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A Cubic Arrangement of DNA Double Helices Based on Nickel−Guanine Interactions

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Abstract: DNA oligonucleotides can be used in order to assemble highly structured materials. Oligonucleotides with sticky ends can form long linear structures, whereas branching is required to form two- and three-dimensional nanostructures. In this paper, we show that when Ni^{2+} is attached to the N7 atom of guanine, it can also act as a branching point. Thus, we have found that the heptanucleotide d(GAATTCG) can assemble into long linear duplex structures, which cross in space to generate a cubic structure. The three-dimensional arrays are stabilized by phosphate−Ni^{2+}−guanine interactions. For the first time, the crystallization of a B form DNA oligonucleotide in a cubic system is reported, space group *I*23. Large solvent cavities are found among the DNA duplexes.

Introduction

Considerable efforts have been developed in order to design nanostructures based on DNA duplexes. In one approach, recently reviewed by Wagenknecht,[1] hydrogen-bonding interactions are replaced by metal-mediated molecules, which mimic the base pairs. The modified DNA duplexes often have a much higher thermal stability. The DNA serves simply as a structural framework. In this approach, the objective is to obtain aligned metal centers in order to prepare nanodevices with novel electric/magnetic properties.

In another approach,[2] the sequence specific base pairing features of DNA duplexes are applied to obtain different types of constructions and nanomachines. Oligonucleotides are used which form double helices and have single-stranded overhangs. The overhangs act as "sticky ends" which may pair with other similar molecules which have complementary sticky ends. The complementarity is based in the classical base pairing scheme A with T and C with G. An important feature of this approach is the need to obtain branched molecules which allow the assembly of DNA in three dimensions, a goal which is easily achieved by using appropriate combinations of sticky ends.

In this paper, we use a different kind of sticky ends and branching points to build a cubic array of DNA double helices. An unpaired guanine at the end of a duplex may work as a sticky end by minor groove binding.[3] As branching points, Ni^{2+}−guanine interactions are used. Ni^{2+} and Co^{2+} interact specifically with guanine residues at their exposed minor groove N7 atom. For this interaction to take place, guanines must be at the end of the oligonucleotide sequence.[4] Two kinds of duplex−duplex interactions are possible: guanine−Ni^{2+}−phosphate[5] and guanine−Ni^{2+}−guanine.[6]

We present the crystal structure of the heptamer duplex $d(GAATTCG)_2$ in the cubic system *I*23 in the presence of Ni^{2+} ions. The oligonucleotide was designed with an extra guanine residue at the 3′-end in order to form guanine−Ni^{2+}−guanine bridges[6] which were expected to give a high stability to the crystal. However such bridges did not form; instead we obtained a cubic structure with guanine−Ni^{2+}−phosphate interactions among neighbor duplexes. Each duplex has a double interaction with one duplex from a neighbor column. Duplexes stack end-to-end in pseudohelical columns stabilized by the 3′ terminal guanine which sticks with the minor groove of the next duplex. The columns run through the whole crystal in four different directions; there is no interpenetration of the DNA duplexes. It should be noted that, in other cases in which DNA columns cross each other in space, a different organization is found: the helical grooves interpenetrate,[7] establishing strong interactions which stabilize the crystal structure.

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Table 1. Crystallographic and Refinement Statistics

	crystal 1 peak	crystal 2 peak	inflection point	remote	merged data[a]
		Crystallographic Data			
λ, Å	1.000 00	1.484 75	1.485 63	1.311 555	
cell parameter, (Å)	$a = 70.87$ (±0.01)	$a = 70.87$ (±0.01)	$a = 70.87$ (±0.01)	$a = 70.87$ (±0.01)	
space group	$I23$	$I23$	$I23$	$I23$	$I23$
resolution range (Å)	25−2.80 (2.90−2.80)	25−2.90 (3.00−2.90)	25−2.90 (3.00−2.90)	25−2.90 (3.00−2.90)	22.4−2.8 (2.95−2.81)
unique reflections	2644	2454	2532	2561	1491
overall redundancy[b]	4.1	18.9	18.4	18.3	
R_{sym} (overall/last shell)	0.024/0.299	0.058/0.761	0.054/0.610	0.052/0.639	0.095/0.273
completeness (overall/last shell)(%)	93.4/54.3	95.6/88.5	99.2/93.3	99.5/100	97.4/82.8
		Refinement Data			
no. of reflections					1388
R_{work}[d]/R_{free}[e] (5% data)					0.282/0.264
asymmetric unit contents					1 duplex 4 Ni^{2+} 20 waters
Weighted Mean PhaseError					27.2

[a] Statistic from the four wavelengths merged using XPREP, with Friedel opposites merged. [b] Total reflections registered divided by the number of unique reflections. [c] $Rsym(I) = \sum_{hkl}\sum_j|_j(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_j I_j(hkl)$ calculated for the whole data set. [d] $\sum_{hkl}||F_o(hkl) - K|F_c(hkl)||/\sum hkl|F_o(hkl)|$. [e] R factor of reflections used for cross validation in the refinement.

