Bulgarian Chemical Communications, Volume 48, Number 4 (pp. 589 – 592) 2016

Crystal structure of a DNA sequence d(CGTGAATTCACG) at 130K

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Received September8, 2016;Revised November7, 2016

The crystal structure of the oligonucleotide d(CGTGAATTCACG)has previously been reported as a B-type double helix at a resolution of 2.5 Å. Here, the structure of this sequence was determined at a higher resolution of 2.00Å in space group $P2_12_12_1$. The adjustmentsin crystal packing between the former and latter are described. The present structure allowed more in depth description of the interactions between the oligonucleotides and with the surrounding solvent: the presence of Mg and Cl ions, a greater number of water moleculesand non-classicalG···G hydrogen bonding interactions between adjacent DNA duplexes.

Keywords:DNA, Single crystal, palindrome

INTRODUCTION

The d(CGTGAATTCACG) DNAduplex is interesting because it features a *Eco*RI restriction site[1]. The crystal structure of the sequence has been previouslyreported at 2.7and2.5 Å resolution[2, 3].Interestingly,data collection has been carried out at 0ºC because the authors state that the useof lowertemperatures resulted in the absence of diffraction, associated with damage of the crystals[2]. In the present manuscript we report the structure of $d(CGTGAATTCACG)_2$ collected at 130 K and at a higher resolution of 2.0Å. This is the highest resolution reported for this structure. Thus, we are able to discern a number of detailsthat were not spotted in the previous reports: the presence of Mg and Cl ions, a greater number of water molecules) and non-classicalG···G hydrogen bonding interactions. One shouldnote that our aim was to co-crystalize the DNAwithDAPI and thus thecrystallization condition includedDAPI. It is unclear whether the presence of an intercalating agent is responsible for the stabilization of the crystal though we observed visually the degradation/destruction of the crystalsinthe drops afew dayslater.

EXPERIMENTAL

Sample crystallization

The dry oligonucleotide sequence CGTGAATTCACG was purchased from "EurofinMWG Genomics",dissolved in a buffer up to 1 mM and annealed at 75°Cbefore usein order to obtain dsDNA. The buffer solution consists of 60mM sodium cacodylate (pH 7.0), 17 mM $MgCl_2$, 2mMSpermine and 1.5 mM DAPI. Crystals were grown from hanging drops (3 μl) at room temperature, equilibratedagainst 50%*v*/*v*2-methyl-2,4-pentanediol (MPD).Large crystals (0.4 x 0.3 x 0.25 mm^3) suitable for single crystal X-ray studies formed within a month(Fig. 1).

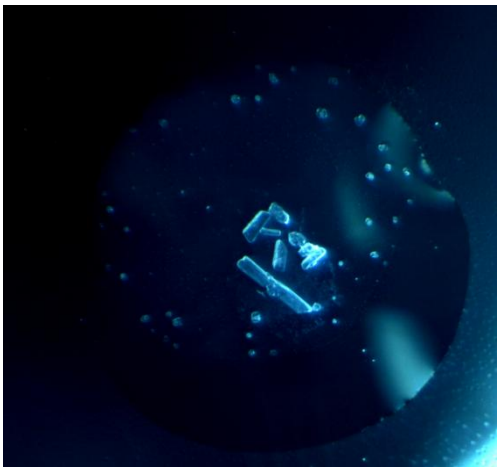

Fig. 1. Observed crystals of d(CGTGAATTCACG)

Data collection and crystal structure refinement

Crystals were mounted on loops and were flash frozen at 130 K directly under the nitrogen cryo stream (Cobra, Oxfordcryosystems). All data were collected at low temperature(130K) on an Oxford diffraction Supernova diffractometer using Cu-Kα radiation (λ = 1.54056 Å) from a micro-focus source. The determination of unit cell parameters, data integration, scaling and absorption correction were carried out using the CrysalisPro[4]. The phases were obtained by molecular replacement with a Phaser[5]using 1D29 [2] as the starting model.The refinement of the structure involved several cycles of refinementusing Refmac 5 [6] and Coot[7]programs.The water and heavier atoms (Mg and Cl) were positioned on the *Fo-Fc*difference

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map using the interface of a Coot program.A summary of the fundamental crystal data and refinement indicators is provided in Table 1.Graphical analyses of the model and the electron-density maps were carried out using Coot[7].X3DNA [8] was used to carry out structural analysis and geometrical calculations of the DNA parameters. PyMOL[9] was used to prepare the figures. The coordinates and structure factors have been deposited in the PDB as entry 5JU4.

