X-ray Diffraction Study of the Mineralization of Turkey Leg Tendon

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X-ray diffraction studies were conducted on calcified turkey leg tendon to establish the effect of mineralization on some of the structural properties of collagen. The principal finding was that the first equatorial reflection of collagen in freshly-excised calcified tendon had a d-spacing intermediate between the values for dried collagen and fresh unmineralized collagen. Since this spacing is a sensitive monitor of moisture levels in collagen, the data suggest that mineralization reduces the amount of water than can associate in vivo with the collagen component in tissue. It was also found that the presence of mineral appears to increase the resistance of collagen to permanent thermal denaturation.

Key words: Apatite—Calcification—Collagen—Tendon—X-ray diffraction.

Introduction

In contrast to a remarkable uniformity in molecular structure, native collagens vary considerably in the complexity of their tissue organization. This complexity is particularly evident in calcified tissues such as bone and dentin. Of the calcifiable collagens, turkey leg tendon has a relatively simple anatomical structure.
In previous studies using electron microscopic and microradiographic techniques (Nylen et al., 1960) and X-ray diffraction procedures (Myers and Engstrom, 1965; Engstrom, 1966), this simplicity in tissue organization proved to be particularly suitable for elucidating collagen-crystal relationships. The most striking observations from these earlier studies were the highly parallel alignment of the apatite crystals with the collagen fibrils (Nylen et al., 1960; Myers and Engstrom, 1965) and the association of initially-deposited crystals with the interband region of these fibrils (Nylen et al., 1960; Engstrom, 1966). Turkey leg tendon was again utilized in the present study to explore these mineral-collagen relationships by X-ray microscopic, and diffraction, techniques.

**Methods**

Leg tendons were obtained from 14 to 22-week-old United States Department of Agriculture Beltsville bronze-white tom turkeys. The tendons were removed by dissection immediately following sacrifice by decapitation and were sealed in the fresh state in 2.0 mm thin-walled glass capillaries.

The X-ray diffraction patterns from the freshly-mounted samples were recorded on film using a flat-plate camera with pin-hole collimation, 127 mm in length and with a defining aperture of 0.3 mm diameter. Zirconium-filtered molybdenum K$_\alpha$ radiation ($\lambda = 0.711$ Å) was employed, and the sample to film distance was 96.6 mm. Exposure times were generally 24 h. The samples were examined with the tendon fiber axis at right angles to the incident beam direction. The diffraction patterns, illuminated from below, were measured on a film measuring scale which permitted readings to within 0.05 mm.

Following X-ray diffraction examination, the samples were removed from the capillaries and vacuum-dried. The pattern of mineral deposition was inspected in a Philips projection X-ray microscope (PXM; Scott et al., 1962) equipped with a titanium target and operated at an excitation potential of 20 kV and emission current of 50 μA. The samples were photographed in vacuo at a magnification of 4X with exposure times of 20 min. Each specimen was radiographed twice with the specimen rotated 90 degrees about its long axis between exposures. Diffraction data were utilized only from those samples which were either free of mineral deposits or which appeared to be fully and uniformly mineralized in both radiographic projections (Fig. 1). These two types of samples will be referred to subsequently in the text as non-calcified and calcified, respectively. Ashing assays (600 °C for 4 h) established the mineral content of the calcified samples to be 43±5% of their dry weight.

At time of sacrifice, additional material was collected, lyophilized, sorted out on the basis of the above described PXM criteria as either calcified or non-calcified, and examined under a variety of experimental procedures as detailed below.

Dried samples were mounted and sealed in capillaries to prevent atmospheric water uptake and their X-ray patterns recorded as above. Distilled water was then introduced into the capillaries to immerse the samples fully. Samples were allowed to equilibrate with the water for 24 h and were then re-examined by X-ray diffraction. Some samples, both calcified and non-calcified, were pretreated in 0.35 M EDTA solution for 18 h and were then washed for one hour with distilled water prior to drying and examination.

Following equilibration in distilled water at room temperature, pieces of leg tendon were heated for up to 2 h in water at various temperatures between 58°C and 100°C. The samples were then either dried at the temperature at which they were thermally treated or, alternatively, were returned to room temperature while still in contact with the solvent. The X-ray diffraction diagrams of the heat-dried samples were recorded on film with a Chesley-Philips microcamera (Chesley, 1947) using nickel-filtered copper K$_\alpha$ radiation ($\lambda = 1.542$ Å). The alternatively treated samples were decalcified in a 0.35 M EDTA solution (as the potassium salt at pH 8.1) for approximately 18–24 h at room temperature followed by thorough washing with distilled water for one hour, then vacuum drying. These samples were examined by X-rays using the flat-plate camera assembly previously described, except CuK$_\alpha$ was employed in place of MoK$_\alpha$ radiation.