Materials and Methods

Synthesis and Crystallization. The deoxyoligonucleotide d(GAATTCG) was synthesized as the ammonium salt on an automatic synthesizer by the phosphoramide method and purified by gel filtration and reverse phase HPLC. Crystals were grown by vapor diffusion at 20 °C in a hanging drop containing 0.5 mM DNA duplex, 20 mM sodium cacodylate buffer, pH 6.5, 5 mM $NiCl_2$, and 10% MPD, equilibrated against a 20% MPD reservoir. Cubic shaped crystals of approximately 150 μm appeared in about 2 weeks.

Data Collection and Structure Determination. Crystals were flash-cooled at 120 K, and diffraction was collected at the ESRF synchrotron beamline BM14 on a MarCCD detector. Data from two different crystals were measured: crystal 1 was collected at $\lambda = 1.00$ Å and diffracted to a maximum resolution of 2.8 Å. Crystal 2 was collected up to 2.9 Å at three different wavelengths in order to use MAD with Ni^{2+} as the heavy atom. Wavelengths were recorded from a fluorescence spectrum measured from the crystal (Table 1). The data were integrated and scaled with the HKL suite.[8] The space group turned out to be cubic $I23$, with $a = 70.87$ Å for both crystals. As crystal 1 achieved a higher resolution, data from the two crystals were scaled together by using XPREP[9] and reflections obtained were used for structure determination. Initial phases were obtained by multiple-wavelength anomalous dispersion (MAD) with the programs SHELXD[10] and SHELXE.[11] Four Ni^{2+} atoms were clearly detected (their final occupancy is 0.7). Best maps appeared when 50% solvent was used, and then an ideal B DNA duplex model of GAATTC (Guanine terminal missing) was fitted into the density with the program XtalView.[12] The model was then refined using the MAD phases with the program REFMAC5[13] of the CCP4 suite (Collaborative Computational Project, Number 4, 1994). After several cycles of maximum-likelihood isotropic restrained refinement, the two terminal guanines were also clearly modeled with a flipped out geometry. Finally, five water molecules (with 0.7 occupancy) were added around each Ni^{2+} ion with an octahedral conformation yielding a final $R_{work} = 28.2\%$ and $R_{free} = 26.4\%$ in the resolution range 50−2.8 Å with a completeness of 97.8% (a set of 5% free reflections was used as an independent cross-validation indicator of the progress of refinement[14]). Notice that

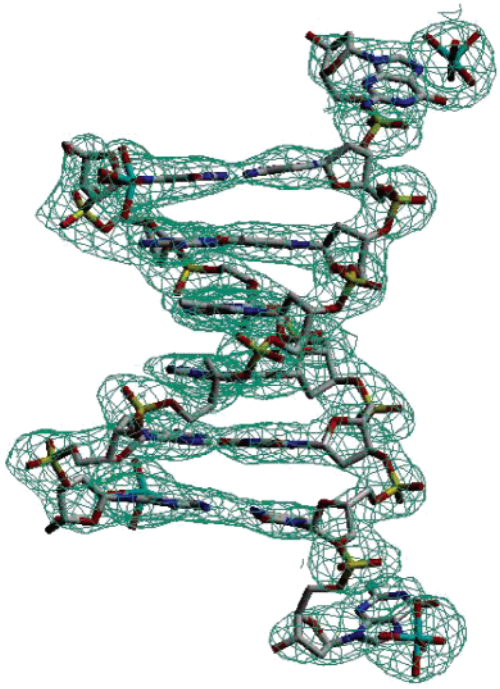

Figure 1. Asymmetric unit of the crystal with its electron density map at 1σ level. The oligonucleotide duplex d$(GAATTCG)_2$ has extrahelical guanines at both ends and hydrated Ni^{2+} ions (cyan) attached to the N7 atom of all guanines.

