is able to bind to ^{32}P-labeled oligonucleotide (II) and produce a gel-retarded complex that has the same migration as that produced by an authentic sample of the complementary PNA (I) (Fig. 4, lanes 2 and 3).

In another experiment crystal material was treated with [γ^{32}P]ATP and polynucleotide kinase, and upon gel analysis a major ^{32}P-labeled component migrating as ^{32}P-labeled oligonucleotide (II), as well as a minor component migrating as the authentic PNA–oligonucleotide complex, were observed.

We therefore conclude that the crystals contain both PNA (I) and oligonucleotide (II).

3.3. Work in progress

So far crystals of the PNA (I)–DNA (II) complex have been obtained diffracting to 5 Å resolution. The experimental conditions for crystallization of PNA–DNA and for data collection are being optimized in order to obtain higher resolution.

Work is in progress to solve the 5 Å structure of the PNA–DNA complex by molecular replacement techniques. A model of a PNA (I)–DNA (II) complex and of an analogous DNA–DNA complex have been constructed by molecular modelling (to be published). These models are being used as search models for molecular replacement.

Acknowledgements: This work was supported by The PharmaBiotec Research Center, The Lundbeck Foundation, The Danish Medical and Technical Research Councils, and The Danish Basic Research Foundation.

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