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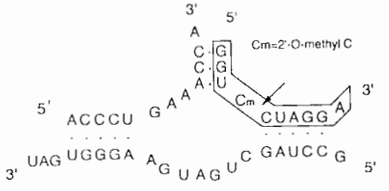
We will present details of the conformation of the d(GATC) sequence and of the individual base-pairs and base steps with particular reference to the conclusions regarding the sequence-dependent conformation of DNA drawn by others (Yanagi, Privé, Dickerson, J. Mol. Biol., 1991, **217**, 204-214).

MS-04.02.07 PRELIMINARY CRYSTALLOGRAPHIC STUDIES ON HAMMERHEAD RIBOZYMES By O.Matsumoto*, Y.Chen, S.Hasegawa and A.Takenaka, *Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan*, A.Ohta, M.Koizumi and E.Ohtsuka, *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 to crystallize 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 cytidine. The crystals grew up to 0.5 × 0.05 × 0.05 mm^3 during two months. It is rather 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=49.6Å and c=53.3Å with trigonal symmetry. If assumed as Z=3, the volume per one nucleotide is 901 $Å^3$. It is in the range of those of tRNAs (780 ~ 979 $Å^3$).



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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Crystals of the domain A of *Thermus flavus* 5S rRNA have been obtained. The space group was found to be $P4_3$ 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 %.

Crystals 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 crystals two data sets were collected. The crystals were mounted in thin-walled glass capillaries with some mother liquor. From one crystal a data set was collected up to 3.0 Å on a conventional sealed tube X-ray source with MoK_α radiation and a graphite monochromator using a MAR 180 mm image plate detector. The space group of the observed crystal was found to be $P4_3$ or $P4_1$ with unit cell parameters of a = b = 30.10 Å and c = 86.80 Å. The packing parameter V_M was 2.6 $Å^3$/Dalton (Matthews, 1968) for one helical fragment per asymmetric unit. This data set was used for themolecular 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 main user mode at 4.7 GeV and 20-40 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 1,477 reflection and shows a completeness of 94 %. The R_{merge} defined as $R(I) = \Sigma|I-\langle I\rangle|/\Sigma I$ is 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 contain 2,170 reflections with R_{merge} of 3.7 %. Finally the two data sets were scaled together. The resulting completeness for all data up to 2.4 Å is 83.5 % 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: $[U(UA)_6A]_2$ (Dock-Bregeon, 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 P43 using all data in the resolution range of 8.0 - 3.0 Å and giving a R-value of 41%. Preliminary refinement confirmed the correctness of this solution by applying restrained least-squares (NUCLSQ; Westhof, 1985) and molecular dynamics refinement (X-PLOR; Brünger, 1989)). The individual steps of data collection and refinement as well as a detailed structure description will be presented.

References

Brünger, A. T., Karplus, M. & Petsko, G.A. (1989). *Acta Cryst.* **A45**, 50-61

Dock-Bregeon, A.C., Chevrier, B., Podjarny, A., Johnson, J., de Bear, J.S., Gough, G.R., Gilham, P.T. and Moras, D. (1989). *J. Mol. Biol.* **209**, 450-474

Kabsch, W. (1988). *J. Appl. Cryst.* **21**, 916

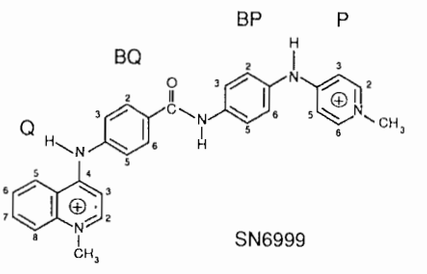
Navaza, J. (1992).'Proceedings of the Daresbury Study Weekend'

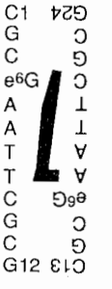
Otwinowski, Z. (1991). DENZO: , Yale University, New Haven.

Westhof, E., Dumas, P. & Moras, D. (1985). *J. Mol. Biol.* **184**, 119-145

PS-04.02.09 ANTITUMOR DRUG SN6999 PERTURBS THE DNA DOUBLE HELIX AND O^6-ethyl-G:C BASE PAIR: CRYSTAL STRUCTURE OF THE d[CGC(O^6-ethyl-G)AATTCGCG]-SN6999 COMPLEX. By Yi-Gui Gao*, M. Sriram, W. Denny+ and Andrew H.-J. Wang, Biophysics Division & Dept. of Cell & Structural Biology, University of Illinois at Urbana-Champaign and +Cancer Chemotherapy Research Laboratory, University of Auckland, School of Medicine, Private Bag, Auckland, New Zealand

4-[p-[p-[4-quinolylamino]benzamido]-anilino]pyridine (SN6999) is a very active antitumor and antiviral 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-NH_2 derivative has already undergone preclinical and toxicological testing.