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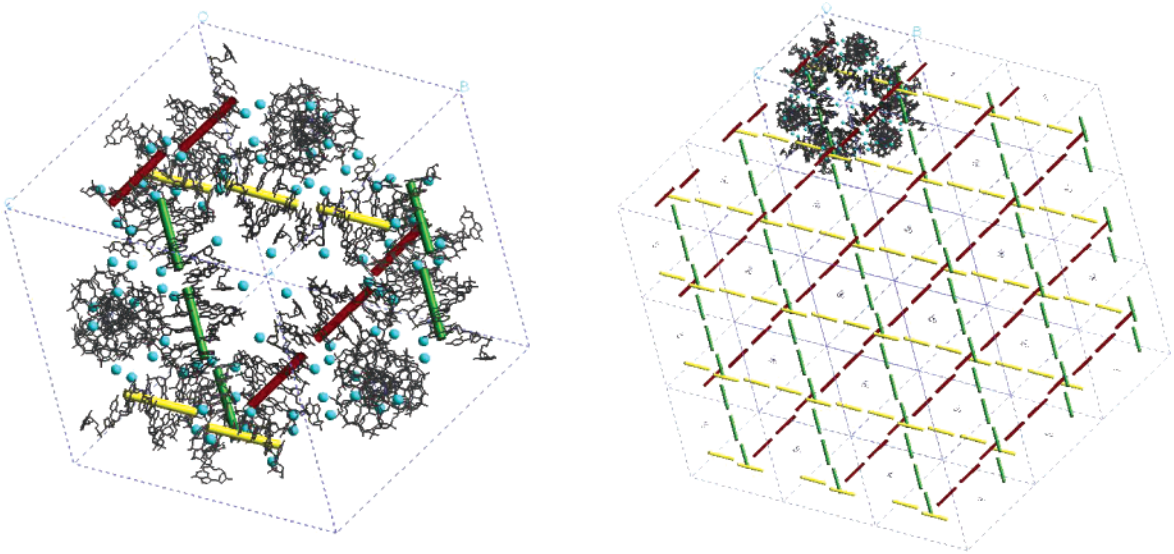

Figure 2. Crystal structure. Oligonucleotide duplexes are organized in columns parallel to the four diagonals of the cubic cell which cross in space. Helical axes are shown as rods of different colors, depending on their orientation. The axes perpendicular to the plane of the figure are barely visible. In this and in the following figures Ni^{2+} ions are given as small cyan balls. A group of 27 cells are shown at the right.

R_{work} has a relatively high value probably due to the high quantity of solvent (76%) present in the crystal, but the fact that we used MAD phases during all refinement and also the good quality of electron density maps confirms that the structure is as completely and correctly modeled as this resolution allows. Through the refinement, noncrystallographic symmetry restraints were applied. Model building was performed using the TURBO-FRODO program.[15] The helical parameters of the duplex were calculated with 3DNA.[16] Figures have been generated with Cerius 2 (Molecular Simulations, Inc.) and SETOR.[17] Coordinates have been deposited in NDB (ID code: BD0069).

Results

The duplex structure and its electron density map are shown in Figure 1. The duplex has standard helical parameters (average twist 35.1°) and conformational angles, with deoxyriboses in the C2′-endo region. Neighbor duplexes stack in columns. Hydrated Ni^{2+} ions are directly attached to the N7 atoms of all guanines in the crystal.

The crystal structure is presented in Figure 2. Twenty-four duplexes and ninety-six Ni^{2+} ions are present in each unit cell. They are organized as columns, which run parallel to the four diagonals of the cube. Such columns cross in space. At each crossing point, there is a double Ni^{2+}−phosphate interaction as described below.

The DNA duplexes are stabilized in the crystal through several interactions, which are presented in Figures 3 and 4. Duplexes stack end-to-end with the extra helical guanines placed in the minor groove of the next duplex (Figure 3). Hydrated Ni^{2+} ions also interact with neighbor phosphates and contribute to the end-to-end interactions. In this way the duplex columns which run through the whole crystal are stabilized (Figure 2).

