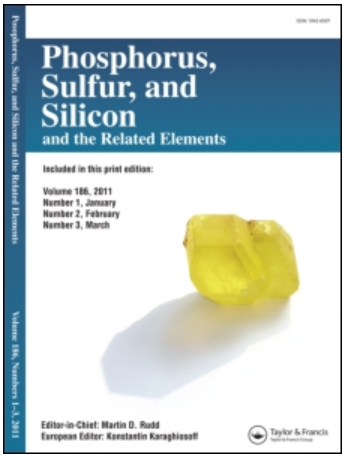

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Physical Chemistry of Osteoclast Resorption of Bone

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In recent years information has developed on the ultrastructure of the osteoclast and the nature of the podosomal seal of the osteoclast to calcified tissue such as bone. The amoeba like movement of the osteoclast to form multiple resorption pits has been documented by electron micrographs. The thermodynamic equations and constants for the ionization of phosphoric acid and the solubility constant for brushite ($CaHPO_4.2H_2O$) was applied to determine by calculation the preferred solid phase of calcium phosphate under the osteoclast. The published literature on the pH and calcium concentration in the fluid space under the osteoclast and the composition of hydroxyapatite (HAp) of bone was used. When the pH is in the range of 3.5 to 5.5 and the calcium concentration is 40mM under the osteoclast, brushite is the preferred phase. From the literature, this means that dissolution under the osteoclast will slow and then effectively stop as brushite covers this HAp surface and it is postulated the osteoclast will migrate to a new spot. If the calcium rises too high and the pH drops to about 3, the osteoclast will probably die (apoptosis). The bisphosphonates will alter this pattern by providing a slightly soluble surface of calcium bisphosphonate on the HAp which will decrease the rate of dissolution of the resorption pit and the depth. The effectiveness of this treatment from a physical chemical standpoint will depend on the individual solubility and particle size of the calcium salts of the bisphosphonates deposited on the calcified surface.

Keywords: osteoclast; resorption; bone; calcium phosphates; thermodynamics

INTRODUCTION

The osteoclast has had an explosion of significant biological and physiologica investigation only in the last 25 years. Before 1975, just very descriptive informatior was available. Since that time ultrastructures[1,2,3,4], membrane and cytoskeleta characteristics[5], and distribution of enzymes have been elaborated[6,7,8]. Th biochemical investigation of the osteoclast response to various substrates[9,10] ha: revealed acidification[11], mineral and matrix dissolution and free radica formation[12,13,14], and acid phosphatase activity[7,12] to name a few. In addition excellent electron micrographs of the amoeba like movement of the osteoclast on

mineral surfaces have been published[15,16]. Instead of the above, the physical chemical processes taking place under the osteoclast in contact with a mineralized surface such as bone will be considered. A tentative explanation of the patterned movement or so called "snail track" lacunae and possible reasons for the observed death of the osteoclast on mineralized surfaces will be attempted. These characteristics of the osteoclast are, in part, the result of highly specific phase changes of the inorganic calcium phosphate under the osteoclast. As the pH and calcium and phosphate concentrations change within the clear zone of the osteoclast during dissolution of the surface of bone, phase changes control the movement and probably the viability of the osteoclast.

Single osteoclasts attach to bone and dissolve out a pit and then can leave the surface and reattach and again can several more times dissolve out a pit in the calcified surface of bone or other calcified system. At times the osteoclast will just partially detach and slide a little across the surface several times creating the so called "snail tracks" on the calcified surface. What is behind this motility of the osteoclast ? Several factors are involved. One is the very tight attachment the cell makes with the surface. Electron microscopy examination has shown that the clear zone of an osteoclast that surrounds the ruffled border delineates the initial resorption perimeter by effectively sealing the osteoclast to the surface[11,12,15]. Another factor that increases the certainty that the volume of fluid encased by the clear zone is effectively sealed, is the hydrogen ion concentration within that fluid can be 100 to 10,000 times higher[17] than the nearby interstitial fluid around the osteoclast (pH about 7.0 – 7.4). In addition, the calcium concentration under the osteoclast has been measured as high as 20 to 40mM under the osteoclast[18] which is 8 to 16 times the extracellular fluid concentration. All these factors suggest the potential for a closed thermodynamic system under the osteoclast based on hydrogen ion concentration and calcium and phosphate thermodynamic equilibria.

