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DETERMINANTS OF COLLAGEN FIBRIL STRUCTURE

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The group of tissues that are classified as connective tissues all contain collagen as a major structural protein that is characteristically found in the form of long, cylindrical fibrils with an axial period of D = 67 nm. Within this group, different connective tissues, such as tendon, bone, cartilage, and skin, show distinct physical and mechanical properties that appear to be related to the presence of specific genetic types of collagen. For example, the collagen of tendon and of bone is almost exclusively type I collagen, the most common of the several different genetic types. Cartilage, notochord, and the vitreous body of the eye contain largely type II collagen. Other tissues contain specific mixtures of different genetic types; for example, skin contains type I collagen mixed with 15–20% of type III.

The tissue-specific properties are accompanied by variations in the lateral structure of collagen fibrils. Variations among different tissue types include differences in the diameter of fibrils, which range from 8 to 600 nm; the distribution of fibril diameters, which vary from monodisperse to highly polydisperse; and the packing of fibrils, which may be tightly or loosely packed. Variations continue at the molecular level where the lateral organization of molecules ranges from crystalline to highly disordered. The range of variation in lateral molecular packing and fibril architecture is being characterized using x-ray fiber diffraction and electron microscopy. Such characterizations are a first step in understanding the interactions and forces that create and stabilize the tissue-specific structures that are observed.

Rat tail tendon (RTT) is the tissue in which the fibril structure is known in most detail. RTT gives excellent x-ray diffraction patterns because the fibrils are well oriented and contain type I collagen molecules organized in large crystalline domains. The discrete Bragg reflections that sample the equatorial molecular transform and the first layer line of the triple-helical pitch at 1/9.5 nm^{-1} have made it possible to define precisely the three-dimensional unit cell of RTT, the orientation of the cell with respect to the fibril axis and the location of the molecules within the cell (1, 2). In addition to the discrete reflections, there is a diffuse background along the equator, indicating that part of the molecules are in a state less ordered than crystalline. Recent analysis by Fraser, et al. (3) has suggested that along each fibril there is an alternation between crystalline domains comprising 0.47 of each D repeating unit (the overlap region) and disordered domains comprising 0.53 D (the gap region).

The notochord sheath of the lamprey, which contains type II collagen, has recently been shown to have well-oriented collagen fibrils of very small diameter (17 nm) in which the collagen molecules are packed in crystalline arrays (4). The axial period is the same as RTT (67 nm) but the lateral structure is considerably different. The unit cell proposed for the lamprey notochord collagen structure is 40% larger in volume than that of RTT, an increase which is largely the result of lengthening of one cell edge. The increase in volume may be caused by the considerably increased level of glycosylation and attendant hydration that type II collagen exhibits in comparison to type I. These specimens also show diffuse scatter along the equator, suggesting the presence of disordered portions of the structure as in RTT. The profile of this scatter indicates that, in comparison to type I, there is a lower density of collagen molecules in the disordered domains of type II as well as in the crystalline regions.

Most tissues, other than RTT and lamprey notochord sheath, do not show crystalline Bragg reflections sampling the molecular transform on the equator; instead they give only diffuse equatorial scattering, indicative of a laterally disordered structure. The scattering is in the form of a single broad maximum which, for most tendons, demineralized bones, and skins, is located at various spacings ranging from 1/1.47 nm^{-1} to 1/1.58 nm^{-1} (5). The breadth of the maximum varies from one tissue to another. Such a maximum represents the product of the molecular transform and the interference function arising from the lateral spatial correlation of the molecular positions. Divi-

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sion of the scattering profile from various specimens by the molecular transform of type I collagen has yielded interference functions that all have a maximum at $1/1.42\ nm^{-1}$, but have various breadths. This indicates that, at closest approach, the molecules have the same nearest-neighbor separation in all of these tissues, a separation comparable to that found in the crystal structure. This finding suggests that the basic molecular interactions are the same in each of these tissues. The differing breadths of the intereference functions show that the variance in molecular position differs from one tissue to another, probably in inverse relation to the packing density. Whether this variance represents static or dynamic disorder is not yet known.

The disordered structures described above at the molecular level have an analog at the fibrillar level in tissues with relatively uniform diameter collagen fibrils, such as lamprey notochord sheath, lamprey skin, and embryonic tendons. From electron micrographs, one can compute the pair distribution function of the fibril locations and determine the degree of short- and long-range order (6). The degree of order seen is less than crystalline, but more correlated than the order of typical nematic liquid crystalline arrays which collagen fibrils resemble. Such tissues give a low-angle x-ray scattering pattern derived from the transform of the cylindrical fibrils multiplied by an interfibrillar interference function. These patterns can be analyzed to determine the diameter of the fully hydrated, native fibrils and their average nearest neighbor center-to-center separation.

Thus connective tissues contain rod-like fibrils which pack in partially correlated arrays in some tissues, and these in turn contain roughly cylindrical molecules that pack with crystalline or less exact lateral order. Calculation of the pair distribution functions from fiber x-ray diffraction data will allow a quantitative description of the lateral structure of these tissues at both the molecular and fibrillar levels and permit us to examine the influence of factors such as composition, ionic strength, pH, pressure, and temperature on these structures.

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TWO-STRANDED α-HELICAL COILED-COILS OF FIBROUS PROTEINS

Theoretical Analysis of Supercoil Formation

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Left-handed "supercoils" (α-helical dimers) (1) are believed to be the dominant structural feature of fibrous proteins such as myosins, α-keratins, viral hemagglutinins, trypanosoma antigens, and cytoskeleton microfilaments and microtubules. Recent experimental work on synthetic oligoheptapeptides of increasing lengths (2) showed that peptides shorter than four repeats of the heptad sequence Lys-Leu-Glu-Ala-Leu-Glu-Gly have disordered structures in aqueous solutions, while longer peptides exist exclusively as α-helical dimers.

Using an empirical potential energy function that incorporates approximate corrections for solvent effects (3), we estimated the differences in Gibbs free energies of extended, α-helical and supercoiled peptides of increasing length. We also examined the effect of the current uncertainties in the calculation on the agreement to the experimentally observed trends of α-helix and supercoil stabilities. Such comparisons are an important first step in calibrating and testing an empirical Gibbs free energy function, which will be essential in the development of algorithms for the prediction of protein folding.

METHODS AND THEORY

Five polyheptapeptides (Ac-(Lys-Leu-Glu-Ala-Leu-Glu-Gly)$_n$-Lys-amide with n = 1–5) were constructed as extended β-strands, single α-helices, and supercoiled, parallel dimers (Fig. 1). The α-helices and