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Oligonucleotides with cyclohexene-nucleoside building blocks: crystallization and preliminary X-ray studies of a left-handed sequence GTGTACAC

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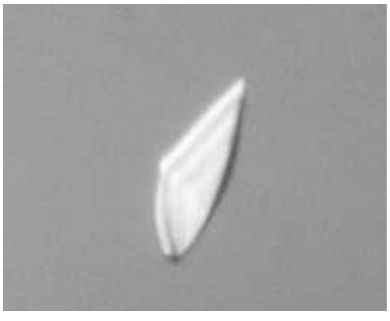
Cyclohexene nucleic acids contain a cyclohexene ring instead of the normal β-D-2′-deoxyribose. The cyclohexene oligonucleotide GTGTACAC was synthesized using phosphoramidite chemistry and standard protecting groups. Crystals of GTGTACAC were obtained at 289 K by the hanging-drop vapour-diffusion technique. The crystals diffract to 1.7 Å resolution and belong to the trigonal space group $R3$, with unit-cell parameters a = 41.434, c = 66.735 Å.

1. Introduction

Unlike traditional drugs, antisense oligonucleotides directly target the genetic code (mRNA or DNA), thereby inhibiting translation (or transcription). Modifications of the classical DNA backbone are necessary because of problems with low stability and cellular uptake. Some of these modifications [hexitol nucleic acids (HNAs; Fig. 1*a*) and D-altritol nucleic acids (ANAs; Fig. 1*b*)] have an extra methylene group between the O4′ and C1′ of the natural β-D-2′-deoxyribose, which has a positive influence on the stability of HNA, ANA, HNA/RNA and ANA/RNA duplexes as indicated by their melting temperatures (Allart *et al.*, 1999).

Cyclohexene nucleic acids or CeNAs (Fig. 1*c*) also form more stable duplexes with RNA than their DNA analogue (ΔT_m per modification +2 K); moreover, they induce RNaseH cleavage of the RNA strand (Wang *et al.*, 2000; Verbeure *et al.*, 2001; Nauwelaerts *et al.*, 2005). In CeNAs, two extreme conformations are possible for the six-membered ring: the $^3H_2$ form, comparable to a C3′-*endo* furanose ring, and the $^2H_3$ form, mimicking the furanose ring in its C2′-*endo* form.

The CeNA sequence GTGTACAC was built from left-handed building blocks and the double-stranded left-handed helix was crystallized in order to explain the stability and to investigate the geometry and hybridization of these new CeNA duplexes.



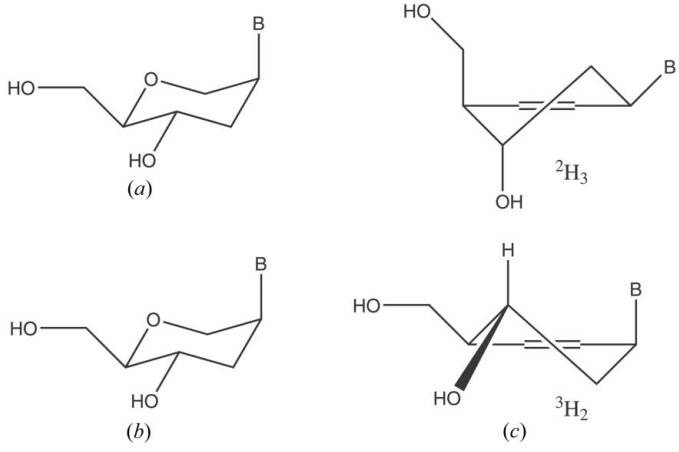


Figure 1
(*a*) HNA building block, (*b*) ANA building block, (*c*) CeNA building block (in two possible conformations).

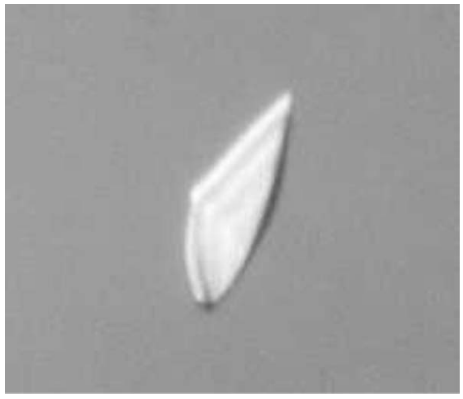


Figure 2
Crystal of the CeNA-containing octamer GTGTACAC obtained by hanging-drop vapour diffusion. The dimensions of the crystal are 0.15 × 0.06 × 0.06 mm.

