

Synchrotron X-ray and Neutron Solution Scattering Studies of the Complex Formed Between the Extracellular Domain of Human Tissue Factor and Factor VIIa

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Blood coagulation has long been recognised as an important process essential for survival. Exposure of the membrane-bound receptor tissue factor to plasma initiates the coagulation pathways. The four domain structure of human factor VIIa and the two domain structure of tissue factor form a very stable enzyme-cofactor complex. In order to elucidate the arrangement of these domains in complex formation, the two soluble recombinant proteins were studied by synchrotron X-ray and pulsed neutron scattering (in 100% ²H₂O buffer). The X-ray and neutron radii of gyration R_G were determined to be close to 3.3 nm (factor VIIa), 2.1 nm (extracellular tissue factor) and 3.1 nm for their complex. The neutron cross sectional radii of gyration R_{XS} were 1.1 nm, 0.6 nm and 1.3 nm in that order. Calculation of the distance distribution function $P(r)$ curves gave maximum dimension of 10 nm, 8 nm and 10 nm in that order. The data show that both proteins have extended structures in solution, which associate side-by-side along their long axes to form the complex. A crystal structure is known for tissue factor, and crystal or NMR structures are known for domains homologous to those of factor VIIa. Using a newly-developed automated computerised search-and-evaluation technique, along with biochemical evidence relating to residues known to lie at the interface between tissue factor and factor VIIa, we are modelling the structures of the free proteins and their complex. We hope to derive insight into the structure of the complex, and obtain an enhanced understanding of how specific residues contribute to complex formation.

Thermotropic Cubic Phases Formed by Cone-Shaped Molecules

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X-ray diffraction experiments of the three derivatives of cone-shaped 3,4,5-tris[3,4,5-tris(dodecyl-1-yloxy)benzyloxy] benzoic acid (**ABG-COOH**) confirmed the formation of a cubic liquid crystalline phase. Powder diffraction data combined with the diffraction patterns of monodomains suggest that the molecules are clustered around special positions of the space-group $Pm\bar{3}n$. We have computed the electron density using the very intense reflections. These results and their implication for the molecular organisation will be presented. The suggested model is different from that proposed for the micellar lyotropic $Pm\bar{3}n$ phase [1,2] which forms in some lipids.

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Automated Modelling of the Multidomain Structure of Carcinoembryonic Antigen

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Carcinoembryonic antigen (CEA) is a cell-surface glycoprotein that is involved in the detection of colonic tumours through the generation of anti-CEA antibodies. Its amino acid sequence reveals that it consists of a single V-type and six C2-type domains in the immunoglobulin superfamily, together with 28 putative N-linked glycosylation sites. X-ray and neutron scattering studies on solubilised CEA have been used to determine the structural arrangements of its seven domains. The radii of gyration R_G of CEA were determined in Guinier analyses to be 8.0-8.8 nm by

X-rays using H₂O buffers and neutrons using 100% ²H₂O buffers. The cross-sectional radii of gyration R_{gs} were 2.1- 2.3 nm by X-ray and neutrons. These results indicate that CEA has an elongated structure with large cross-sectional dimensions. Molecular models of CEA were constructed from atomic coordinates. Sequence comparisons of CEA with the homologous proteins CD2 and CD4, for which crystal structures are available, show that the peptides which link the seven CEA domains are similar in length to those found in the CD2 crystal structures. The human CD2 crystal structure consists of a V-type and a C2-type domain, and was used to model the seven CEA domains. An atomic model of an averaged carbohydrate structure was constructed and 28 were added to the CEA model at each position to correspond to the glycosylation sites. Two-density sphere models were generated for X-ray and neutron curve simulations. An automated computer search procedure explored the arrangement of the seven glycosylated domains in CEA by generating a representative range of structures, and testing the scattering curves calculated from these against the X-ray and neutron data. The models which best satisfied the experimental data have domains which are twisted and tilted relative to their neighbouring domains in an orientation that is similar to the conformations of domain pairs observed in the crystal structure of CD2. The carbohydrate chains were found to have extended conformations in order for the CEA models to satisfy the experimental data. The gross morphology of the best models are analogous to the rod-shaped molecules observed by electron microscopy. This model may provide insight on the interaction of CEA domains with anti-CEA antibodies.

