Interpreting the Equatorial Diffraction Pattern of Collagenous Tissues in the Light of Molecular Motion

Sidney Lees
Forsyth Dental Center, Boston, Massachusetts 02115 USA

ABSTRACT The equatorial diffraction pattern associated with collagenous tissues, particularly type I collagen, is diffuse and clearly unlike that from crystals. Hukins and Woodhead-Galloway proposed a statistical model that they termed a "liquid crystal" for collagen fibers in tendons. Fratzl et al. applied this model to both unmineralized and mineralized turkey leg tendon, a model that ignores the organization imposed by the well-known cross-linking. The justification for adopting this model is that the curve fits the data. It is shown that the data can be equally well matched by fitting a least-squares curve consisting of a second-order polynomial plus a Gaussian. The peak of the Gaussian is taken as the equatorial spacing of the collagen. A physical explanation for this model is given, as is a reason for the changes in the spacing with changes in water content of the tissue. The diffusion is attributed to thermally driven agitation of the molecules, in accordance with the Debye-Waller theory including the Gaussian distribution. The remainder of the diffusion is attributed to other scattering sources like the mineral crystallites.

INTRODUCTION

Fratzl et al. (1993) showed that the equatorial diffraction pattern in turkey leg tendon could be calculated (from the liquid crystal model proposed by Hukins and Woodhead-Galloway, 1977) for unmineralized tissue with different amounts of water and for dry, fully mineralized tissue. The independent variable is the packing fraction. From this, a model for mineralized tissue was proposed to account for the decrease in the spacing with mineral content. The correlation between the calculated curves and the experimental data is reasonable, although no measure of goodness of fit was given. The peak in the pattern, usually interpreted as the equatorial spacing between collagen molecules for the crystal case, is considered to be an incidental result, providing information on the intermolecular spacing. For the liquid crystal model the diffraction pattern is a continuous function, whereas for the crystal model it should be a set of delta functions. Because the meridional pattern is highly ordered but the equatorial pattern is not, Hukins (1981) has described collagen as a one-dimensional crystal.

The authors based their model on a completely random distribution of the collagen molecules, represented as cylinders with no long-range lateral order. They specifically and explicitly avoided any reference to an underlying structure. In particular, no reference was made to the cross-linking between collagen molecules, although the cross-linking is well known and is an important factor in the properties of collagen, whether or not the tissue is mineralized (Tanzer, 1973; Eyre et al., 1984). The bond angle and bond spacing for each cross-link must be fixed and well defined, so that at certain points the structural relationship among the molecules is set. For collagen the bonds are at the ends of the molecules.

In contrast to the model of Fratzl et al., it is shown here that the tissue, mineralized or not, can be considered to be a disordered molecular crystal in the sense described by Amoros (1968), and a curve can be calculated that fits the data as well as or better than the model of Fratzl et al. The cross-linking bonds provide a reference framework. The lateral spacing between molecules as determined from the peak in the x-ray or neutron diffraction pattern is real. The disorder is presumed to be primarily the consequence of thermally driven molecular vibration, and the diffraction pattern should be described by application of the Debye-Waller theory (Amoros, 1968), although some structural disorder is not precluded.

Fratzl et al. note and recognize that the spacing between collagen molecules decreases with mineral content for wet tissues (Lees et al., 1984; Bonar et al., 1985; Lees et al., 1987). But the one example they calculate is for dry mineralized tissue, where the spacing is about the same for all type I collagen tissues, 1.1 nm (Fig. 1), whether or not they are mineralized. The reason for concluding that mineral is absent between molecules is that the spacing in mineralized tissues decreases as the tissue is dried. For fully mineralized turkey leg tendon, the spacing changes from 1.33 nm for wet tissue to 1.03 nm when fully dried, a change of 19% (Lees and Page, 1992). The lateral molecular spacing is computed from the equatorial diffraction pattern. The reason that the equatorial spacing is the same for all dried tissues is that the collagen molecules are packed as closely as they can get (Lees, 1986).