2. Methods and results

2.1. Synthesis

The synthesis of the left-handed CeNA oligonucleotide sequence GTGTACAC is described in Gu *et al.* (2003).

2.2. Crystallization

Crystallization conditions of the left-handed CeNA sequence GTGTACAC were screened at 289 K by the hanging-drop vapour-diffusion technique using a 24-matrix screen for nucleic acids (Berger *et al.*, 1996). Crystals appeared after 1 d using the condition 10%(*v*/*v*) MPD, 12 m*M* spermine tetrachloride, 80 m*M* $SrCl_2$, 40 m*M* LiCl, 20 m*M* $MgCl_2$ and 40 m*M* cacodylate buffered at pH 7. The best crystals were obtained using 4 µl droplets (2 µl screen and 2 µl CeNA oligonucleotide at 1.5 m*M*) equilibrated against 500 µl MPD reservoir. The crystal form is somewhat bent (Fig. 2), with average dimensions of 0.15 × 0.06 × 0.06 mm; the crystals remain stable over time. The quality of the crystals was tested on a Bruker Smart 6000 CCD system, showing the necessity of using synchrotron radiation.

2.3. Data collection and processing

Data were collected on an MAR Research image plate at the EMBL synchrotron facility in Hamburg (BW7b, λ = 0.84140 Å). The crystals were flash-cooled at 100 K and data were collected over a 90° φ range with an increment of 2° and a crystal-to-detector distance of 300 mm (1.63 Å). Crystals showed no sign of decay during irradiation. Data-collection statistics are given in Table 1.

The data set was processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski & Minor, 1996).

Table 1
Data-collection statistics.

Values in parentheses are for the outermost shell.

No. of reflections used	23788
No. of unique reflections	4707
Resolution range (Å)	20–1.7 (1.76–1.70)
$I/\sigma(I) > 3$ (%)	54.2 (35.1)
$\langle I/\sigma(I)\rangle$	6.57 (3.05)
Completeness (%)	99.4
$R_{\rm merge}$ (%)	7.2 (29.4)
Multiplicity	5.0

The crystals belong to the trigonal space group *R*3, with unit-cell parameters a = 41.434, c = 66.735 Å (hexagonal setting) and one duplex in the asymmetric unit, resulting in a volume per base pair of 1378 Å^3. The Matthews coefficient ($V_{\rm M}$) is 2.2 Å^3 Da^{-1} for one double helix in the asymmetrical unit, giving a solvent fraction of 42.4%.

The default settings of *DENZO* initially indicated a smaller cell (*c* halved) owing to alternating strong and weak layers in the diffraction patterns.

Structure determination is currently ongoing using a model based on the HNA double helix as a molecular-replacement target. The distribution of the strong base-stacking reflections suggests that the double helix is orientated along the *z* axis.

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References

Allart, B., Khan, K., Rosemeyer, H., Schepers, G., Hendrix, C., Rothenbacher, K., Seela, F., Van Aerschot, A. & Herdewijn, P. (1999). *Chem. Eur. J.* **5**, 2424–2431.
Berger, I., Kang, C., Sinha, N., Wolters, M. & Rich, A. (1996). *Acta Cryst.* D**52**, 465–468.
Gu, P., Schepers, G., Rozenski, J., Van Aerschot, A. & Herdewijn, P. (2003). *Oligonucleotides*, **13**, 479–489.
Nauwelaerts, K., Lescrinier, E., Sclep, S. & Herdewijn, P. (2005). In the press.
Otwinowski, Z. & Minor, W. (1996). *Methods Enzymol.* **276**, 307–326.
Verbeure, B., Lescrinier, E., Wang, J. & Herdewijn, P. (2001). *Nucleic Acids Res.* **29**, 4941–4947.
Wang, J., Verbeure, B., Luyten, I., Lescrinier, E., Froeyen, M., Hendrix, C., Rosemeyer, H., Seela, F., Van Aerschot, A. & Herdewijn, P. (2000). *J. Am. Chem. Soc.* **122**, 8595–8602.