Magnetic Alignment of Microtubules

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Several attempts to obtain high resolution X-ray fibre diffraction patterns of microtubules can be found in the literature. The main problem so far has been to obtain highly aligned samples of microtubules of which the structure was not altered in a significant way either through dehydration, mechanical or radiation damage.

For suitable diamagnetic macromolecules it is possible to use high magnetic fields to prepare an aligned sample. We have applied this alignment method to microtubules. The dynamic behaviour of the microtubules in the magnetic field has been studied with magnetic birefringence. Some of these results will be discussed. Also the results of X-fibre diffraction experiments will be shown.

CCP13 Program Developments

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Modifications made to existing CCP13 programs and some new programs will be described. The 2-D background and peak fitting program LSQINT has been modified to cope with the presence of intensity from multiple lattices on a single diffraction pattern. FIX has been modified to enable simultaneous refinement of pattern centre, rotation and specimen tilt parameters. A prototype program TBACK has been written which fits the central broad background peak which is present in many diffraction muscle patterns. This is done by utilizing a penalty function which ensures the fitted peak does not cut through desired signal. A routine, SAMPLE, to smooth noisy continuous layer line intensity using the Fourier-Bessel interpolation formula given by Makowski (1982) has also been included in the suite. Attempts at producing a practical routine for deconvoluting the main beam profile from small angle diffraction patterns will also be discussed.

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Simultaneous X-ray & Optical Techniques in Liquid Crystals & Polymeric Systems

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Concurrent experiments involving synchrotron radiation offer detailed and accurate information on a minimal time scale. In the study of liquid crystal devices and polymeric systems two key measurements are X-ray scattering and optical/electro-optical

responses. This paper describes novel apparatus[1] allowing:

(i) The simultaneous observation of time dependent small angle X-ray scattering and birefringence changes of liquid crystal devices. This apparatus is incorporated into the X-ray system at station 2.1, Daresbury Laboratory.

(ii) The simultaneous measurement of time resolved small angle and wide angle X-ray scattering with Raman spectroscopy. This apparatus was designed to operate at station 8.2, Daresbury Laboratory.

Electro-optic responses are determined using a specially mounted diode laser and photodiode detector, a hot stage and temperature controller are also incorporated into the apparatus to allow the study of liquid crystal devices. The Raman spectrometer is mounted in a unique geometry defined by the space available and includes an air cooled Argon Ion laser and a high resolution monochromator. Both sets of apparatus are described in detail and simultaneous acquired results are presented.

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Conformations of AMiC, the Amide Sensor Protein of *Pseudomonas Aeruginosa*: A Preliminary Study with SAXS and SANS

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domain structures with acetamide and butyramide, the two forms were studied by both neutron and X-ray scattering. The neutron and X-ray radii of gyration R_G values were each in good agreement with each other for both forms. Values close to 3.2 nm for neutrons in 100% $^2\text{H}_2\text{O}$ and 3.4 nm for X-rays were obtained under conditions where the molecular weight of AmiC was shown to be similar from the $I(0)/c$ values. These data suggest that if there is a conformational change it is too small to influence the observed R_G values. AmiC is normally thought to be dimeric. Despite this, we have X-ray data which corresponds to AmiC monomers. From these, a good curve fit could be obtained by calculation from the monomeric "closed" AmiC coordinates, which is distinct from that for the "open" LivJ crystal structure. Other X-ray data correspond to AmiC dimers, and these give good agreement with the curve calculated from the crystal structure of dimeric AmiC. At higher AmiC concentrations, the scattering curves change again to a putative trimeric or tetrameric form. Gel filtration experiments suggest however that only the dimeric form is present. These anomalies are under further investigation. At present, it can be concluded that for similar oligomeric forms in AmiC, no conformational changes between the acetamide-bound and butyramide-bound forms can be detected.

