Distribution of Bone Crystallites in Mineralized Collagen Fiber

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Characteristics of bone crystallites at the insertion of the distal phalanx of the horse in the stage of fibrous bone formation were investigated using electron microscopy. In this tissue, most of the collagen fibrils are ordered in the axial direction and crystallized. The longitudinal section of the collagen fibril showed periodic bands approximately 640–670A apart and with interperiod bands A1, A2, A3, B, C1, C2, D, E and F. In general, these crystallites seemed to adhere to the surface of the collagen fibrils and in the fibrils on the bone surface, needle-or plaque-shaped crystallites were oriented in the same axis as collagen fibrils and arranged on the cross bands. In the bone, many needle-shaped crystallites lay between the parallel collagen fibrils, and the crystallites infiltrated into the collagen fibrils where the interperiod bands are dense. These dense interperiod bands extended approximately 380–400A between two periodic bands 640–670A apart. Infiltration of the crystallites seemed to start at the fibril edge and spread reticulately into the interior. Lucent spots diameters of 50–80A were observed within the collagen fibrils. The crystallites filled the space (20–30A in width) between the spots.—Key words: Bone crystallite, Collagen fiber.

Characteristics of formation of bone crystallites during the healing of bone fractures and other ossifications are not fully understood as yet. Katz and Li [8] studies the distribution of demineralized bone collagen and concluded that 70% to 80% of the mineral is contained in the fibrils. The most of the crystals are rod-shaped, approximately 300–1,000A in length and 25–75A in diameter, and the crystalline apatite rods orient parallel to the collagen fibrils. X-ray diffraction studies [4] [15] indicate that the c-axis of bone crystallites is parallel to the collagen molecular axis. A model of crystallite distribution presented which reconciles with the two types of crystallite orientation. The collagen is known to be an array of parallel rod-like molecules, 13A in diameter and 3,000A in length. The Hodge-Petruska scheme [5] [6] postulated that there is a chain of gaps, 400A in length, between the colinear molecular ends and that adjacent molecules are staggered to overlap the gaps. Lees [9] presented a model of the distribution and orientation of the crystallites in the bone. It is generally agreed that the crystals are distributed along the collagen fibrils with the same 670A periodicity as the collagen. However, there is still debate whether they are confined to the periphery or penetrate inside the collagen fibrils. Furthermore, there is little knowledge about the axial location of the crystals in respect to the collagen period.

We investigated these questions using the insertion of the extensor tendon to the distal phalanx of the horse by electron microscopy.

MATERIALS AND METHODS

The insertion of the extensor tendon to the distal phalanx was obtained from 5 horses in the stage of fibrous bone formation (from 3 months to 1.5 years old) [11]. At this stage, the connection between the tendon and the bone is complete. At the insertion, the distal
phalanx consists almost completely of spongy bone, but the fibrous bone formation is observed at the distal end. The material used in the present study was this fibrous bone. The specimens were fixed two hours in ice cold 1% osmium tetroxide. The tissue was washed, dehydrated in increasing concentrations of ethanol and embedded in Epon. Sectioning was done with JUM-5 and LKB ultramicrotomes using glass or diamond knife. Sections, 1 or 2 μm in thickness, were mounted on glass slides and stained with aqueous toluidine blue. These sections were examined by light microscopy to judge whether they were correctly oriented. The acceptable specimens were stained with uranyl acetate and lead citrate. Microscopy was performed with JEM-7 and HU-12A.

RESULTS

The collagen fibrils of the tendon: The tendon consisted almost completely of bundles of fibres lying nearly parallel to its long axis. The fibre was composed mainly of collagen fibrils. Longitudinal section of individual fibril exposed the monotonously regular, repeating periodicity and the characteristic of the macromolecular aggregation state. The average period of the collagen fibrils was about 640 to 670Å. A1, A2, A3, B, C1 and C2 intraperiod bands were clearly observed (Fig. 1). In uranyl acetate and lead citrate stained fibrils, the dark portion of the 640 to 670Å period consisted of the A1, A2, A3, B, C1 and C2 intraperiod bands and the light portion consisted of the D, E and F bands. In cross sections, collagen fibrils ranged from 1,000 to 1,500Å in diameter. Closely packed

Fig. 1. Longitudinal section of tendon showing parallel collagen fibrils with their 640–670 Å period. A1, A2, A3, B, C1, C2, D, E and F intraperiod bands standout clearly. Adjacent collagen fibrils run in opposite directions. ×55,000

Fig. 2. In cross sections, collagen fibrils range from 1,000 to 1,500 Å in diameter. Closely packed filaments form the fibril. ×100,000.
filaments, either rods or tubes, formed a fibril (Fig. 2). There was space between the collagen fibrils. Thirty collagen fibrils were observed in an area of 5,000×5,000A (Fig. 2). One third of the whole area consisted of the space between the collagen fibrils.