Duplexes in neighbor columns present pairwise interactions which involve the hydration waters of Ni 101 in one duplex[18] and the phosphate of adenine 3 in the next duplex (Figure 4). The two interactions are identical and related by symmetry. They determine a very precise mutual orientation of the two interacting duplexes, which cross in space. They define the spatial organization of duplex columns. There is no interpenetration of the DNA helical grooves as found in other crystal structures.[7]

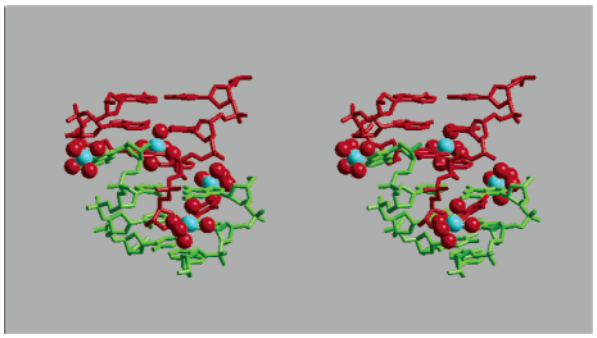

Figure 3. Stereoview of end-to-end interactions. The extra-helical guanines penetrate the minor groove of the neighbor duplex and form an N3−N2 hydrogen bond with its terminal guanine. Ni 103 and 104 also interact with neighbor phosphates. Ni^{+2} hydration waters are shown as red spheres.

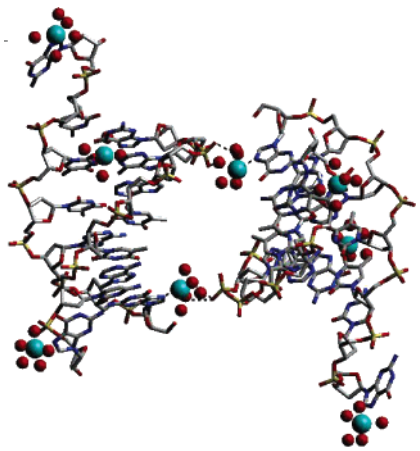

Figure 4. Interaction between two neighbor duplexes which cross in space. It is mediated by two symmetrical Ni 101−phosphate interactions.

The combination of both types of interactions (Figures 3 and 4) determines the organization of the duplex columns in the

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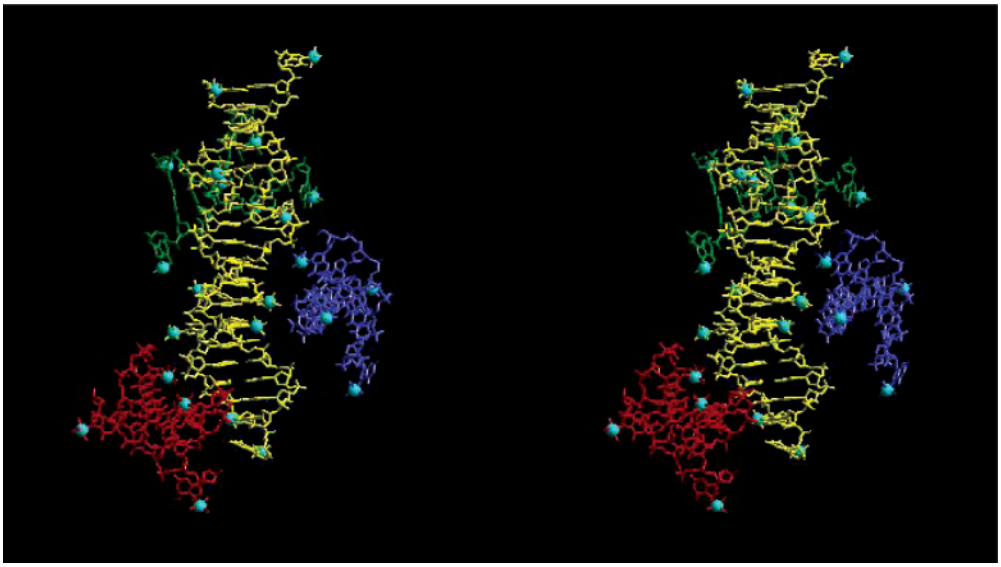

Figure 5. Stereoview of three duplexes in a column. Another three duplexes which interact with the central column are also shown. The colors correspond to those of the helical axes in Figure 2.

crystal as shown in Figure 5. Consecutive duplexes in a column interact with neighbor duplexes which are rotated 120°. Thus every duplex column interacts with the other columns in the crystal in a very precise manner, which defines the overall geometry.

At the center and corners of the unit cell large solvent cavities are present, as shown in Figure 6. The amount of solvent in the crystal structure is very large; DNA atoms only occupy about 24% of the available volume, as calculated by current volume estimates.[19] In fact a distorted icosahedron can be formed by joining together twelve Ni^{2+} ions (Ni 104) in the center of the unit cell. Identical icosahedra can be formed at the corner of the unit cells. The icosahedra define a large solvent region of about 50 Å diameter, in which neither DNA atoms nor other Ni^{2+} ions are found. Additional solvent channels run through the edges, and the center of each face of the cube and interconnect the icosahedral cavities.

