03-Crystallography of Biological Macromolecules

03.10 – Macromolecular Assemblies

PS-03.10.01 STUDY OF GUANOSINE DERIVATIVES BY X-RAY DIFFRACTION

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Guanosine derivatives in water solution have been shown to self-associate forming macroaggregates in both isotropic (I) and liquid crystalline states (P. Mariani et al, J. Am. Chem. Soc., 1989, 111, 6369-6373; 1991, 113, 5809-5816; Liq. Crystals, 1991, 10, 495-506) (L.Q. Amaral et al, Liq. Crystals, 1992, 12, 913-919). The common structural unit is a planar disc-shaped tetramer formed by four hydrogen bonded guanosine residues. Self-association through stacking of the tetramers produces rod-shaped chiral aggregates leading to cholesteric N* and hexagonal H liquid crystalline phases with decreasing water content.

In a recent paper on the H phase in a micellar system (L.Q. Amaral et al, Phys. Rev. A, 1992, 46, 3548-3550) we analysed the distance "a" between cylinder axes in the H phase as a function of the volume concentration of solute c_v obtaining $a \alpha c_v^{-1/3}$, typical of finite cylinders, while $a \alpha c_v^{-1/2}$ is expected for infinite cylinders. This method of analysis is now used to study a series of guanosine derivatives, with following results:

- the dinucleoside phosphate G2 presents a N* - H transition and a change of functional behavior $a \alpha c_v^{-n}$ at the transition (n = 1/3 in N* phase and n = 1/2 in H phase), evidencing cylinder grow.

- the derivatives G1, G3 and G6 show instead n = 1/3 in the H phase, evidencing smaller aggregation process.

- the derivative G2 cyclic presents n = 1/2 in H phase.

- results obtained in folic acid salt, that forms aggregates similar to the four-stranded helices given by guanosine derivatives, evidence a direct I - H transition and n = 1/3 in H phase.

These results are discussed in terms of differences in the aggregation process of the several derivatives and the phase transitions analysed in terms of recent statistical mechanical theories that take self-association into account (M.D. Taylor and J. Herzfeld, Phys. Rev., 1991, A43, 1892-1905).

PS-03.10.02

COLLAGEN FIBRIL STRUCTURE AND MINERAL CRYSTAL GROWTH IN NORMAL AND PATHOLOGICAL BONES

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The major vertebrate hard tissues bone, dentin and mineralized tendon are composed of crystals of carbonate apatite (dahllite) in a matrix of type I collagen fibrils, together with small amounts of other macromolecules and water. The crystals are small plates organized in parallel layers along grooves through the collagen fibrils. These grooves arise from contiguous collagen 'gap' regions and are separated by the 67nm D repeat along the length of the fibrils and some 4nm in the orthogonal direction.

In mineralized turkey tendon and bone the crystals form initially in the gap regions, appearing as short needles near the surface of the collagen fibrils at the 'e' bands. The needles grow along the length of the fibrils till temporarily constrained by the boundaries of the grooves. They then grow laterally to form belts, and ultimately push their way out of the grooves into the 'overlap' regions.

The mineralized collagen fibrils with their 3-dimensionally ordered arrays of flat crystals may be regarded as building blocks, assembled in various ways in different mineralized tissues. In turkey tendon they are all generally parallel to the tendon axis, and there is a tendency for adjacent fibrils to be coherently aligned so that their crystals are coplanar. This is even more pronounced in cortical bone where crystals from many fibrils grow together to form continuous planes, but only within the confines of the lamellar sub-structures. In alternate lamellae the orientations of the collagen fibrils and the crystal planes are very different, giving the bone a kind of plywood structure. The relative widths and orientional relations between lamellae appear to vary with the bones mechanical functions.

Preliminary studies have been made of bones from patients affected by osteogenesis imperfecta (O.I.) a condition generally atttributed to mutations in the collagen. Some of the mineralized fibrils appear to have quite normal structures even in severe forms of O.I. However, others have small poorly aligned crystals, sometimes encrusted onto seemingly normal fibrils. Fused crystal aggregates, apparently unrelated to any collagen fibrils, were also seen. These observations could be attributed to a scarcity of collagen in the extracellular matrix rather than to the presence of structurally abnormal molecules, which are less likely to be extruded by the cells than the normal collagen.

PS-03.10.03

DATA COLLECTED AND EVALUATED FROM CRYSTALS OF RIBOSOMAL PARTICLES.

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X-ray diffraction data have been collected at cryotemperatures, using intense synchrotron radiation sources, from crystals of ribosomes, their complexes with components of protein biosynthesis and their natural, mutated, selectively depleted and modified subunits, as well as from ribosomal particles specifically labelled with a gold cluster (composed of 11 gold atoms). The best crystals, those of the large subunit (50S) from Haloarcula marismortui, diffract to almost atomic resolution (2.9Å). The best resolution to date of the whole ribosome (70S) is 15Å and that of the small subunit (30S) is 7.3Å, both from Thermus Thermophilus.

Crystals of ribosomal particles exhibit special features: large unit cells, high mosaicity, extremely weak diffraction, beam sensitivity, a limited internal order and an extremely steep descent of the intensities of the reflections as a function of resolution.

A continuous feed-back mechanism between the process of data collection and that of data reduction has been developed. It enables modification of these procedures and their adjustment to account for the specific problems of ribosomal crystals.

