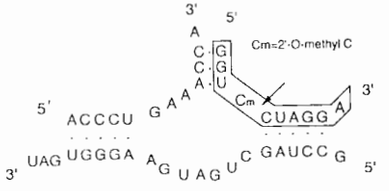
04-Crystallography of Biological Small Molecules

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.

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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.