Fratzl et al., in their one example of mineralized turkey leg tendon, show collagen molecules, packed together without a pattern, surrounding mineralized regions. They suggest that the molecules are pressed together by the deposition of mineral within the fibrils so as to surround clumps of

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Address reprint requests to Dr. Sidney Lees, Forsyth Dental Center, 140 Fenway, Boston, MA 02115. Tel.: 617-262-5200, ext. 330; Fax: 617-262-4021; E-mail: slees@forsyth.org.

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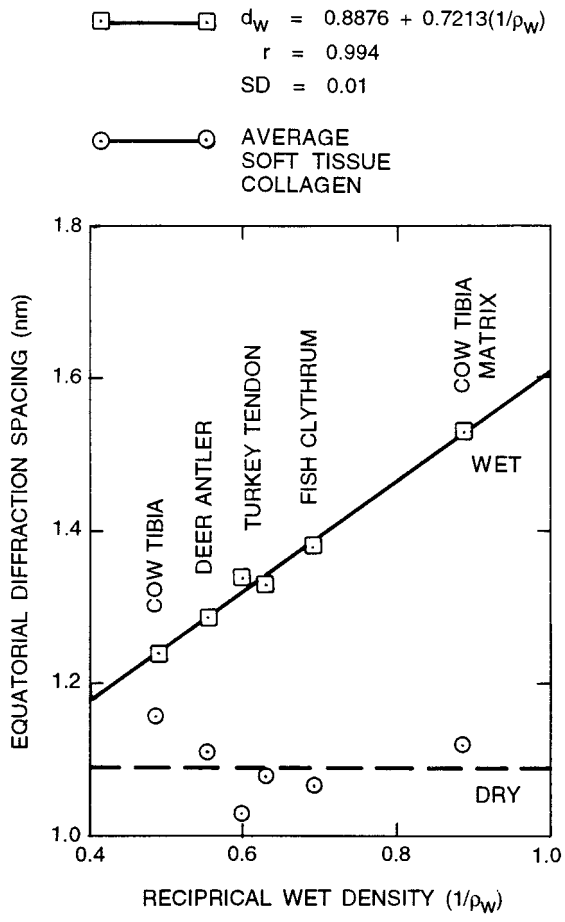

FIGURE 1 The equatorial diffraction spacing versus the reciprocal density. The upper plot is for wet tissues, the lower for dried tissues. The spacings for the dried tissues are more scattered, but the spacing is about the same for all tissues. The equation was fitted to the wet tissue values, and the lower horizontal line is the average spacing for rat tail tendon (Lees et al., 1984).

mineral. While the Fratzl et al. model provides a possible way for the equatorial spacing to decrease with mineralization, it does not show why the spacing in wet tissue, corresponding to the diffraction pattern peak, is exactly inversely proportional to the density, with no perceptive deviation, as seen in Fig. 1. Nor does it account for the linear decrease in the equatorial collagen spacing as the water is removed for both mineralized and unmineralized tissue (Lees, 1986; Lees and Mook, 1986), or why the spacing in the dried tissue is about the same for all tissues (1.1 nm). As shown in these last references, it is the loss of water within the fibrils that accounts for the decrease in the equatorial spacing, and not the deposition of mineral.

Electron microscopy shows that a major part of the mineral is in the space between fibrils, and a lesser amount is within the fibrils (Lees et al., 1994; Prostak and Lees, 1996; Landis and Song, 1991). The model of Fratzl et al. assumes that most of the mineral is within the fibrils. Most reports of mineral in collagenous tissues show only the mineral within the fibrils (Lee and Glimcher, 1991), because the commonly accepted model of mineralized tissue assumes that the mineral locks up the collagen molecules within the fibrils. Moreover, it has been shown that the extrafibrillar mineral is oriented differently from the intrafibrillar mineral, and that their dimensions are very different (Lees et al., 1994; Prostak and Lees, 1996). As a consequence, the elastic properties of mineralized tissue cannot be just that of mineralized collagen; the extrafibrillar mineralized component must play an important part.