The collagen fibrils of the bone surface: The collagen fibrils extended without substantial change in arrangement or direction from the tendon to the bone surface. Many particles are observed in the space between the collagen fibrils (Fig. 3). In the long axis of the fibrils, needle-or plaque-shaped crystallites were oriented in the same axis as the fibrils and appeared to be arranged on the cross bands (Fig. 4). Sometimes, they extend over the interval between the cross bands. In general, the crystallites seem to adhere to the surface of collagen fibrils (Figs. 3, 4).

The bone side: Where the collagen fibrils enter into the bone side, many needle-shaped crystallites lay between the parallel collagen fibrils (Fig. 5). In this region, the crystallites infiltrated into the collagen fibrils where the interperiodic bands are dense (Fig. 5). These dense interperiodic bands span about 380 to 400A between pair periodic bands 640 to 670A apart. In cross section, infiltration of crystallites seemed to start at the edge of fibril and spread reticulately into the interior (Fig. 6). Lucent spots with diameter of 50 to 80A were observed within the collagen fibrils (Fig. 6). The crystallites filled the space (20 to 30A in width) between the spots.

DISCUSSION

The material used in the present experiment was the insertion of the extensor tendon to the distal phalanx of the horse in the stage of fibrous bone formation. This tissue was chosen because the collagen fibrils in this area were generally ordered in the axial direction.
Further, this system has been well studied using optic microscopy in our previous work [11].

Uranyl acetate and lead citrate stained fibrils showed high and low density region; the length of the former is, on the average, 400A and that of the latter was about 270A. Lees [9] proposed a model of the distribu-
tion and orientation of the crystallites in the bone. The crystallites filling the gap are needle like with the c-axis parallel to the collagen molecule axis. The pores are filled with axially oriented mineral particles in an incomplete crystallites state. The interfibril spaces have larger crystallites lying between the fibrils with the c-axis perpendicular to the fibril axis with some dislocations along the midline.

When the collagen fibrils near the bone surface were observed at lower magnifications, the crystallites were seen along the collagen fibrils as elongated elements with their longitudinal axis nearly parallel to the axis of collagen fibril. This is the characteristics of the early stage of ossification, as has been noted [2] [3]. Robinson et al. [12] [13] also described the needle-shaped objects following path of the collagen fibril and interpreted them to be crystal platelets on the edge of the fibril.

In the bone region, the crystallites lay between the parallel collagen fibrils which may correspond, in the Lees' model, to the larger crystallites lying in the inter fibril spaces. The minerals in the collagen fibrils formed relatively dense periodic bands approximately 380 to 400A in length. These mineral bands had a close correlation to the intraperiod bands. Such location of mineral bands seems to correspond to the water-containing hole zones postulated in the Hodge-Petruska's model [5] [60] of collagen macromolecular aggregation also to the hole of the Lees' model. White et al. [15] have shown that a close axial relationship exists between the mineral and the collagen in calcified turkey leg tendon using low-angle X-ray diffraction. The most attractive feature of their model is that the size of the mineral block precisely fits the gap region. Cooper and Misol [2] described that, deep in mineralized fibrocartilages at the tendon insertion, the collagen fibrils remain parallel, intraperiod bands remain well defined, and minerals are well organized in relation to the intraperiod bands.

Cross sections of the mineralized collagen fibrils of the bone region showed reticulately arranged lucent spots, the diameter of a spot being from 50 to 80A. It is suspected that the lucent spot is the domain occupied by a collagen molecule. It has been recognized that several collagen molecules form a packing of collagen molecules. The nature of the precise way of the package of collagen molecules into a collagen fibril is still debatable [1] [7]. The pentafibrillar model, originally proposed by Smith [14], has been widely accepted as the fundamental building unit of the fibril. This model, based on the quarter-stagger end-overlap hypothesis of Hodge and Petruska, has been supported by the X-ray diffraction data of Miller and Wray [10].

The crystallites between the lucent spots are 20 to 30A in width. This corresponds to the intrafibril crystallites in the pore of the Lees's model, and the pore of the Katz and li's model which are the lateral space between adjacent molecules.

REFERENCES


要約
骨化膠原線維における骨結晶の分布について：大友助十郎・小池寿男（北海道大学医薬学部医学部）
骨の治癒過程における骨結晶の析出状態を組織学的に観察した。比較的多数の骨原線維が平行状態で化骨する部位として、骨の錐骨の関節付着部を選定し、未脱灰標本の縦断面、横断面の超薄切片を作製して観察した。観察した骨原線維の縦断像では、明瞭な640～670Åの周期が観察され、その中にさらにA1、A2、A3、B、C1、C2、D、E、Fの小周期が区別される。骨の移行部で骨原線維の表面に針状または板状の結晶が観察された。骨側の縦断像では、多数の針状結晶が認められ、骨原線維の周期構造と密接な関係があるように見えた。この部分の横断像では、骨原線維の間隔は完全に骨化した。さらに骨原線維の骨化は表層から内層に向けて進むように見えた。骨結晶は骨原線維内で径50～80Åの空間を形成しながら網目状に侵入しており、線維内の空間は骨原線維を構成するフィラメントの専有領域と考えられる。