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.

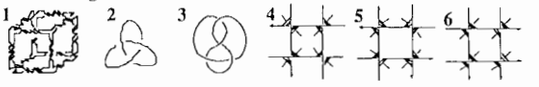
MS-04.02.04 THE CRYSTALLIZATION OF DEOXYOLIGONUCLEOTIDES. G. P. Schroth, T. K. Kagawa, K. Tseng, B. Basham and P. Shing Ho*, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA.

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.