DYNAMIC CONSIDERATIONS

It is well established that the collagen molecules are in constant, thermally driven agitation. Brillouin light scattering was used to determine the sonic velocity of collagenous tissues at gigahertz frequencies (Cusak and Miller, 1979; Cusack and Lees, 1984; Lees et al., 1990), a technique that depends on the thermal motion of the molecules. Presumably it is transverse linear motion of the molecules that scatters the light (Amoros, 1968). Furthermore, Torchia (Batchelder et al., 1982; Jelinski et al., 1980; Sarker et al.,

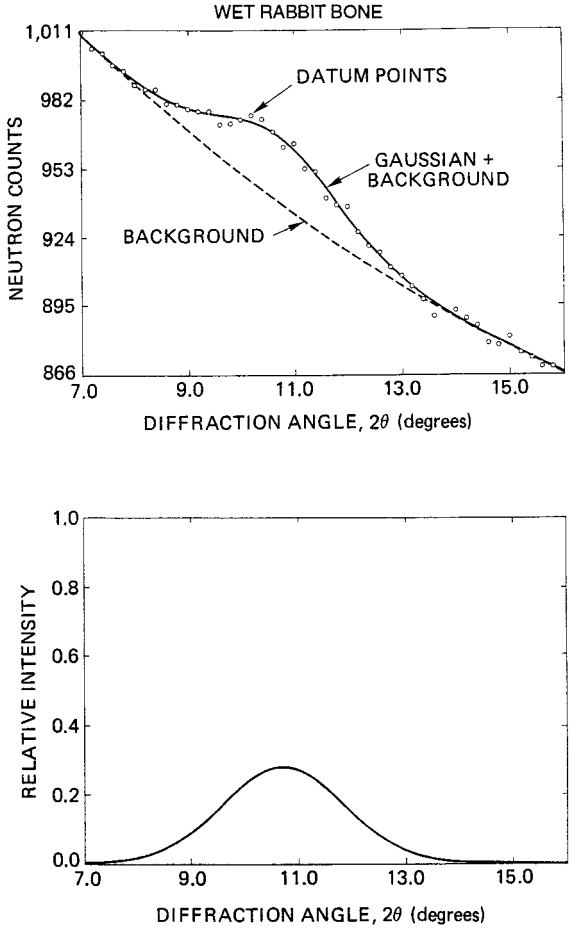

FIGURE 2 An example of a curve fitted to the data by least squares. The curve is a second-order polynomial plus a Gaussian. The peak of the Gaussian is taken as the equatorial spacing (wet rabbit bone) (Lees et al., 1987).

1983, 1985) showed that the collagen molecules oscillate in rotation around the long axis and that the maximum angular displacement depends on the condition of the tissue. Where there are no cross-links, the amplitude is 41°. For cross-linked rat tail tendon it is 31°, and for bone it is 14°. For frozen tissue there is no rotation. The variation is illustrated in Lees (1989, figure 7.7). This is further evidence that in bone, the molecules must be free and cannot be bound by mineral. The collagen molecules clearly engage in every mode of vibration, and presumably the energy of vibration is equally partitioned among all modes.

Collagen molecules look like strings fixed at the ends, where the cross-links are located. Consider only the transverse modes, in which the collagen molecules vibrate like strings, with each molecule vibrating independently. The spacings between molecules are therefore continually changing very rapidly, at gigahertz frequency. Because it takes an appreciable amount of time to record, the diffraction pattern should correspond to a continuously but randomly distributed arrangement of the molecules. However, each molecule is vibrating string-like in a complex pattern about the same reference position that is set by the cross-links. The cross-links tie colinear collagen molecules in strings, but also bond the strings laterally, so that there is a reference state for the collagen, embedded in the tissue for any given condition. When the condition is changed, by drying or by mineralization, the reference state changes. An illustration of what the cross section of collagen might look like when the molecules are disturbed from their reference state is shown by Lees (1986, figure 6).

The spacings reported by Lees et al. (1984) were obtained by representing the curve as the sum of a second-order polynomial and a Gaussian and fitting the curve by least squares. The peak of the Gaussian was taken as the equatorial spacing. Fig. 2 is an example of the fitting process for wet rabbit bone, where the radiation was neutrons (Lees et al., 1987). In the upper part, the fitted curve is shown overlying the data. The fitted polynomial may also be seen. The resultant Gaussian is seen in the lower figure. Fig. 3 shows several additional examples where the radiation again was neutrons (Lees et al., 1984; Bonar et al., 1985). The data fall very closely on the calculated curve in every instance. The Gaussian distribution corresponds to the temperature function in the Debye-Waller treatment of thermally agitated disorder in crystals (Amoros, 1968).