Simultaneous SAXS/WAXS/DSC Studies of Linear and Cyclic Poly(ethylene oxide)

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The amidase operon consists of 5 genes, *amiE*, *amiB*, *amiC*, *amiR*, and *amiS*, and is involved with amide metabolism in bacteria. AmiC is the amide sensor protein, and is the negative regulator of the amidase operon. The crystal structure of AmiC shows that the overall fold of AmiC is very similar to that found in the crystal structure of the leucine-isoleucine-valine binding protein (LivJ) of *Escherichia coli*, despite only a 17% amino acid sequence identity. AmiC has two domains, which interestingly are in a substantially closed conformation, compared to an open one seen in LivJ. The AmiC amide binding site is extremely specific for acetamide, and the anti-inducer molecule butyramide ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CO.NH}_2$) binds 100-fold more weakly to AmiC than acetamide ($\text{CH}_3\text{CO.NH}_2$). To test whether AmiC has different

The crystallisation behaviour of several poly(ethylene oxide) samples has been studied using DSC, hot-stage optical microscopy and a time-resolved SAXS/WAXS/DSC technique developed at Daresbury Laboratory. Linear PEO molecules with nominal molecular weights (\bar{M}_n) of 1500, 2000 and 3000 have been produced for this study, some of which were then cyclised using an acetal condensation, to give cyclic molecules [1,2].

Simultaneous SAXS/WAXS patterns were obtained from rings of molecular weight 2000, 3000, 4000, and 10000 and the 1500, 2000, and 3000 linear molecules during melting and recrystallisation. While the wide-angle patterns confirm the same helical

structure is present for each of the samples, it has been observed experimentally from the SAXS patterns that the long spacings of linear molecules are double those of the corresponding molecular weight cyclic samples. In addition, during heating of the 3000 and 10000 rings below their melting points, first order peaks in Iq^2 from the small-angle patterns, have been observed to shift to a lower value of q which is consistent with a doubling of the long spacing. This change has been attributed to the unfolding of the molecules, that is, that the sample originally contained folded molecules which were able to unfold on heating.

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Using Synchrotron Radiation to Examine the *in-situ* Processing of Long-Chain Hydrocarbons

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With support from the EPSRC Structured Materials Initiative, Cadbury Ltd and Unilever Research we are involved in two parallel investigations:

- In order to elucidate the crystallisation behaviour of cocoa butter, which is the main ingredient of chocolate we want to characterise the polymorphic structures and phase transitions of fat systems. In chocolate manufacturing, careful control of the solidification processes is quite important because it significantly influences both rheological and physical properties of end products.
- Processing and in-use behaviour of detergent products is extremely sensitive to surfactant crystalline morphology. As environmental pressure leads to changes in permissible surfactant choice, a detailed understanding of surfactant crystallisation will aid reformulation and redesign of the process. Straight-chain saturated sodium soaps were chosen as model systems for our studies as

they represent typical examples of natural surfactants.

Our research program seeks to characterise and interrelate the crystal structure, external morphology and growth kinetics of triglycerides and surfactant systems. We have developed a new *in-situ* cell for X-ray studies which permits the examination of long-chain hydrocarbon samples under conditions of well controlled stirring/agitation and has the capability for very rapid cooling/heating (*ca.* 10°C/min). This rheometer cell design incorporates an optical light probe to detect nucleation. We have used this and other X-ray cells for time-resolved combined small and wide angle X-ray scattering studies on station 16.1 in order to investigate *in-situ* the crystallisation process of fats and surfactants. A polymorphic phase transition was observed for cocoa butter under conditions of shear during a cooling/heating cycle. For anhydrous sodium myristate at least 8 phase transitions were observed. Sodium laurate was crystallised from a hexagonal liquid crystalline phase in order to study the descent through the liquid crystalline phases to the resulting coagel phase. The poster will outline our *in-situ* crystallisation data together with a description of the X-ray cells used.

Two-Dimensional Detector Calibration and Data analysis with FIT2D

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Detector calibration and data correction is of utmost importance for extracting high quality quantitative results from modern area detectors. Non-linearity of intensity response, spatial distortion, and non-uniformity of intensity response, and for imaging plates decay of latent signal during scanning, can all be corrected using the program FIT2D [1,2].

