The Histochemical Localization of Acid Phosphatase Activity in BMU

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ABSTRACT

In order to clarify the coupling mechanism (or phenomena) of the bone resorption–formation in the remodeling process, this study was attempted to observe the histochemical location of acid phosphatase (ACPase) and tartrate-resistant ACPase (TRACPase) activity of the newly formed cement lines and the mononuclear cells in BMU (basic multicellular unit) using JB-4 embedding technique for the demonstration of ACPase and TRACPase activity on the tibial metaphysis of rat.

The ACPase activity could principally be observed both at the sites where the onset of the newly cement line formation of the trabecular bone and the endosteal bone surfaces, and the resorbed bone surfaces. However, the ACPase reactive cement line was never observed at the erosion zone of the tibial metaphysis, where the osteoblastic bone formation does not occur.

Other than osteoclasts and cement lines, the ACPase activity was also observed either in the mononuclear cells closely located to the ACPase positive resorbed bone surfaces or the osteocytes near the osteoclasts. They were also positive for the TRACPase reaction.

It was concluded that the active osteoclasts are moving on the bone surface as secreting ACPase to form the cement line, which may influence to induce osteoblasts from adjacent preosteoblasts. It was also suggested that the mononuclear cells and the osteocytes may also relate to the bone remodeling.

Introduction

Bone is constantly reconstructed and it has been classically thought that osteoclasts resorb the preexisting bone (resorption) and osteoblasts synthesize the new bone matrix (formation).

In the remodeling, resorption and formation of bone does not involve the independent event, but there is the coupling phenomena between bone resorption by osteoclasts and formation by osteoblasts: that is, osteoclastic bone resorption is always followed by osteoblastic bone formation (Frost, 1964 (1)).

The mechanism which underlies the coupling phenomenon of bone remodeling remains obscure, but it has been known that the coupling is an interaction between osteoclasts and osteoblasts being controlled locally in the bone microenvironment and can be observed as a cellular event sequence in the same place but at different time (Frost, 1964 (2), Parfitt, 1976 (3)) after the activation of osteoclast precursors (activation phase), osteoclasts resorb the old bone (resorption phase), following that, preosteoblasts differentiate into osteoblasts and form the new bone at the same place (formation phase) (4).

Between the resorption and the formation phase, the reversal phase is postulated, in which the mononuclear phagocytes (MNP) can be assumed (Baron, 1977 (5)). Although the structure and function of MNP is still obscure, it is supposed that these
cells may scavenge the bone surface following the osteoclastic bone resorption and secrete the cementing substance of the cement line (Baron et al., 1980 (6), Tran van et al., 1982 (7)).

The cement line is a 1–2 μm thick layer of mineralized ground substance rich in glycosaminoglycans and contains few or no collagen fibers, and separates pieces of bone at different times (Parfitt, 1983 (8)). It has been described that the cement line is stainable for PAS and positive ACPase activity (Parfitt, 1983 (8), Baron et al., 1983 (9), Wergedal et al., 1969 (10)). However, the precise location of ACPase activity on cement lines, especially cement lines at the beginning of their formation area, has not been demonstrated.

The purpose of this study is, therefore, to demonstrate more precise location of the cement lines and their formation processes, and also to observe the mononuclear cells on the remodeling bone surface using the histochemical technique for demonstration of ACPase activity after embedding in JB-4 resin.

Materials and Methods

The tibiae of 7 days old Wistar strain rats were sacrificed by intracardiac perfusion with the 1% gluteraldehyde, 1% paraformaldehyde mixture in a 0.07 M cacodylate buffer, pH 7.4, containing 0.05% CaCl₂, for 15 minutes at room temperature. The tibiae were excised and immersed in the same fixative for 2 hours at 4 °C.

After fixation, the specimens were washed in a 0.1 M cacodylate buffer, pH 7.4, containing 7% sucrose and then decalcified in 5% EDTA at 4 °C for 3 days. Following decalcification, they were dehydrated through graded ethanols and embedded in JB-4 (Polysciences) (11). Embedded specimens were polymerized at 4 °C or 60 °C (12, 13, 14, 15, 16).

Semithin sections (2 μm) were prepared with Porter–Blum MT–1 ultramicrotome and were incubated in the following solution for the histochemical demonstration of ACPase activity. Naphthol AS-BI phosphate (Disodium, SIGMA) was used as the substrate. Incubation was performed at pH 5.0 for 30–60 minutes at 37 °C. A complete medium (Barka and Anderson, 1962 (17)) containing 10 mM sodium fluoride or a section pretreated with 50 mM L(+)–tartric acid (12) was used as a control experiment. In addition, the medium lacking substrate was also used as a control.

After incubation, the sections were washed in a 0.1 M cacodylate buffer, pH 7.4, stained with 0.1% toluidine blue and examined under the light microscope.

Results

An intense reaction of ACPase activity was observed in the osteoclasts, resorbed bone surfaces and mononuclear cells only in the case of JB-4 polymerized at 4 °C (Fig.1), but no reaction was seen on sections of JB-4 polymerized at 60 °C.

![Fig.1](image1.png)

**Fig.1** Light micrograph of the location of acid phosphatase (ACPase) activity in the endosteal bone surface of rat tibia. ACPase activity is observed in the osteoclasts (OC), resorbed surface (RS), cement line (CL) and mononuclear cells (MNC). OB: osteoblast. Toluidine blue staining. X880

![Fig.2](image2.png)

**Fig.2** High magnification light micrograph showing mononuclear cells (MNC). OC: osteoclast. OB: osteoblast. RS: resorbed surface. CL: cement line. Toluidine blue staining. X1300