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DOES THE GENETIC TYPE OF COLLAGEN DETERMINE FIBRIL STRUCTURE?

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A number of genetic types of collagen, all triple-helical but with significant variations in their amino acid sequences, have been found and the distribution of these genetic types is tissue specific. For example, tendon is composed only of type I collagen, while cartilage contains largely type II collagen. Skin contains a large amount of type I, but has a significant fraction, ~15%, of type III. Each of these types can form fibrils, but it is not known whether they form distinctive fibril structures that are important in determining tissue organization. We are using x-ray diffraction to analyze a variety of tissues with different collagen genetic types to compare the fibril structures and thus investigate whether genetic type is an important determinant of this structure.

In connective tissues collagen is organized into cylindrical fibrils with the molecules parallel to the fibril axis. The x-ray diffraction pattern of a well-oriented connective tissue specimen, such as a tendon, typically shows a meridional series of Bragg reflections with a 67-nm periodicity, denoted as D, arising from the axial stagger of the molecules and an equatorial pattern dominated by reflections from the intermolecular spacing. The intensities of the meridional reflections contain information on the axial electron density distribution. Comparison of the intensities obtained from different tissues should indicate the degree to which the electron density distributions in the tissues are similar to a resolution of 1.5 nm if, as is typical, 40 orders are observed. Different tissues also contain various kinds and amounts of noncollagenous material, and these components may influence fibril structure or bind regularly to the fibrils, either of which may be expected to influence the meridional intensities. If a set of phases is available, the intensity data can be interpreted directly in terms of electron density and then it may be possible to relate differences in electron density to features of the different genetic types or their organization in tissues.

Tendons consist almost exclusively of type I collagen which is organized into parallel fibrils with diameters ranging from 30 to 400 nm in adult specimens. Wet tendons from a variety of sources consistently show a D periodicity of 67 nm and show the same orders to be strong in their meridional patterns. Among weaker orders there are small differences in the relative intensities, especially in the 14th through 18th orders. Some tendons, such as rat tail tendon,

show sharp lattice reflections on the equator, but other tendons do not seem to show this feature.

Skin is composed of collagen fibrils oriented randomly in the plane of the skin. In addition to type I, skin contains a significant amount of type III collagen and a larger amount of proteoglycan than found in tendon. The relative intensities of the meridional reflections show a strong resemblance to those in tendon but there is a decrease in the D period to ~65 nm. This decrease in the D period appears to be due to some feature in the native skin environment and not to the collagen types present.

We have obtained well-oriented x-ray patterns of lamprey notochord, a tissue with components which are homologous to those in cartilage. A type II-like molecule is the main collagenous component and there are minor collagenous components which also appear to be homologous with those found in cartilage. Unlike most cartilages the notochord contains little proteoglycan, although that which it does contain seems to be homologous to the proteoglycan of cartilage. The D period and the intermolecular spacing are similar to those found in tendon, but the relative intensities of many reflections are different.

It is clear from our studies that although fibrils of tendon, skin, and lamprey notochord look very similar by electron microscopy, the x-ray patterns of the first two are very similar, while the notochord is quite different. The presence in skin of type III and the increase in the noncollagenous materials in comparison with those found in tendon does not influence the axial arrangement of collagen at the resolution of our studies, but some factor in the native tissue does alter the D period. The differences found in notochord could be due to the genetic type of collagen present or to the proteoglycan content. To clarify these factors, and to provide reference specimens for further comparisons, reprecipitated fibers of purified genetic types are being examined. Reconstituted fibers of type I collagen give a pattern very similar to that of rat tail tendon or skin, while preliminary data on reprecipitated type III fibers show significant differences in the meridional intensities.

Simple comparisons of intensity data and repeat distances are useful in assessing the degree of similarity between tissues, but are not useful in interpreting changes in terms of specific structural features. To get more quantitative data, one needs a set of phases so that difference electron density distributions can be calculated. Using the phase set calculated theoretically by D. Hulmes (1) for rat tail tendon, we have calculated difference Fourier transforms using the intensity data from a variety of specimens. This work is in progress; thus far the observed intensity changes have indicated electron density changes widely spread within the D period, rather than confined to a particular site.

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