We will present details of the conformation of the (d(GATC)) sequence and of the individual base-pairs and base steps with particular reference to the conclusions regarding the sequence-dependent conformation of DNA drawn by others (Yanagi, Privé, Dickerson, J. Mol. Biol., 1991, 217, 204-214).

MS-04.02.07 PRELIMINARY CRYSTALLOGRAPHIC STUDIES ON HAMMERHEAD RIBOZYMES By O. Matsunaga, T. Chen, S. Masayoshi and A. Takahara, Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan; and Moto, M. Kosumi and E. Ohnishi, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido 060, Japan

The discovery of ribozymes induced a new aspect in nucleic acid chemistry. The structural basis is essential not only for understanding the mechanism but also for design of new functional molecules. We synthesized several kinds of hammerhead ribozymes and succeeded in crystallizing one of them (shown in the figure) by the hanging drop vapor diffusion method with MPD precipitant at 25°C. The sample is composed of three RNA chains, the substrate chain of which is methylated to prevent cleavage at the reaction site of ytidine. The crystals grew up to 0.5 x 0.05 x 0.05 mm during two months. It is either stiff for handling and stable for X-ray radiation. Using synchrotron source we obtained the diffraction pattern with more than 5 Å resolution. The crystallographic data could be evaluated to a=b=40.6 Å and c=53.3 Å with trigonal symmetry. If assumed as Z=3, the volume per one nucleotide is 901 Å³. It is in the range of those of tRNAs (780 ~ 979 Å³).

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sealed tube X-ray source with MoKα radiation and a graphite monochromator using a MAR 180 mm image plate detector. The space group of the observed crystal was obtained from those X-rays with unit cell parameters of a=b=30.1 Å and c=86.80 Å. The space group is P2₁. The packing parameter VM was 2.6 Å/Dalton (Matthews, 1968) for one helical fragment per asymmetric unit. This data set was used for molecular replacement calculations. A second data set was collected to 2.3 Å resolution with synchrotron radiation using a MAR 300 mm image plate detector at the EMBL beam line X11. The storage ring was operated in max user mode at 7.7 GeV and 26.40 mA. The wavelength was 0.92 Å. The images of the first data set were processed using the program DENZO (Otwinowski, 1991). The reduced data set contains 1,777 reflection and shows a completeness of 94%. The K refine defined as R(I) = Σ(Iobs-Icalc)/Σ(Iobs) was 6.6%. The images collected using synchrotron radiation were processed using a modified version of the XDS program package (Kabsch, 1988). The unique data up to 2.3 Å contains 2,760 reflections with Rmerge of 3.7%. Finally, the two data sets were scaled together. The resulting completeness for all data up to 2.3 Å is 83% and for all data up to 2.3 Å 77.3% due to the limited completeness of only 50% in the resolution shell between 2.3 and 2.4 Å caused by radiation damage. The structure solution was achieved by molecular replacement using the coordinates of the synthetic RNA helix: [1UUA]₆[A]₂ (Duck-Bergen, 1989) as starting model and a new rotation and translation function program AMORE (Navaza, 1992). The rotation function gave a clear solution for the orientation of the molecule. In the following translation search the space group was assigned to be P4₁ with all data in the resolution range of 8.0 ~ 3.0 Å and giving a R-factor of 41%. Preliminary refinement confirmed the correctness of this solution by applying restrained least-squares (NCLLSQ, Westhof, 1985) and molecular dynamics refinement (X-PLOR; Brüger, 1989). The individual steps of data collection and refinement as well as a detailed structure description will be presented.

References


MS-04.02.08 REFINED STRUCTURE OF HELIX A FROM THERMUS FLAVUS 5S rRNAs AT 2.3 Å RESOLUTION USING SYNCHROTRON RADIATION

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Cryostals of the domain A of Thermus flavus 5S rRNA have been obtained. The space group was found to be P4₁ with unit cell dimensions a=b=30.10 Å and c=86.80 Å. Data to 2.3 Å have been recorded and the structure was solved by means of molecular replacement techniques and refined to R = 18%.

