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Research Paper

Osmotically driven tensile stress in collagen-based mineralized tissues

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ABSTRACT

Collagen is the most abundant protein in mammals and its primary role is to serve as mechanical support in many extracellular matrices such as those of bones, tendons, skin or blood vessels. Water is an integral part of the collagen structure, but its role is still poorly understood, though it is well-known that the mechanical properties of collagen depend on hydration. Recently, it was shown that the conformation of the collagen triple helix changes upon water removal, leading to a contraction of the molecule with considerable forces. Here we investigate the influence of mineralization on this effect by studying bone and turkey leg tendon (TLT) as model systems. Indeed, TLT partially mineralizes so that well-aligned collagen with various mineral contents can be found in the same tendon. We show that water removal leads to collagen contraction in all cases generating tensile stresses up to 80 MPa. Moreover, this contraction of collagen puts mineral particles under compression leading to strains of around 1%, which implies localized compressive loads in mineral of up to 800 MPa. This suggests that collagen dehydration upon mineralization is at the origin of the compressive pre-strains commonly observed in bone mineral.

1. Introduction

Collagen is an essential constituent of many mammalian tissues and its mechanical function is critical for bone, tendons, blood vessels, skin and other organs. Water is an integral part of these tissues and its (partial) removal has dramatic effects on collagen and on bone properties (Buehler, 2006, 2007; Fratzl, 2008; Gautieri et al., 2011; Spiesz et al., 2012a; Utku et al., 2008; Weiner and Wagner, 1998). Many studies have addressed the macroscopic and microscopic changes occurring in collagen-based tissues as a function of the state of osmotic pressure in the surrounding environment. In the seventies, for example, the group of Katchalsky and Oplatka (1971) observed that reconstituted collagen undergoes significant length changes (up to 40%) and can generate very high tensile forces (up to 10 times those of human muscles) when exposed to concentrated

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solutions of LiBr and proposed a design principle for a turbine able to convert chemical energy into mechanical work. Later on, Maroudas et al. (1991) and Katz et al. (1986) showed that the amount of intermolecular water in cartilage can be tuned by cells by controlling the concentration of osmotically active components in the extrafibrillar space. Also, by using a balance of forces approach for the cartilage, they described how osmotic and mechanical pressures modify the swelling equilibria of the tissue. Interestingly, also the presence of a mineral in a collagenous tissue affects the packing density and the mesoscopic structural features of collagen molecules: In bone, for instance, the staggering period and the lateral spacing of collagen are very close to the one of the dry collagen. It has become then clear that the state of water surrounding collagen critically affects its supra-molecular structure. Recently, we have extended these studies focusing on the molecular level changes occurring when the molecule is subjected to a hydration change. We have shown that, in collagen type I, the removal of water is associated with a conformational change of the molecule leading to its shortening (Masic et al., 2015). Collagen fibrils are based on an assembly of these triple-helical molecules arranged in a staggered fashion. In mineralized tissues such as bone, beside collagen and carbonated apatite mineral, water is the third most important component adding up to 10% by mass even in fully mineralized tissue.

A wide range of experimental methods have been employed to explore the hierarchical organization of structural collagen-based materials and other tissues. In the context of this study, microfocus X-ray scattering permits the acquisition of structural and chemical information across multiple length scales spanning from the molecular to the micron scale (Paris, 2008). Wide-angle X-ray scattering (WAXS) provides information concerning crystallographic (organic or inorganic) structure and texture, while small-angle X-ray scattering (SAXS) gives access to electron density variations corresponding to larger scale structural features. Using these approaches, we studied the structural changes associated with dehydration in mineralized tissues to elucidate the influence of water on mechanical behavior. Indeed, dry tissue is known to be stiffer and more brittle than hydrated tissue, but in general very little is known about the mechanical role of water in collagenous mineralized tissues. For tendon collagen, it has been shown that water plays a fundamental role in stabilizing the structure of the collagen molecule and that its removal results in conformational changes, producing large tensile stresses (Masic et al., 2015). In this work we extend the study to collagen-based mineralized tissues by studying fibrolamellar bovine bone and mineralized collagen from turkey leg tendon (TLT). The TLT material has been studied over many years as a model system for bone, with the advantage that collagen is strongly aligned and that the tissue exists with varying degrees of mineralization (Landis et al., 1995, 1991; Landis and Silver, 2001, 2002a, 2002b; Siperko and Landis, 2001; Spiesz et al., 2011, 2012a, 2012b).

We find the behavior of both mineralized tissues (bone and mineralized TLT) to be similar to that observed for rat tail tendon (RTT) indicating that collagen plays a crucial role in force generation mechanisms independent of the presence of the mineral. This force generation is already significant at relatively low osmotic pressure changes which might occur even inside a fully hydrated environment, such as those found in vivo. This conclusion was ascertained by measuring force generation in mineralized TLT and in fibrolamellar bovine bone immersed in water and subjected to the osmotic pressure from polyethylene glycol surrounding the mineralized tissue specimens.

2. Materials and methods

2.1. *Parallel fibered fibrolamellar bone*

Fibrolamellar bone is made up of tissues with different degree of organization. To study the effects of the changes collagen molecules undergo during dehydration on in mineralized tissues, we extracted the parallel fibered component of fibrolamellar bone as described by Gupta et al. (2006). Collagen 50 to 100 μm thin fibrolamellar bovine bone samples were prepared from the mid-diaphysis of a 12 month old calf obtained from the slaughterhouse. The bone was cut in radial-longitudinal sections perpendicular to the long axis with an inner-hole saw (Leica SP1600, Leica Mikrosystem Vertrieb GmbH, Bensheim, Germany). These slides were then polished by means of an automatic polisher (Logitech PM5, Logitech Ltd., Glasglow, UK) with 3 μm grit-sized diamond particles (DP-Spray P, Struers A/S, Ballerup, Denmark) and, subsequently, with 1 μm sized particles until the sheets achieved the right thickness. Before the measurements the sheets were cut in 1–5 mm wide and 5–10 mm long stripes along the fibrolamellar orientation. For storage the samples were kept at −20 °C wrapped by 1% sodium azide in phosphate buffered saline (PBS) solution soaked gauze.

2.2. *Turkey Leg Tendons (TLT)*

The turkey legs originate from a turkey farm in Germany. The legs from 20 weeks old domestic male (BUT 6) animals were stored until sample preparation in a freezer (−20 °C) and gently unfrozen in an ice bath at 4 °C. The tendons (flexor digitorum) were then removed manually and sectioned with a cryomicrotome into slices with a thickness of about 140 μm. After cutting, the slices were directly placed on a specimen slide and freeze dried overnight.

2.3. *Mechanical testing.*

2.3.1. *Measurement chamber for in situ X-rays scattering experiments*

Samples were tested in a sealed chamber of volume of about 140 cm^3. The chamber was kept at a constant temperature of 23° or 25° by means of cooling bath circulation thermostat (Huber). The humidity inside the chamber was controlled by means of a "Wetsys (Setaram)" humidity generator, which was working with a flow of 200 ml/min. Temperature and humidity were monitored via a SHT75 digital humidity and temperature sensor (SENSIRION) that was placed in the vicinity of the sample. Samples were clamped to two aluminum holders. The strain was controlled by one of the holders which connects to a PI (Physik-Instrumente) M-126.DG1 linear