Discussion

The crystal structure we have described is unique, not only because it is the first oligonucleotide crystal of B form DNA obtained in the cubic system but also mainly because of its organization. Pseudo continuous oligonucleotide columns of duplexes cross in space stabilized by phosphate−Ni^{2+}−guanine interactions. Large solvent cavities are found among the oligonucleotides in well-defined positions. Ni^{2+} ions define the icosahedral solvent region shown in Figure 6. Their mutual repulsion may force the oligonucleotides to form interstitial regions between large solvent domains, which are not populated by any oligonucleotide atoms. It is interesting to consider the possibility to crystallize this oligonucleotide with a protein or other substances, which could occupy the large solvent cavities (about 50 Å diameter).

A geometrical feature of interest in this structure is the distorted icosahedra defined by the Ni^{2+} ions. Due to the *I*23 space group, the icosahedra cannot be regular, but they still have

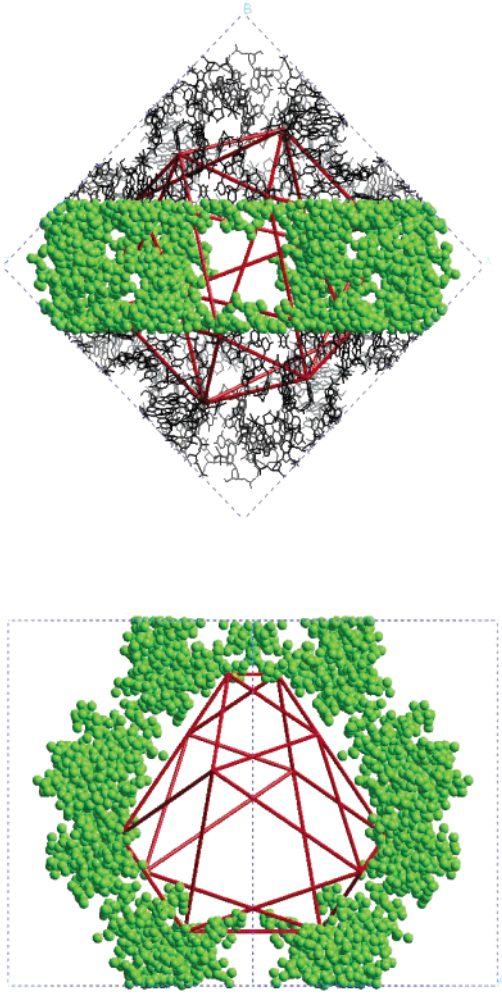

Figure 6. Solvent cavities are found at the center and the corners of each unit cell. No oligonucleotide atoms are found inside the cavities. They have the approximate shape of distorted icosahedra (shown in red) in whose vertexes are found Ni^{2+} ions (Ni 104 in the PDB file). Identical cavities are found at the eight corners of each unit cell. A complete cell is shown in the upper part of the figure in which the atoms in a central slice are given as green spheres and the rest as black sticks. In the lower frame, only the central slice is shown, demonstrating the large central solvent cavity.

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a remarkable symmetry: all twelve vertexes are identical, edges and faces of only three different sizes are present. This observation allows us to wonder whether some viruses might use this kind of symmetry. A small number of different subunits would be required to build an icosahedral capsid with *I*23 symmetry, not much greater than those required to form normal icosahedral viral capsids.

The use of extrahelical guanine interactions introduces a new approach for the development of nanodevices based on DNA structure. In the work of Seeman and co-workers,[2] three-dimensional structures are developed based on the specificity of base pairing in DNA. Here we have used different tools. As sticky ends, the interaction of a terminal guanine with the minor groove of a neighbor duplex allows the formation of continuous columns of DNA duplexes.[3] This interaction is shown in detail in Figure 3, but it should be noted that Ni^{2+} ions are not required for such an interaction to be effective.

Branching points have been obtained through guanine−Ni^{2+}−phosphate interactions, as shown in detail in Figures 4 and 5. Alternatively guanine−Ni^{2+}−guanine bridges could be used. It should be noted that Co^{2+} has similar properties. These types of branches could act as hinges, since the interaction does not define a precise orientation of the duplexes which interact, which may be either parallel[20] or perpendicular.[6]

In conclusion, our work shows that by using extra helical guanines with tightly bound Ni^{2+} ions a new type of three-dimensional organization is achieved. By this approach, novel nanostructures may be designed, complementary to those previously described and summarized in the reviews presented by Wagenknecht[1] and Seeman.[2]

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