METHODS AND RESULTS

The basis for considering these unique osteoclastic resorption pits has been published for a closed pure hydroxyapatite/acid buffer system[19]. This work has documented that pure HAp in contact with acid buffers, such as acetic and lactic acid, readily dissoloves but dissolution slows and then stops. This blocking of HAp dissolution in the presence of excess acid buffer has been determined by both thermodynamic data[19] and by x-ray[20] to be caused by formation and adsorption of brushite overlying the surface of HAp which produces an effective diffusion and hence dissolution barrier[19,20]. It is also the reason why within that closed thermodynamic system, the calcium concentration will rise sharply, well above the molar Ca/Pi = 1.67 of hydroxyapatite[19] as shown by the following equations.

$$\underline{Ca_{10}(PO_4)_6(OH)_2} + 8H^{+1} \longrightarrow 10\,Ca^{2+} + 6HPO_4^{-2} +$$

$$2H_2O \xrightarrow{10H2O} 4Ca^{+2} + \underline{6CaHPO_4{\cdot}2H_2O}\downarrow$$

Thus as the solubility product of brushite is exceeded in the sealed acidic buffer zone of the osteoclast and brushite begins to precipitate out epitaxially on the HAp surface[21], excess calcium ion must accumulate in the solution phase as has been observed in pure systems[19] and under the osteoclast[18]. Thermodynamic calculations can now be made on the fluid – solid system under the osteoclast. The most accepted pH values measured by several techniques are 4.5 to 5.0 after attachments of the osteoclast to the mineralized surface[17,18] and the calcium concentration has been measured from 8 to 40mM[18]. For calculations of this biological thermodynamic closed system of the osteoclast values of pH from 3.0 to 5.0 and calcium concentrations of 40mM were utilized. The theoretical Molar Ca:Pi = 1.67:1 is used for solid hydroxyapatite (in some calcified substrates containing carbonate apatite the ratio may be slightly higher and in immature apatite the ratio may be lower than the above). From the thermodynamic literature, the equilibria to consider in these calculations are shown below:

$$H_3PO_4 \rightleftharpoons H^{+1} + H_2PO_4^{-1} \qquad Ki = 7.52 \times 10^{-3}$$

$$H_2PO_4^{-1} \rightleftharpoons H^{+1} + HPO_4^{-2} \qquad Ki = 6.23 \times 10^{-8}$$

$$HPO_4^{-2} \rightleftharpoons H^{+1}\ PO_4^{-3} \qquad Ki = 4.22 \times 10^{-13}$$

$$\underline{CaHPO_4{\cdot}2H_2O} \rightleftharpoons Ca^{+2} + HPO_4^{-2} + 2H_2O \qquad Ksp = 2.77 \times 10^{-7}$$

$$Ca^{+2} = 1.67 \times Pi \text{ (for hydroxyapatite)}$$

$$Pi = [H_3PO_4] + [H_2PO_4^{-1}] + [HPO_4^{-2}] + [PO_4^{-3}] \text{ (In conditions of pH = 3 to 5 } [PO_4^{-3}] \text{ is negligible.)}$$

Using these equilibria and equations, precipitation of brushite must occur within the clear zone of the osteoclast at all pH values from 4.0 to 5.0 and a calcium concentration of 40mM. At a pH of 5.0, brushite formation will occur if the calcium concentration rises above 8.7×10^{-3}m/L. At a low pH of 4.0, brushite will only form if the calcium concentration rises above 27×10^{-3} m/L. At pH of 3.0, the solubility product of brushite is not exceeded even at a calcium concentration of 40mM. The system is undersaturated due to the suppression of the HPO_4^{-2} species at this low pH. If the pH has dropped from the initial attachment of the osteoclast, brushite will have been deposited during this drop (pH approximately 7 to 4). If now the pH continues to drop due to the proton output of the osteoclast, the combination of the low pH and the high calcium concentration could account for the death of the osteoclast (apoptosis) before it can release the seal and so raise the pH and lower the calcium concentration.

The bisphosphonates will alter the above pattern in two ways by providing a slightly soluble surface of calcium bisphosphonate on HAp which will decrease the rate of dissolution of the resorption pit and the depth of the pit. The decreased rate of dissolution will decrease the calcium and phosphate accumulation under the osteoclast by blocking or reducing the brushite formation. This should result in a decrease in migration of the osteoclast and so decrease the destruction of the calcified surface which has been observed. The effectiveness of this treatment will depend on the individual solubility and adsorbed particle size of the calcium salt of the various bisphosphonates and their individual metabolic effect on the osteoclast[22].

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