Table 1. Selected crystallographic data-collection statisticsand refinement indicators for 5JU4

Crystal system	Orthorhombic
Space group	$P2_12_12_1$
cell dimensions	
a, b, c, Å	24.503, 41.09, 65.184
α, β, γ, °	90, 90, 90
independent molecules	2
diffraction data	
wavelength, Å	1.54056
resolution, Å	2.0
reflections	4796
completeness, *%*	99.5
I/σ(I) at 2 Å	*2.77*
redundancy	8.2
R*merge* %	7.5(36.8)
Refinement	
reflections used	4524
resolution, Å	2.0
R (R*free*)%	21.8 (29.0)
no. of atoms	544
DNA	488
Mg, Cl/ion	2
Waters	74
average B fatcor, Å ^{2}	31.01
R.m.s.d.	
bond lengths, Å	0.009
bond angles,°	1.776
PDB code	5JU4

RESULTS AND DISCUSSION

Single crystal data collection has been attempted for several different crystals. One should note that the dataset collected up to a resolution of2.00 Å was from a crystalthat was harvestedfromthedrop five(5) daysafter it was spotted. Crystals withsimilar or even bigger dimensions (size)that were allowed to "stabilize" for more than a week in the crystallization drop diffracted usually at a resolution up to 2.5 Å.Attempts for datacollection at roomtemperature (19^{o}C) were performed on afew crystals, however the observedquality of the diffraction was not comparable with that for experiments conducted at130K.The presence of DAPI in the crystallization conditions may have played a role for crystal structure stabilization.

The asymmetric unit of 5JU4 consists of two chemically equivalent self-complementary strands (each strand is twelve base pairs in length)forming an anti-parallel right-handed DNA(Fig. 2).The B-type DNA duplex is formed by classical Watson-Crick (W-C) hydrogen bonding base-pairing interactions between the two strands: basesC1 to G12 from the first strand interact with basesG13 to C24from the opposite (second) strand (thenumbering corresponds to a sequence of thecrystal structure).Theminor grooveof the present double stranded oligonucleotide features a central TpAstep (AATT) surrounded by C or G rich regions. Its overall secondary structure is comparable to the previously reported structureswith the same sequence, PDB entry 1D29[2], with an *rmsd* of 1.61Å when the two structures are superposed (Fig. 2b).

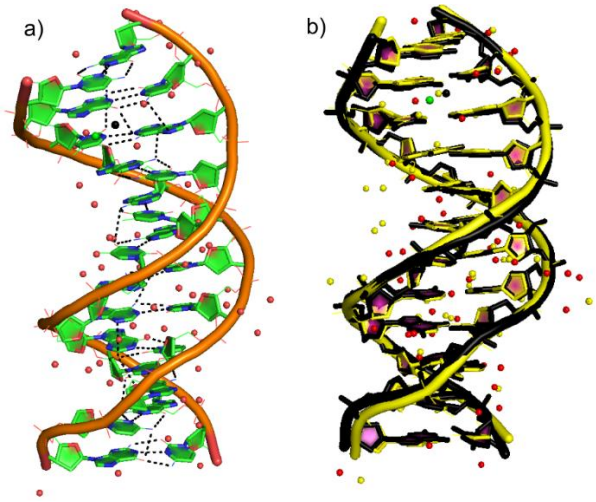

Fig. 2. View of the asymmetric unit of **a)**5JU4 including hydrogen bounds (dottedlines) and water moleculesand**b)** structural alignment of 1D29[2] (shown in yellow) and 5JU4 (the backbone is shown in black color, Mg in green, Cl in black and the water molecules in red).

The base-pair morphology values for theshear, stretch, stagger, buckle, open and propeller twist obtained using X3DNA[8] for 5JU4 and 1D29are shown in Table 2.While for the shear, stretch and staggerthe variationsbetween the two structures areminimal (average differences are 0.15, 0.23 and 0.12 respectively)the averaged differences for the

buckle, open and propeller twist values in the structures are moreevident(2.61, 5.07 and3.46 respectively). The mostpronounced differences are not in the core TpAregion butare seen mostlyat thetwoC-G ends, e.g.“propeller” differences of 11.97 and 10.45 in C-G pairs 12-13 and2-23 and differences for “opening” of 8.01 and6.24 forC-G pairs 1-24 and 11-14.Consequently, althoughthe DNA sequence is slightlyalteredthe intrastrand interactions of 5JU4 produces a motifthatis in agreement withthe Dickerson-Drew dodecamerandclassical right-handed B-DNA duplex structuralfeatures[10, 11].

