Studies on Fish Scale Formation and Resorption

III. Fine Structure and Calcification of the Fibrillary Plates of the Scales in Carassius auratus (Cypriniformes: Cyprinidae)*

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Summary. Electron microscopic investigation of scales of the goldfish Carassius auratus revealed that the lamellae of fibrillary plates contain sheet-like structures composed of vertically oriented collagen fibers embedded in an organic matrix. The fibers (TC fibers) are smaller in diameter (35–45 nm) than those of the lamellae and the matrix is stained intensely with lead citrate.

The sheet-like structures as well as the lamellae are formed by fibroblasts located beneath the lamellae. The orientation of the collagen fibers of the sheets and the lamellae seems to be controlled by the orientation of the ridges and invaginations of the surface of the fibroblasts.

The fibrillary plate of C. auratus was found to be partially calcified. Calcification was initiated by the deposition of needle-like or flaky crystals of hydroxyapatite in the organic matrix of the sheet-like structure and proceeded into the TC fibers and the matrix region of the lamellae. The potassium pyroantimonate-osmium tetroxide method showed a heavy concentration of calcium in the osteoblasts, fibroblasts, and in the matrix regions of the fibrillary plate. Calcium-containing precipitates were also present in the “hole zone” of the collagen fibers in the lamellae, but the significance of this location in calcification remains to be elucidated.

Key words: Fish scale – Fine structure – Calcification – Fibrillary plate – Carassius auratus.

Scales of teleost fish are composed of two layers, the upper osseous layer and the lower fibrillary plate. The fibrillary plate is known to be composed of multiple layers of lamellae, each of which is filled with parallel collagen fibers and an organic matrix.

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The fibrillary plate is partially calcified in some species, such as *Leuciscus rutilus* (Wallin, 1957), *Fundulus majalis* (Cooke, 1967), *Hippoglossoides elassdon* (Brown and Wellings, 1969), *Cyprinodon variegatus* (Olson, 1976) and *Fundulus heteroclitus* (Yamada and Watabe, 1979), and uncalcified in other species, e.g., *Salmo gairdnerii irideus* (Maekawa and Yamada, 1970), *Brachydanio rerio* (Waterman, 1970) and *Oncorhynchus keta* (Yamada, 1971). Yamada and Watabe (1979) reported that calcification of the plate in *Fundulus heteroclitus* proceeded by invasion of needle crystals of hydroxyapatite in the interfibrous spaces of collagen from the upper calcified osseous layer.

During the study of the fibrillary plate of the scale of the goldfish *Carassius auratus*, the present authors have found a new structure within the lamellae that seems to play a significant role in calcification. This paper describes the detailed morphology of this structure and calcium distribution, and discusses the process of calcification of the fibrillary plate.

**Materials and Methods**

Goldfish, *Carassius auratus*, weighing 2.0 to 4.8 g were obtained commercially and kept in a 10-gallon glass aquarium with dechlorinated tap water maintained at 19–21°C. They were fed on fish food pellets. From each fish, 5 to 10 scales were removed from the two rows on both sides of the anterior lateral line, usually at the left side of the body. A total of 60 to 70 scales were sampled from 10 fish. A regenerated scale which was formed after the removal of a scale was included in the study for observation of active fibroblasts. The scales were fixed in 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) for 2 h, postfixed with 1% osmium tetroxide in the same buffer for 1 h, dehydrated through an ethanol series, and embedded in Spurr resin (Spurr, 1969). Ultrathin sections were cut with a Sorvall Porter-Blum MT-2 ultramicrotome using a diamond knife. Sections were unstained, or stained with uranyl and lead citrate, and observed with JEOL 100-B or Zeiss EM-9S electron microscopes. Some sections were decalcified by floating on a drop of 0.13 M 2 Na EDTA. For identification of the crystal type, electron diffraction analysis was performed on unstained sections.

For localization of calcium, scales were fixed in 1% osmium tetroxide containing 2% potassium pyroantimonate following the method of Simson and Spicer (1975). They were then dehydrated, embedded in Spurr medium and sectioned as described previously. Unstained sections were observed with a JEOLJSM-U3 scanning electron microscope equipped with a transmission detector, and elemental composition of the precipitates was analyzed with EDAX-EDIT energy dispersive x-ray microanalysis system.

**Results**

The fibrillary plate of the goldfish is composed of stratified lamellae. Each lamella is 1 to 3 μm thick and made up of tightly packed collagen fibers (Figs. 1, 6), which will be called LC fibers (lamellar collagen) in the present report. The directions of orientation of the LC fibers within each lamella were identical. The directions of orientation of the LC fibers between any two adjacent lamellae differed, but were not aligned at right angles to each other. The LC fibers are embedded in an amorphous matrix and spaced at the intervals of 8 to 15 nm. In cross section, the LC