Consequently, the quality of the data has significantly improved: Currently the evaluated data sets are of completeness and quality comparable to those obtained from crystalline proteins of average size (Rmerge 5-9%).

Special emphasis is put upon the accurate measurement and evaluation of the very low resolution reflections (below 150Å). These reflections are believed to be very important for low resolution phasing by methods other than MIR.

References:
Berkovitch-Yellin, Z., Bennett, W.S. and Yonath, A. CRC Rev. Biochem and Mol. Biol. (1992), 27 403-444.
Von Bohlen, K., Makowski, I., Hansen, H.A.S., Bartels, H., Berkovitch-Yellin, Z., Zaytzev-Bashan, A., Meyer, S., Paulike, C., Franceschi, F. and Yonath A. (1991), J. Mol. Biol. 222, 11-15.

PS-03.10.04 CRYSTALLOGRAPHY OF A MULTIENZYME COMPLEX CONTAINING RUBISCO. **By M.V.Hosur and K.K.Kannan**, Solid State Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay-400085, INDIA.

The existence of multienzyme complexes functional inside living cells has been postulated lately, and crystal structures of two such complexes have been reported so far. We report here our results on one multienzyme complex isolated and purified from spinach leaves . The purified sample is assumed to contain the following enzymes: (1) RUBISCO (2) phosphoribulose isomerase and (3) phosphoribokinase. Single crystals grown by the hanging drop method, have the orthorhombic space group P21212 with a=173, b=134 and c=112Å, and contain two copies of the multienzyme complex in the unit cell. Diffraction data to 2.5Å resolution has been collected at the photon factory in Japan on Prof. Sakabe's oscillation-Weissenberg camera and the Image Plate system. The L4S4 part of the high resolution structure of spinach RUBISCO was the search model in the Molecular Replacement calculations carried out using the software package, MERLOT. The cross rotation function was calculated by including data between 10 and 5Å resolution limits, and for an integration radius of 29Å. The highest peak in this rotation function map was 7.8 times the standard deviation, and for this solution, the translation function calculations gave a consistent set of strong peaks (about 18 sigma) for all symmetry related pairs of molecules. These solutions to the rotation and translation problems led to an initial R-factor of 35.9% for all data between 10 and 3.5Å resolution. Preliminary electron density maps were then calculated using X-PLOR. There is a good deal of continuous electron density on the outside of the input RUBISCO molecule. This extra density represents the other components of the multienzyme complex. Refinement of the model and atomic interpretation of the extra density is currently under progress.

PS-03.10.05 A STUDY OF THE INTERACTION MODES FOR BILE SALTS. By A.R. Campanelli, S. Candeloro De Sanctis*, M. D'Alagni, A. D'Archivio, L. Galantini, E. Giglio, L. Scaramuzza, Dipartimento di Chimica, Università di Roma "La Sapienza", Roma, Italy.

Bile salts, the most important natural detergents, form molecular aggregates in aqueous solutions, which interact in bile and in the small intestines with several important biological compounds as, for example, bilirubin-IXα, cholesterol, phospholipids, glycerides and fatty acids.
There are many indications that the structures of the aggregates in solution are sometimes similar to those found in the crystals and, for this reason, the crystal structure of these compounds is of great interest.
The crystals, difficult to grow, are very seldom single and very often grow like a bunch of thin needles, sometimes unstable in the air. However, we have succeeded in solving a number of crystal structures and we have found that in the crystals the molecules are held together into very stable structural units, sometimes loosely bound to one another, this being a further indication that the aggregation scheme can be similar in the aqueous solutions. For two bile salts, sodium and rubidium deoxycholate, such similarity has been proved unambiguously.
We have found that different bile salts can have very similar aggregation patterns in the crystals and the structures examined so far can be grouped into three basic aggregation schemes.
Generally, the structures are helical (hexagonal, trigonal, twofold) stabilized by hydrogen bonds and ion-ion and ion-dipole interactions. Similarities and differences will be discussed, in particular concerning the hydrogen bond network, compaired with the other interactions through which the structures are stabilized.

PS-03.10.06 X-RAY ANALYSIS OF YEAST LIPOAMIDE DEHYDROGENASE AT 3.5Å RESOLUTION.
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The pyruvate dehydrogenase complex is one of the highly organized multienzyme complexes which catalyze serial reactions accurately and efficiently. To elucidate the reaction mechanism, we are analyzing the crystal structure of lipoamide dehydrogenase which is a component of the complex from yeast.
Crystals, newly obtained by a desalting method, have the same space group as, but are slightly different in the cell parameters from previous ones, the structure of which has already been X-ray analyzed at 4.5Å resolution[1]. Diffraction data were collected up to 2.98Å resolution (max. 2.49Å, 65%) using synchrotron radiation. The crystal structure was solved by molecular replacement with glutathione reductase. Three programs (X-PLOR, MERLOT, MOLREP) gave a significant unique solution with reasonable crystal packing. After several refinements of the poly peptides, validity of the structure was verified using omit maps. The R-factor was further improved by 2% with molecular dynamics. The present R-factor is 30.5% at 3.5Å resolution.
In the electron density map, the main chain could be easily traced, detecting the insertions and deletions of amino acids. Some residual densities are assignable to FAD and side chains. The whole molecular model is in construction.

1) Takenaka, A., Kizawa, K., Hata, T., Sato, S., Misaka, E., Tamura, C., Sasada, Y., J. Biochem. (1988). **103**, 463-469