04-Crystallography of Biological Small Molecules

MS-04.02.03 CONFORMATION OF BASIC PROTEINS AND CHROMATIN STRUCTURE. By Juan A. Subirana, Departament d'Enginyeria Química (ETSEIB), Universitat Politècnica de Catalunya, Diagonal 647, 08028 Barcelona, Spain.

When the negative charges of DNA are partially neutralized with basic counterions (histones, protamines, polivalent cations, spermine, etc.) the complex condenses into rods, fibres or toroidal particles with a rather uniform diameter in the range 20-40 nm. Surprisingly the morphology of the complexes does not depend on the counterion used. In the case of chromatin the system is rather complex, since it contains nucleosome cores, spacer DNA and histone H1. These elements interact within the chromatin fibres and give rise to different levels of secondary/tertiary structure (solenoids, bundles of DNA, zig-zags, etc.). Their spatial organization as a function of the local histone and DNA composition is not yet understood.
In our laboratory we have been working on the structure of these complexes by several methods, in an effort to understand the various structural components involved in the system:
a) Fiber diffraction of DNA/protein complexes shows that DNA is always in the B form. The proteins, depending on their sequence, may be in the β or α conformation, with the latter being favoured in most cases.
b) Single crystal work with oligopeptides which contain lysine confirms their tendency to acquire a helical conformation. Detailed studies with several lysine peptides show that its side chain conformation is very versatile.
c) Circular dichroism studies and structure prediction methods have been applied to histone H1 and related proteins, showing that they tend to interact with DNA through their C-terminal regions in the α-helical form.

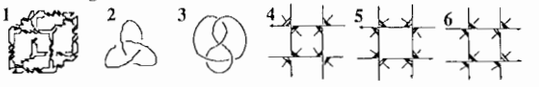
MS-04.02.04 THE CRYSTALLIZATION OF DEOXYOLIGONUCLEOTIDES. G. P. Schroth, T. K. Kagawa, K. Tseng, B. Basham and P. Shing Ho*, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA.

The crucial step in any crystallographic study is to obtain a single crystal of the desired molecule. Yet, this process is generally treated by a 'hit-or-miss' rather than a scientific approach. We have previously shown that the conditions required to crystallize a hexanucleotide in the left-handed Z-conformation is dependent on the conditions that stabilize this DNA form, and that these conditions can be predicted by comparing the free energies required to hydrate the exposed surfaces of that sequence in the B- versus the Z-forms (Ho, *et al*, *Science*, **254**, 1003-1006). We have recently applied this method towards the crystallization of various oligonucleotides in the B- and A-conformations. Our studies show that in fact the specific conformation of DNA in the A-family of structures can also be predicted from the analysis of the hydrated surfaces of the DNAs.

Within the crystal itself, we have been able to manipulate the specific orientations of DNAs by slight alterations in the exposed hydrophobic surfaces of the asymmetric unit. Thus we are developing a general approach to understanding how a DNA sequence crystallizes, and the most stable conformation of the sequence once it has crystallized.

MS-04.02.05 THE CONTROL OF DNA STRUCTURE AND TOPOLOGY. J. Chen, J.E. Mueller, Y. Zhang S.M. Du, Y. Wang, T.-J. Fu, H. Wang, H. Qiu, S. Zhang, and N.C. Seeman*, Chemistry Dept, New York University, New York, NY 10003, USA.

A significant goal of crystallography is the type of structural control sought for nanotechnology. We are pursuing this end with synthetic DNA, whose sequence is selected by sequence symmetry minimization (SSM) algorithms, so that it can form branched junctions. These structures can be ligated together in the same way that linear DNA is ligated together in molecular cloning. Ligating branched structures generates stick-figures whose edges are duplex DNA, and whose vertices are branch points. We have developed a solid-support-based procedure to implement these constructions. We have built a DNA molecule whose helix axes have the connectivity of a cube (**1** below). It is possible to attach proteins to edges of the cube. The vertices are separated by two helical turns of DNA; hence, the plectonemic nature of DNA makes this molecule a hexacatenane, each of whose cyclic strands corresponds to a face. The close topological relationship between catenanes and knots suggests that the synthesis of particular knots can also be directed by SSM: Equating a node in a knot projection with a half-turn of duplex DNA permits one to do this. We have constructed trefoil (**2**) and figure-8 (**3**) knots from DNA, as small as 70 nucleotides. B-DNA is used to generate negative nodes, and Z-DNA is used for positive nodes. Knot construction has led to new multistranded structures, extrapolating from junctions (**4**) to antijunctions (**5**) and mesojunctions (**6**); these are less stable than conventional branched junctions, but nevertheless they have been built and characterized. Control of topology in this system is strong, but 3-D structural control remains elusive. Our key aim is the formation of prespecified 2-D and 3-D periodic structures from these materials, for use in diffraction studies. A number of theoretical and practical problems pertaining to lattice construction have been solved. Applications envisioned include nanomanipulators and scaffolding for MED-BCC devices.



MS-04.02.06 THE STRUCTURE OF d(CGTAGATCTACG) AT 2.25Å RESOLUTION. By G.A. Leonard* and W.N. Hunter, Department of Chemistry, University of Manchester, Manchester M13 9PL, England, U.K.

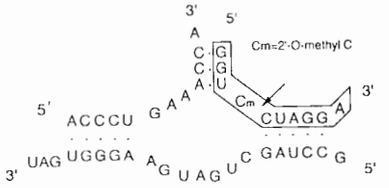
The synthetic deoxydodecamer d(CGTAGATCTACG) which contains the d(GATC) sequence required for the efficient repair of base-pair mismatches (Laengle-Roualt, Maenhaut-Michel, Radman, 1986, EMBO J., 2009-2013) crystallises as B-DNA. The monoclinic unit cell, dimensions a=64.8, b=35.4, c=25.3Å, β=92.2°, space group C2 represents a new crystal form for B-DNA. The structure which represents only the third distinct type for B-DNA dodecamers was solved using molecular replacement techniques and was refined to an R-value of 0.14 for an asymmetric unit consisting of the DNA duplex, one Mg^{2+} ion and 137 water molecules. The metal ion and one of the solvent molecules sit on crystallographic dyads.
The mode of packing of the duplexes in the unit cell is similar to that observed in the structure of d(CGCGAATTCGCG) and, like that sequence, the duplex is curved, thus providing evidence that the bending of DNA duplexes is not confined solely to those containing large A•T-rich tracts. The minor groove width of the duplex ranges from 3.2Å to 8.5Å and the structure is unique in that it represents the first DNA dodecamer structure in which both the spine of hydration characteristic of narrow minor grooves and the double ribbon of water molecules so often found when the minor groove is wide are observed. Another important feature of the structure is the high propeller twist observed for the base-pairs in the central part of the duplex even though none of these can be involved in major groove cross-strand hydrogen bonding.

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.