The asymmetric unit of 5JU4 contains 74 water molecules (whileonly 36 are located in 1D29). Many of these waters are first hydration-shell, ordered and welldefinedand may be responsible for providing additionalstabilization to the DNA duplex. In addition the *Fo-Fc*difference map, suggests thepresence ofheavier atoms (than water)e.g.Mg^{2+} and Cl^{-} ions. No such ions, compensating the DNA negative charge are present in the 1D29 structure.The 5JU4 model shows thatMg^{2+}interactsattwo levels – firstly withthe DNA moleculepresent in the ASU, near the ends of the strands and secondly with a neighboring DNA molecule (via symmetry operation), near the minor groove (Fig. 3).One should note that the position of this particular Mg^{2+} is evident in earlier studies of $d(CGCGAATTCGCG)_2$and it has been concluded that the binding to the minor groove does not drastically affectthe DNA helical parameters [12]. The above mentioned interaction led us to the conclusion that this Mg^{2+}may have implicationsforthe DNAstabilization and the three-dimensionalarrangement of the DNA molecules.

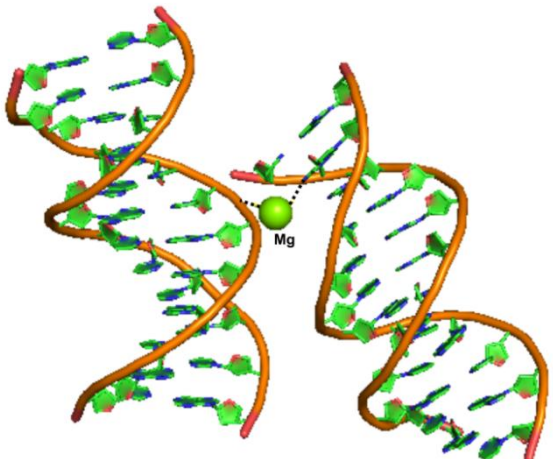

Fig. 3. Positioning of Mg^{2+} compensating the negative charge of the DNA phosphate backbone and acting as a bridge between the DNA molecules.

The 5JU4 crystal structure reveals a nonclassicalinterstrand hydrogen bonding interactionsinvolving G bases. The base pairs C1-G24, G2-C23and G12-C13, C11-G14,located at the ends of the duplexes form G...G bonds with the adjacent DNA duplexes. The discerned G…G hydrogen bonding does not correspond to Hoogsten[13]. Representative electron densities and hydrogen-bonding interactions are shown in (Fig. 4).

Based on the donor acceptordistances(*D*…*A*) the observed G…G hydrogen bonds are probably slightly weaker[14] thanclassical W-Cones(the *D*…*A*distance forG…G is around 3.0Å while in C…Git is around 2.85 Å). The G…Ginteractions are located at theends of the DNA strands whilethepreviously mentionedDNA … Mg^{2+} …DNAbridge (Figure 3) involves the AATT domain.

Table 2. X3DNA [8]results for Base-Pair morphology:shear, stretch, stagger, buckle, opening and propeller twist values in5JU4 and 1D29DNA crystal structures.