Major progress has been made in the correction of non-uniformity of intensity response (flat-field correction). A fluorescence sample at the crystallographic sample position provides a "flood-field" illumination of the detector from a very small source volume (similar to the sample). The source intensity distribution, which is not quite isotropic, is characterised with a two-theta scan. FIT2D allows "flood-field" detector images to be corrected to "flat-field" images, which are used for flat-field correction of scientific data [3].

New data analysis developments have been made in the field of Powder Diffraction (with 2-D detectors). These developments can also be interesting for fibre diffraction e.g. arbitrary pixel size re-binning (transformation) to polar coordinates.

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Fast Time-Resolved X-ray Diffraction Studies of Fish Muscle using the Fast 1-D Multiwire Detector

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In order to improve the time and spatial resolution of our X-ray diffraction studies of the bony fish equatorial pattern (Harford & Squire (1992) Biophys.J. **63**, 387-396; Harford *et al* (1994) J.Mol.Biol. **239**, 500-512), as part of our study of the molecular events involved in force production (Squire *et al* (1994) Biophys. Chem. **50**, 87-96), measurements were carried out on beam line 16.1 at the SRS using the new fast 1-D multiwire detector. We have previously produced a 10ms time-resolved sequence of the density changes viewed down the muscle fibre axis by Fourier synthesis using the first five orders of equatorial diffraction, corresponding to about 13nm spatial resolution. It has been possible using the CCP13 software developed at Daresbury to extract meaningful intensities to about 6nm so far from multiwire area detector images. Counting statistics for the 10 equatorial reflections from one muscle summed over 30 tetani recorded at 1ms time-resolution were just greater than 1%. There is a small decrease in the spacing of the 10 reflection (0.3%) accompanying contraction and this parameter and the increase in the peak width show a 5 to 10ms lead with respect to the intensity. It should be possible to improve the time-resolution of our 2-D molecular movie by at least a factor of two and with phases from our present modelling of the 3-D structure to improve the spatial resolution by a similar factor. [Work Supported by BBSRC & MRC].

Solving Frame 1 of "Muscle - the Movie": Myosin Head Arrangement in Relaxed Bony Fish Muscle.

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Low-angle 2-D X-ray diffraction patterns from bony fish muscle (Harford *et al* (1994) J.Mol.Biol. **239**, 500-512) contain sufficient information to allow modelling using the known myosin head shape. A series of 2-D diffraction patterns has been recorded at 5 ms time intervals during a typical tetanic contraction of plaice fin muscle (Harford & Squire (1992) Biophys.J. **63**, 387-396). The problem of separating the well-defined Bragg peaks from the asymmetric, smooth background to give reliable intensities ($I(hkl)$) for the Bragg peaks has been tackled using new CCP13 software. A new program has also been written to read in the stripped $I(hkl)$ values and the head shape and to search over parameter space (head orientation, tilt, rotation, radius etc) to give the best R-factor or correlation coefficient values between observed and calculated intensities. Refinement procedures are being used to optimise the modelling. Results to date indicate that, although quite good agreement with the observed data can be obtained from the myosin heads alone. When the basic modelling is established, structure refinement will proceed by combining model phases and observed amplitudes in a Fourier difference synthesis. The final relaxed fish muscle structure can then be used as part of a boot-strapping exercise to define the fish muscle unit cell contents as force is gradually generated through the tetanic contraction. This will provide a time sequence of images from which to compile 'Muscle - The Movie'. Included in this will be modelling of the actin filament structure during activation, including both the tropomyosin shift and actin sub-domain movements (Squire *et al* (1994) Biophys. Chem. **50**, 87-96). [Work Supported by BBSRC & MRC].

Neutron Fibre Diffraction Studies at the ILL

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At the beginning of this year, after a temporary shut-down of almost four years, the ILL restarted as the world's most intense dedicated neutron source. A brief review of the wide range of neutron diffraction

techniques being used to study fibre structures and dynamics will be given, with particular emphasis on high angle diffraction.

