

Experiment title:	Experiment
Three dimensional structure and packing of collagen	number:

LS1271

Beamline:	Date of experiment:			Date of report:
ID22	from: April 99	to:	May 99	Aug 99
Shifts:	Local contact(s):			Received at ESRF:
18	Drakopoulos			

Names and affiliations of applicants all were experimentalists:

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Report:

The beam time was a block allocation of two sets of 3 days grouped under the title the three dimensional packing of collagen.

Please note that this is a provisional report and a full report with figures will be lodged with the user office before the deadline of March 2000.

A number of experiments were conducted successfully, progress was made in others:

Mineralisation: at the growth edge of calcifying tendon.

Calcifying tendon is a model system for studying the process of calcification; the crystallites deposited are well aligned and relatively easy to study. They also have the advantage that the axial ordering of the mineral crystallites in the collagen matrix can be observed since the collagen fibrils are well aligned.

Thin sections (1 micron) of calcifying turkey leg tendon we examined to detect the size of the nascent calcifying region. Tendons of different ages were used to examine the mineralising edge of tendon that was not fully calcified (age 12-20 weeks) and also tendons judged to have completed the calcification process (34-45 weeks). Preliminary investigation shows that the size of the crystallite changes in the samples from younger birds where the crystal thickness increases with distance (up to 100microns) from the edge of mineral deposition. The effect is less marked in the older birds where the average crystallite size is larger.

Skin: reorientation of collagen fibrils as a toughening mechanism.

The dynamic toughening mechanism of a collagen matrix is a process of collagen fibril realignment in the 10x micron lengthscale. It serves to prevent the propagation of fractures within the material, although the degree of realignment required to stop fracture propagation is not well understood. Normal collagen fibrils in skin form an isotropic two dimensional feltwork that resists the lateral stresses imposed.

An incision was made in a piece of bilaterally stretched skin and the fibril orientation measured by the degree of arcing of the meridional collagen diffraction pattern. Measurements were made from the edge of the incision at 10 micron intervals, we observed in all cases a marked change in the degree of orientation in collagen fibrils at the edge of the incision, this effect decayed as the distance from the incision increased. The decay in fibril orientation was measured to be with the second power of the distance from the incision. This is the first time this data has been recorded in order to compare experimental data with theoretical models of natural toughening mechanisms.

Single collagen fibril: diffraction of a 1 micron wide structure.

We produced collagen fibrils by extraction from rat tail tendon and also using preliminary in vitro methods for fibril production using acid soluble collagen. Initial experiments used thin sections of wax embedded collagen fibrils to give sections of 2 to 5 micron thick embedded tendons. The initial experiments with wet collagen fibres indicated that the effects of beam damage due to a very high localised flux were causing problems. We used a cryostream to successfully protect the sample for over five minutes. Although we were able to obtain diffraction from thin dry samples, we were unable to locate single fibrils due to alignment of a sample of 1-2 microns. Improvements have now been made in sample alignment procedures for ID22.

Fibrillin: mapping heterogeneity in zonular fibrils.

Zonular fibres from mammalian eyes are rich in fibrillin and present an excellent system to study the structure and extensibility of fibrillin, a long ubiquitous multi domain calcium binding protein. Fibrillin is thought to provide elastic properties in all extracellular matrix structures. The molecular packing of this essential elastic component of the extracellular matrix has recently been determined by our group.

Following enucleation of a bovine eye lens, thin aluminium strips with internal rectangular apertures (typically 3 x 5 mm) were glued with cyanoacyrylate to the lens capsule at one end and the ciliary body at the other, such that the zonular filaments were positioned in the aperture and aligned parallel to its long axis. Fibrillin diffraction patterns were obtained which correspond to Bragg reflections indexing on a 56nm periodicity. The effects of extending the sample was also investigated and showed the Bragg reflections to change to an axial unit cell with longer periodicity (75nm). Alterations in the level of Ca²⁺ ions in the tissue have revealed different supramolecular packing architectures. Although we were able to record diffraction patterns from different regions of zonular fibrils, there is significant room for improvement that will enhance differences in the diffraction between supramolecular packing of fibrillin within a zonular fibril bundle.