Cryostals suitable for X-ray analysis of the domain A of Thermus flavus 5S rRNA were obtained by vapour diffusion followed by repeated seeding. From these cryostals two data sets were collected. The cryostals were mounted in thin-walled glass-capillaries with some mother liquor. From one cryostal a data set was collected up to 3.0 Å on a conventional

PS-04.02.08 ANTITUMOR DRUGS SN6999 NITRILES THE DNA DOUBLE HELIX AND O'-ethyl-G'-C' BASE PAIR: CRYSTAL STRUCTURE OF THE d([CGC]₂[O'-ethyl-G]-[AATTGCG]₁) SN6999 COMPLEX. By Yi-Gui Cao*, M. Simam, W. Denny and Andrew H.-J. Wang, Biophysics Division & Dept. of Cell & Structural Biology, University of Illinois at Urbana-Champaign and * Cancer Chemos research Laboratory, University of Auckland, School of Medicine, Private Bag, Auckland, New Zealand

4-[(p-quinonylaminobenzamido)-anilino]pyridine (SN6999) is a very active antitumor and universal drug both in vivo and in vitro. The drug binds along the minor groove of DNA and the binding site ranges approximately five base pairs. SN6999's 6-NH₂ derivative has already undergone preclinical and toxicological testing.
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The crystal structure of S9995 determined from the DNA sequence d(5'-GC(GG)-3') has been reported at high resolution. The space group is P2_12_1 and the unit cell dimensions are a= 28.48 Å, b=36.11 Å, c= 69.6 Å. A new lattice packing is observed and the structure was solved by molecular replacement using ULM. A canonical d(5'-CC(AGGAAUCC)-3') dodecamer crystal structure was used as the search model. The asymmetric unit contains one dodecamer duplex, one S9995 and 67 water molecules. The structure was refined against 16185 symmetrically independent reflections extending to 1.23 Å resolution by constrained least squares using XSCALO to an R-factor of 7.1%. S9995 distorts the double helix at the C1 or C2 end. The new lattice retains the interlocking interactions between the unpaired GC base pairs in the minor groove. The C6G.C2I backbone pair is distorted while C10T.C9 shows a normal base pair.

Supported by NIH grants GM-41612 and CA-52206 to A.J.W.

PS-04.02.11 AMBIGUOUS PACKING OF DNA HELICES IN CRYSTALS OF d(5'GGAGGCAAGCAAG)-d(5'CAGCAGCGCA) by C. Sadasivan and N. Gautham, Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Madras-600 025, India

DNA modelers are known to pack in a few well recognized patterns in single crystals. We report here an unusual space group ambiguity in the crystals of the non-self-complementary duplex d(5'GGAGGCAAGCAAG)-d(5'CAGCAGCGCA) grown from a drop containing 1μM DNA, 10mM sodium cacodylate at pH 6.8, 10mM sodium cacodylate, the hanging drop method against 25X PD. The X-ray diffraction pattern from a crystal of size 0.2X0.2X0.5 mm was ambiguous and indicated that the X-ray pattern could be indexed in the space groups given in table 1. All of them can be approximately explained by the same packing mode, viz., that of hexamer duplexes stacked on top of each other in an infinite continuous Z-DNA helix. A continuous helix can be formed by the repetition of two types of arrangement

Table 1

<table>
<thead>
<tr>
<th>CELL</th>
<th>ABSENCE</th>
<th>SPACE GROUP</th>
<th>R (merge)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a=17.67</td>
<td>b=17.66</td>
<td>P61</td>
<td>0.18</td>
</tr>
<tr>
<td>c=20.56</td>
<td>e=90.2</td>
<td>P2_1</td>
<td>0.22</td>
</tr>
<tr>
<td>t=9.99</td>
<td>y=11.9</td>
<td>P2_1</td>
<td>0.22</td>
</tr>
<tr>
<td>a=17.67</td>
<td>b=30.60</td>
<td>P2_1 x 2</td>
<td>0.21</td>
</tr>
<tr>
<td>c=42.65</td>
<td>e=90.0</td>
<td>C2</td>
<td>0.22</td>
</tr>
<tr>
<td>t=90.0</td>
<td>y=90.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This symmetry is revealed by the systematic absences occurring in data set 1. Similar sequences have been crystallized in space group P2_1x2. For the same packing, a suitable selection of the unit cell will yield the cell parameters shown in the data set 2. The systematic absences of these data showed the presence of three mutually perpendicular Z screw axes and a C-centering. The volume of the unit cell does not permit a C-centered orthorhombic cell. Therefore, the space group can either be P2_1x2 or C2. In the case of P2_1x2, a full turn of helix could be forced as shown in arrangement 1, while arrangement 2 can yield C2. The Rmerge showed more or less equal but unconvincing possibilities for all the four space groups. Therefore, a constrained refinement has been carried out in the space group P1. Present R factor is 0.22 for 612 reflections of 1.35Å within the resolution limit 7 to 3 Å.

The nucleotide functional moieties in the electronic zones or channels. This structure may suggest some plausible rationale for the interaction of proteins and nucleic acids in the biological system.