The potential of this technique is dependent on developments in a number of areas, two of which will be discussed;

1. The efficient coverage of large continuous volumes of reciprocal space simultaneously. For this reason fibre studies now account for around 25% of beam time on the high flux single crystal diffractometer which is equipped with a large position sensitive detector, D19.

2. The exploitation of the difference in scattering length of hydrogen and deuterium. In particular novel deuteration techniques for biological molecules are opening up exciting possibilities.

An X-ray Diffraction Study of the 14.34nm Meridional Reflection during the Contractile Cycle of Live Frog Muscle

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We have been investigating the two-dimensional high resolution low angle X-ray diffraction patterns from whole frog sartorius muscles in isometric and isotonic contractions and at rigor. One-dimensional time resolved meridional data were also collected.

As has recently been shown (Bordas et al, *Biophys.J.*, in press) at the peak of isometric contraction the third order myosin meridional reflection is resolved into two closely overlapping peaks at 1/14.42nm and 1/14.62nm, indicating that the myosin heads occupy two distinct head configurations. During isometric contraction and quick releases two peaks are visible, one of which follows the same time course as tension generation. But when the release is extended to suppress tension generation, the reflection returns to a single peak at the rest periodicity.

After studying the spacing and intensity time courses of the individual peaks during these experiments, we believe that this split is not caused by interference effects, but is a genuine feature arising from two

individual myosin head populations with slightly different but distinct axial configurations.

Structure & Crystallisation Kinetics of Diblock Copolymers Containing a Crystallisable Block.

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As part of our research programme into the phase behaviour of diblock copolymers [1] we have initiated a programme of study into semicrystalline block copolymers. Time-resolved simultaneous synchrotron small-angle and wide-angle X-ray scattering (SAXS and WAXS) and DSC experiments have been performed on poly(ethylene)-poly(ethylene) [2,3], poly(ethylene)-poly(ethylene-propylene) [2,3] and poly(ethylene)-poly(hthpropylene) [4] diblock copolymers quenched from melts with lamellar and hexagonal-packed cylinder structures. A series of polyether based copolymers close to the order disorder transition have also been synthesised by anionic polymerisation and studied by SAXS/WAXS/DSC [5]

We find that the original microphase separated morphologies are destroyed due to poly(ethylene) (PE) chain folding upon crystallization. Below the melting temperature, samples with a volume fraction $f_{PE} \approx 0.5$ form lamellar structures distinct from that in the melt. On quenching hexagonal $f_{PE} = 0.25$ samples form a lamellar structure similar to that of the $f_{PE} = 0.49$ sample, whilst the hexagonal-packed cylinder structure of an $f_{PE} = 0.75$ sample is also destroyed by crystallization but does not form well ordered lamellae.

The evolution of structure from the disordered melt to the ordered melt and then to the semicrystalline solid has been studied. The SAXS profiles from crystallized materials are shown to correspond to the sum of scattering from block copolymer lamellae, with up to four orders of reflection, plus a broad peak arising from semicrystalline PE. Analysis of scattering density correlation functions calculated using the SAXS data shows that the PE lamellae thickness is

(45±10)Å for all samples, similar to that observed for PE homopolymer. The WAXS data reveal that PE crystallizes in its usual orthorhombic form in all samples. The relative degree of crystallinity as a function of time after a quench, determined from the SAXS invariant, is fitted by Avrami equations for spherulitic crystallite growth. The Avrami exponent is found to be $n=(3.0±0.1)$ for all samples (14 polymers), consistent with crystallisation to form a spherulitic morphology by a nucleation and growth process.

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Morphology of bulk and oriented gels of triblock copolymers as observed by small-angle scattering.

