SHORT COMMUNICATION

ALKALINE PHOSPHATASE IN BONE: INSIGHT FROM ZINC MAPPING STUDIES

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Alkaline phosphatase (ALP) is present in all skeletal and dental tissues. In bone tissues, histochemical studies using the azo-dye method that eliminates diffusion artifacts show that ALP is present on the outer membrane of the osteoblasts as well as focally in the osteoid before mineralization. When the osteoid mineralizes ALP enzyme activity is not longer revealed at the mineralization nodules despite the fact that ALP is buried at these sites, as some immunohistochemical studies have detected (1, 2, 3). As ALP is a metalloprotein containing two Zinc atoms, we aimed to localize ALP in bone by its zinc content using sensitive spatial methods for zinc detection (4). Firstly, micro particle-induced-X ray-emission (μPIXE) analysis at a nuclear microprobe with a 3.1 MeV proton beam from a Tandetron accelerator was applied to study bovine cortical bone. The μPIXE analyses of calcium and zinc scan lines, at 7.4 μm spatial resolution, showed that in cortical bone there were two types of zones particularly rich in zinc. One of them was characterized as to have the higher zinc and lower calcium content. The other had the higher calcium and medium zinc content. Whereas the first zone was readily assigned to the mineralization front, either in the periosteum, endosteum, or growing osteons, the second zone was imprecisely located somewhere within the primary bone. To better identify such zones in cortical bone, backscattered electron imaging for calcium, and various sulfide-silver staining procedures for zinc were performed. We found calcium and zinc maps that matched the μPIXE results. The zones rich in zinc were identified as the mineralizing surfaces, whilst the highly mineralized zones were located in the periphery of the primary osteons. At the mineralizing surfaces, zinc was abundant at the boundary between the osteoid and mineral. In the primary osteons, the highly mineralized zones were also rich in proteoglycans; they corresponded to the initial bone tissue deposited during osteon formation. Taken together, these data point out that ALP concentrates at the mineralization front especially during initial bone mineralization, and that ALP remains as a matrix protein bound to proteoglycans in mineralized bone.

1. de Bernard B et al. J Cell Biol 1986;103:1615-1623. 2. Gomez S at al. Bone 1999; 25:33-38. 3.Hoshi K et al. J Bone Miner Res 2001; 16:289-298. 4. Gomez S et al. Nucl. Instr. and Meth. in Phys. Res. B 2006, 249: 673–676.