Electron Microscopic Study of the Intracellular Activity of Alkaline Phosphatase in the Hypertrophic Chondrocytes of Epiphyseal Cartilage

by

Minoru TAKAGI, Hideo TAKAGI, Noboru KOJIMA, Kenji KASUYA and Yoshihisa TODA

In the present investigation the intracellular activity of alkaline phosphatase was observed in the hypertrophic chondrocytes of epiphyseal cartilage from rat tibia.

The proximal epiphyseal cartilage of a tibia from Wistar rats weighing 100 g each was removed under Nembutal anesthesia. All specimens were reduced to small pieces, fixed for 3 hours at 4°C in 2% glutaraldehyde solution buffered with 0.1 M cacodylate buffer (pH 7.3) to which was added 0.2% ruthenium red in order to avoid the removal of the proteoglycan[1-3]. Commercial ruthenium red was purified by a method of Brooks[4]. Subsequently, the slices were rinsed in 0.1 M cacodylate buffer containing 7.5% sucrose and 0.1% ruthenium red at 4°C and sections 30-40 μ in thickness were made by Sorvall TC-2 Tissue sectioner. To demonstrate alkaline phosphatase activity, these sections were incubated in a medium (pH 9.3) by a method of Mayahara et al.[5]. As controls, some sections were heated at 60°C for 30 minutes prior to incubation. Incubation was carried out for 20 minutes at 37°C and terminated by rinsing the sections at 4°C for 10 minutes in sucrose-containing cacodylate buffer. Subsequently, the sections were postfixed in 1% OsO₄ in 0.1 M sodium cacodylate plus 0.05% ruthenium red for 1 hour at 4°C. The sections were dehydrated in a graded series of ethyl alcohol, and embedded in Spurr’s resin[6]. Thin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi H-500 electron microscope.

Figure 1 shows the chondrocytes in the upper part of the hypertrophic zone. Lead deposits demonstrating alkaline phosphatase activity may be seen in the Golgi apparatus of chondrocytes. The Golgi apparatus seen in the chondrocytes is composed of flattened sacs, variable sized vesicles and large vacuoles. Some of the Golgi vacuoles contain filamentous material, while the remainder contain filamentous and finely granular materials. The former (Fig. 1, F) show distinct lead precipitates in the membrane and the latter (Fig. 1, FG) in the membrane and also inside the vacuole. Occasionally, the Golgi vacuoles that exhibit an definite membrane show distinct lead precipitates in the inner leaflet (Fig. 2, arrow). Reaction products are also seen in vesicles and flattened sacs.

The reaction products were also found in association with spherical or ovoid cytoplasmic bodies with a diameter of 0.3-0.6 μ in the peripheral cytoplasm of
Fig. 1. The chondrocyte in the upper part of the hypertrophic zone. F: the Golgi vacuoles containing filamentous material. FG: the Golgi vacuoles containing filamentous and granular materials. ×21,600.

Fig. 2. A high magnification view of the Golgi area in Fig. 1. ×43,200.
chondrocytes (Fig. 3). These bodies were enveloped with one exception (Fig. 3, B) by unit membrane. Reaction products were seen in the inner leaflet of these bodies and inside them. It is noted that reaction products were found in the inner leaflet of a multivesicular body and on the membranes of the vesicles found inside a multivesicular body (Fig. 3, F). In the present experiment, however no reaction products were seen in the chondrocytes of the control specimens.

There have been many reports [7-15] on the electron microscopic observation of alkaline phosphatase activity in calcifying cartilage. But there have been few reports [14,15] which observed alkaline phosphatase activity in the Golgi apparatus of chondrocytes. In the present investigation the authors noted the presence and distribution of alkaline phosphatase in the Golgi apparatus of chondrocytes as shown by distinct precipitates in the inner leaflet of Golgi vacuoles exhibiting an unit membrane.

Cytoplasmic bodies positive for alkaline phosphatase have been described in other calcifying tissues [16-18], but this is the first report of their presence in cartilage. The bodies, observed in this study, resemble the fine structure of the Golgi vacuoles, suggesting that they may originate from the Golgi apparatus. It is possible that they could fuse to the plasma membrane and discharge their contents into the pericellular matrix.

Further detailed study is necessary to clarify the function of these bodies.

References