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Gels of triblock copolymers display superstructures consisting of domains of associated endblocks, connected by the midblocks dissolved in an extender oil (physical network). The endblock used is polystyrene (PS), while the rubber midblock is either polyethylene/propylene (PS-P/EP-PS) or polyethylene/butylene (PS-P/EB-PS). The ordering of the PS domains is a function of block copolymer concentration, molar mass, end/midblock ratio, deformation and/or external pressure and temperature. The scattering patterns of the isotropic samples are fitted with two different models: a hard sphere liquid model and

a model of a disordered solid with local coordination. At moderate (25-100%) stretching two different scattering effects are derived from experimental observations. The first one is the change from a circular interdomain interference ring to an elliptical band which represents the stretching of the first coordination spheres of the PS domains, regarded here as "basic units". The second effect is the appearance of diffraction spots which are assigned to an increase of correlation between the basic units (formation of a layered structure). At higher (150-1000%) deformations the SAXS pattern is gradually transformed into a "butterfly" type scattering pattern with a pronounced scattering-free stripe perpendicular to the stretching direction. Preparation of gel films by high-pressure (40 MPa) molding at elevated temperatures can cause a preorientation effect which further develops by high-pressure (0.1-0.7 GPa) treatment. These observations point to the cluster character of the physical network in the gel.

The Effect of Fibres on the Solidification Processes That Occur During The Reactive Processing of Polymer Composites

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Simultaneous SAXS/WAXS has been used to study the structure development in polyurethanes. Reactive materials based on polyurethane elastomers and rigid isocyanurate materials have been investigated as a function of mould temperature with and without fibres in the X-ray path.

The poly(urethane-urea)-forming system has a two stage reaction, which is characterised by macrophase separation followed by microphase separation. The presence of glass fibres appeared to accelerate the rate of structure development in this system at temperatures of between 105 and 120°C. The most probable mechanism responsible for this increase in growth-rate is surface-induced crystallisation around the fibres, which will be further studied by a combination of 2D SAXS/WAXS, optical microscopy and

birefringence measurements.

Cyclo-trimerisations used in the formation of poly-isocyanurates are very fast and, as such, the material experiences a deep quench. It is shown that, as a result of this, spinodal decomposition, rather than nucleation and growth, is the mechanism responsible for microphase separation during the formation of the poly(isocyanurate-urea) system

X-ray Diffraction Studies of Lyotropic Liquid Crystals

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Arguably the most important class of lyotropic liquid crystals is that comprised of ordered solute aggregates formed by self-association, usually in water. There are two major types, amphiphilic mesophases formed by surfactants and chromonic mesophases formed by flat, polar, polarisable, polyaromatic materials (which frequently are used as dyes or drugs). Both types are commercially important as well as offering the opportunity to study fundamental questions concerning molecular interactions and the nature of phase transitions.

For surfactant systems, major outstanding questions are:

- (i) The structure of mesophases with aggregate curvature intermediate between hexagonal and lamellar phases.
- (ii) The structure and swelling behaviour in very dilute systems, including those of semi-solid "gel" phases.
- (iii) Partial miscibility within mesophases.

With the chromonic systems many basic problems remain to be solved. These include:

- (i) Long range mesophase structure
- (ii) Molecular packing within aggregates
- (iii) The nature of phase transitions.

Examples of these problems investigated using the Synchrotron diffraction facilities will be discussed.

Refinement of the fd filamentous bacteriophage coat protein using maximum entropy

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The virion of fd filamentous bacteriophages is a flexible nucleoprotein rod 6nm in diameter and 880nm long, with a shell of α -helical protein subunits surrounding a core of DNA. X-ray diffraction from magnetically aligned fibres of the native and iodinated Y21M mutant of the fd strain of bacteriophage gives patterns with layer-lines that can be measured to a resolution of about 3.3Å. Quantitative intensity data have been extracted from such patterns and used to calculate electron density maps of the fd major coat protein using a maximum entropy method. The helical symmetry of fd is such that on a given layer-line at a given resolution there are a number of overlapping Bessel function terms. The maximum entropy method that we are using can separate and phase the Bessel function terms using only native intensity data and a low resolution prior electron density map. We have tested the performance of the maximum entropy method in this task using simulated data from the current model of the fd major coat protein. We have also tested by simulation various methods for using maximum entropy in the refinement of the fd major coat protein model against fibre diffraction data. In the light of the results of these simulations we are now refining the fd model against the observed fibre diffraction data.