There is a physical explanation. In the experimental arrangement, the specimen is contained within a thin-walled plastic container, and there is some gas, usually moist air, whereas the rest of the optical path is in a vacuum. Some scattering is induced by these materials. Furthermore, the mineral crystals in the tissue cause considerable scattering. The other scattering is due to the diffraction by the collagen molecules. Hence there are two major independent scattering processes, and the results are additive.

All of the modes vibrate about the reference position (Amoros, 1968), so that the most common and most frequently observed spacing must be the reference spacing. The least frequently observed spacing is the maximum displacement from the reference spacing.

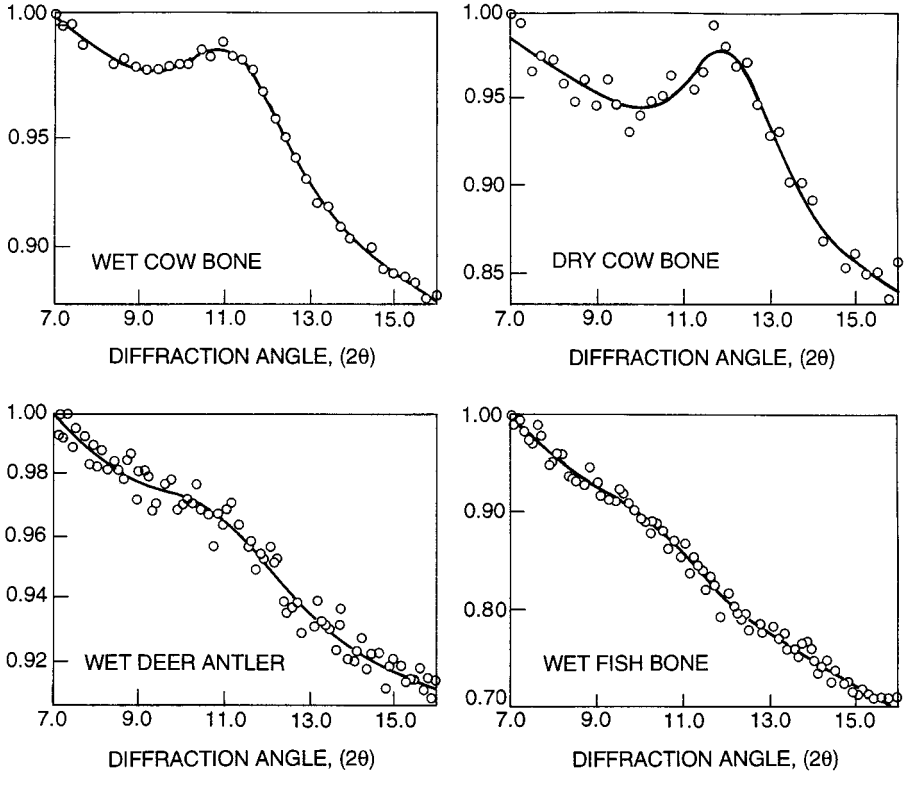

FIGURE 3 Additional examples of least-squares curves fitted to wet bone diffraction patterns, showing close fit to the data (Lees et al., 1984).

CONCLUSIONS

Two very different models have been compared that closely fit the small-angle, diffuse equatorial scattering pattern observed for type I collagen, whether exposed to x-rays or neutrons. Fratzl et al. employed a statistical model with no long-range lateral order based on overlapping parallel cylinders. Our model is a disordered molecular crystal. Lees et al. discerned two scattering processes, one due to the collagen molecules and another attributed to background. The equatorial motion of the thermally driven collagen molecules, in accordance with the Debye-Waller theory, presents a range of continually changing spacings. A reference spacing based on the stereochemical restraints imposed by the cross-links is identified by the peak of the Gaussian function. Different conditions modify the bond angle and the bond spacing, and therefore the reference collagen spacing.

The model of Fratzl et al. does not provide a physical interpretation for the observed peak, whereas our model does. Our model explains why the spacing is linearly dependent on the water content. Neither model explains why the spacing is so closely related to the bone density. Our model explains why the equatorial spacing is the same for all type I collagen tissues in the dried state.

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