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The crystal structure of SN6999 bound to the DNA sequence d[CGC(O^6-ethyl-G)AATTCGCG] is presented. The space group is $P2_12_12_1$ and the unit cell dimensions are a=28.48Å, b=36.11Å, c=69.6Å. A new lattice packing is observed and the structure was solved by molecular replacement using ULTIMA. A canonical d[CGCGAATTCGCG] dodecamer crystal structure was used as the search model. The asymmetric unit contains one dodecamer duplex, one SN6999 and 67 water molecules. The structure was refined against 1618 2σ(F) reflections extending to 2.25Å resolution by constrained least squares using NUCLSQ to an R-factor of 17.0%. SN6999 distorts the double helix at the C1 to e^6G4 end. The new lattice retains the interlocking interactions between the terminal CG base pairs in the minor groove. The e^6G4:C21 base pair is distorted while e^6G16:C9 shows a bifurcated base pair. Supported by NIH grants GM-41612 and CA-52506 to A.H.-J.W.

PS-04.02.10 CRYSTAL AND MOLECULAR STRUCTURE OF A HEXADECAHYDRATED 2:1 CO-COMPLEX OF INOSINE 5'-MONOPHOSPHATE WITH L-SERINE, $2C_{10}H_{13}N_4O_8P.C_3H_7NO_3.16H_2O$ by Sreya Ghosh, S.Pain, I.Dey, G.Biswas, B.P.Mukhopadhyay and Asok Banerjee* Biophysics Department, Bose Institute, Calcutta 700 054 INDIA

Interactions between protein and nucleic acids are ubiquitous and are of fundamental importance in molecular biology. Till now, the structure of a few protein-nucleic acid co-complexes have been reported at low resolution. There is, therefore, need for elucidation of protein-nucleic acid model interaction at atomic resolution since high resolution precise information of unique, simple nucleotide-amino acid/peptide complexes can throw deeper insight on the general recognition rules involved in those broad range of DNA-protein interactions in the biological systems. Recently, we have been successful in getting the single crystal of the co-complex of Inosine 5'-Monophosphate with L-serine in adequate hydrated environment. The physicochemical and IR spectral investigations have clearly indicated the presence of both the parent molecules in the single crystal with other several hydrogen bonded water molecules. The co-complex has crystallised in space group $P2_1$ with cell dimensions a=8.690(1),b=21.898(2),c=12.378(1)Å and ß=110.59(3)° respectively. The structure has been solved by direct methods and the water molecules have been located from the successive difference Fourier analysis. The electron density map of the asymmetric unit has clearly shown the complexation of the two Inosine 5'-Monophosphate with one L-serine and sixteen water molecules. The structure has been refined to current R value of 0.11.

The structure consists of polar hydrophilic columns (made up of the phosphate, sugar hydroxyl, serine, and water molecules) parallel to the a-axis, surrounded by hydrophobic columns (generated by the stacking of the nucleotide bases). An intricate network of hydrogen bonding among the serine groups, nucleotides, and water molecules are present in the structure. The phosphate, 2'- and 3'-hydroxyl, and keto-oxygens (O6) as well as the purine nitrogen (N7) of the nucleotide molecules are involved in the H-bonding scheme with the serine molecules through phosphate oxygens... amino and 2'-hydroxyl...carboxyl groups. Strong H-bonding is observed involving the water molecules themselves and their participation with serine and the nucleotide functional moieties in the hydrophic zones or channels. This structure may suggest some plausible rationale for the interaction of proteins and nucleic acids in the biological system.

PS-04.02.11 AMBIGUOUS PACKING OF DNA HELICES IN CRYSTALS OF d(CGCACG).d(CGTGCG). By C. Sadasivan and N. Gautham , Department of Crystallography and Biophysics, University of Madras, Guindy Campus Madras-600 025, India.

DNA oligomers are known to pack in a few well recognised patterns in single crystals. We report here an unusual space group ambiguity in the crystals of the non-selfcomplementary duplex d(CGCACG).d(CGTGCG) grown from a drop containing 1mM DNA, 50mM sodium cacodylate at pH 6.8, 10mM $BaCl_2$, equilibrated by the hanging drop method against 25% MPD. 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 all 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 infinitely continuous Z-DNA helices. A continuous helix can be formed by the repetition of two types of arrangement
1) (CGCACG CGCACG).(CGTGCG CGTGCG) and
2) (CGCACG CGTGCG).(CGCACG CGTGCG). In case 1, one hexamer duplex is related to the other by a 2_1 screw along the longest axis, while both the arrangements can give a disordered 6_1 screw, with a dinucleotide duplex as the asymmetric unit. Disordering arises because of the absence of every 6th phosphate group as well as the presence of a T-A base pair instead of C-G base pair. This symmetry is revealed by the systematic absences occuring in data set 1. Similar sequences have been crystallized in space group $P2_12_12_1$. 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 2_1 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 $P2_12_12_1$ or C2. In the case of $P2_12_12_1$, a full turn of helix could be formed as shown in arrangement 1, while arrangement 2 can yield C2. The $R_{(merge)}$ 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 $I>3\sigma(I)$ within the resolution limit 7 to 3 Å

Table 1.

	CELL	ABSENCES	SPACE GROUP	R (MERGE)
Set 1	a=17.67 b=17.66 c=42.65 α=90.2 β=89.9 γ=119.9	0 0 1, l≠6n	P6₁	0.18
			P2₁	0.22
Set 2	a=17.67 b=30.60 c=42.65 α=90.0 β=90.0 γ=90.0	h 0 0, h=2n+1 0 k 0, k=2n+1 0 0 1, l=2n+1 h k 1, h+k=2n+1	$P2_12_12_1$	0.21
			C2	0.22