X-ray fibre diffraction and molecular models of Pf3 filamentous bacteriophage.

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The major coat protein of the Pf3 strain of filamentous bacteriophage has been extensively studied in membrane environments but knowledge of the virion structure is underdeveloped relative to that of other strains of filamentous phage. However, the current data indicate that the Pf3 virion has a number of unusual properties. We have collected wide-angle diffraction patterns from magnetically aligned fibres

of Pf3 and these have confirmed earlier suggestions that this strain has a class II helix symmetry. In fact the observed patterns are very similar to those of the high temperature form of the well-studied Pfl strain. Despite the similarities to Pfl as regards the coat protein symmetry and structure, the packing of the DNA in the two strains must be very different as Pf3 has a nucleotide/coat protein subunit ratio about 2.4 times that of Pfl. Furthermore Pf3 does not show a temperature dependent phase transition analogous to that in Pfl, as determined from both DSC and fibre diffraction. We have built initial molecular models of the Pf3 major coat protein, based on the high-temperature model of Pfl, and checked their validity by various methods including the simulation of fibre diffraction patterns. We will refine these models against the observed fibre diffraction data using techniques developed for the refinement of the fd strain of filamentous bacteriophage.

The Structure of an Avian Cartilage. A Combined X-ray and Biochemical Analysis

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Cartilage is susceptible to degenerative changes, this is of commercial and welfare importance in the poultry industry. X-ray diffraction has been used to determine the orientation of collagen fibrils at a variety of depths in cartilage. A complimentary biochemical assessment of collagen type with respect to penetration from the articulating surface towards the bone at 20 micron intervals has been made. X-ray diffraction of antitrochanter (hip) joints was conducted using synchrotron radiation at DRAL and ESRF synchrotrons with a beam size of 200 microns to examine the antitrochanter of turkeys aged from 8-60 weeks at 10 week intervals. The arced distribution of intensity from equatorial reflections was used to determine the parameter $g(\phi)$, a probability distribution for the fibril orientation. Biochemically two main regions of the antitrochanter were found. The surface region of the articulating surface to a depth of 1.2 mm was type I collagen as judged by the CNBr digest pattern on PAGE gel electrophoresis. This corresponded with X-ray diffraction patterns indicating relatively well aligned fibrils of collagen (half height of $g(\phi)$ +/- 30 degrees). The surface portion showed a higher

degree of disorientation indicating that a different architecture of fibril organisation may occur at the surface. The change from type I to type II collagen was abrupt (less than 20 micron) and the underlying cartilage exhibited a much reduced orientation profile indicating that fibril orientation was close to isotropy. Further changes in fibril organisation were observed close to the interface with the ossified

Novel Experiments in Polymer Science by Means of Synchrotron Radiation: On-Line Fiber spinning, Microfocus X-ray Diffraction and Grazing Incidence Diffraction.

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Three types of experiment in the application of synchrotron radiation to polymer science have been performed by us recently. (i) An extruder and a take-up device suitable for spinning of fibers at speeds up to 4000 m/min has been set up at HASYLAB in Hamburg. The development of wide-angle X-ray scattering (WAXS) and small angle X-ray scattering (SAXS) along the fibre was measured and related to the temperature and diameter profile. It was shown that SAXS appears before crystal reflections become visible. It was also shown that crystallization starts at the beginning of the neck-like deformation. (ii) By using a microfocus camera at the ESRF in Grenoble, the local change of molecular orientation in a nematic liquid crystalline polymer was determined. It was shown that it is possible to investigate the nature and arrangement of the disclinations by using this method. Furthermore one can determine local changes in chain orientation in oriented conventional polymers as well as local changes of structure within spherulites. (iii) A goniometer for measuring the grazing incidence diffraction in vacuum was constructed at HASYLAB. This scattering from 400 Å thick films obtained by spin coating of different polymers was measured before and after crystallization. Among other things, the influence of the spin coating speed on the orientation of the crystals was determined.