	Pair	Shear		Stretch		Stagger		Buckle		Propeller		Opening	
		1D29	5JU4	1D29	5JU4	1D29	5JU4	1D29	5JU4	1D29	5JU4	1D29	5JU4
1-24	C-G	-0.4	0.39	-0.15	-0.22	-0.1	-0.13	3.49	4.99	-11.84	-10.24	-6.69	0.45
2-23	G-C	-0.25	-0.32	-0.33	-0.31	0.04	-0.04	-7.03	-4.24	-13.6	-3.12	-11.8	-6.15
3-22	T-A	-0.17	-0.3	-0.56	-0.07	0.19	-0.27	-5.65	4.58	-10.97	-6.3	-7.79	-2.78
4-21	G-C	-0.53	-0.48	-0.56	-0.18	-0.12	-0.07	7.21	10.06	-11.47	-5.61	-0.33	0.62
5-20	A-T	0.68	0.13	-0.38	-0.05	-0.26	-0.12	4.26	8.06	-12.17	-11.91	6.81	2.01
6-19	A-T	0.08	-0.08	0.06	-0.12	-0.01	0.09	2.83	3	-18.58	-17.08	-3	3.61
7-18	T-A	0.41	-0.02	-0.01	-0.03	-0.23	0.08	0.95	-0.63	-21.35	-15.28	2.34	1.8
8-17	T-A	0.32	0.02	-0.47	-0.11	-0.13	0.09	0.66	-3.97	-18.33	-15.32	5.65	3.81
9-16	C-G	0.39	0.26	-0.41	-0.06	-0.35	0.09	-8.39	-15.17	-18.68	-12.21	-3.77	-0.49
10-15	A-T	0.09	-0.08	-0.28	-0.22	0.33	0.16	-0.23	-0.06	-15.29	-6.92	5.49	3.8
11-14	C-G	-0.17	0.04	-0.44	-0.08	0.51	0.15	3.35	1.79	-16.27	-16.8	-8.1	0.09
12-13	G-C	-0.12	-0.19	-0.47	-0.16	0.12	-0.06	3.31	10.34	-4.5	16.47	-7.9	-4.39

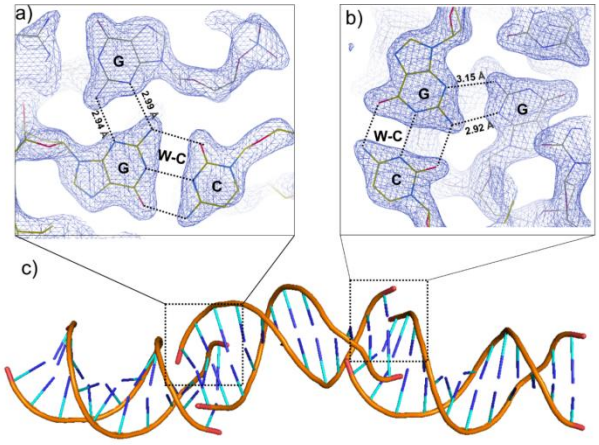

Fig.4. Representation of non-canonical base pairings within the adjacent DNA duplexes; (*a*) G…G Interaction I (*b*) G…G Interaction II and (*c*) Head-to-endarrangement of DNA duplexes generating interactions a) and b).

One can assumethat the "bulky" Mg^{2+}requiresmore space and thusoccupies the outside of theminor groove.On the other hand, the upper and lower surfaces of thepurine and pyrimidine rings are hydrophobic and the G…G interactions exploit the interaction of the edges of the bases (which are hydrophilic) thus eliminating the need of water molecules. Of course, whenno suitableinteraction is achievable thewater molecules interact with the available donors andacceptors. Thus theinterstrand interaction and stabilization of the three-dimensional crystal structure isachievedby evenly distributedweak interaction involvingDNA, ionsand waters molecules.

CONCLUSIONS

The crystal structure of the oligonucleotide d(CGTGAATTCACG)at 2.0 Åresolution is described.In addition to the classical intrastrandWatson-Crickhydrogen bonding interactions the present structure disclosed some noncanonicalG…Ginterstrandinteractions, which had not been previously reported. The presence of a Mg^{2+} ion acting as a charge compensating ion has alsobeen discovered.The positioning of the Mg^{2+} is comparable to similar higher resolution structures of the Dickerson-DrewDNA dodecamer.Data collection showed that the time of crystal growth is crucial for the crystal quality.

Acknowledgments:*The authors wish to thank the Bulgarian Fund for Research Investigations (FNI)for the financial support, with grantT02/14 and DRNF02/1.*

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Кристалнаструктурана ДНК последователностd(CGTGAATTCACG) при 130K

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Получена на 8 септември, 2016 г.; коригирана на 7 ноември, 2016 г.

Кристалната структура на двойно верижната ДНК секвенцияd(CGTGAATTCACG) е отснета и разшифрована с резолюция of 2.00Å в орторомбичната кристална система и пространствена група $P2_12_12_1$. Описани са разликите между настоящата структура и отснетите преди това с резолюция 2.5 и 2.7 Å на стайна температура. Забелязва се наличие на Mg и Cl йони както наличие на нетипични G…Gвзаимодействия между съседни ДНК дуплекси.