The Role of Phosphorylated Proteins in the Chemistry of Bone

Nicole E. Drummer Literature Seminar October 27, 1998

Bone is a complex biological system composed of an organic framework of the protein collagen (20% by weight), small hydroxyapatite-like crystals (69%), water (9%), and other non-collagenous proteins (2%), of which some are highly phosphorylated.[1] Collagen, a triple helix of a-helical chains consisting of repeat units of glycine-X-Y units, where X and Y are often proline and hydroxyproline, respectively, is the macromolecule that defines the shape and structure of the mineralizing compartment.[2,3] This matrix interacts with cells, mineral, and other components of the matrix.[4]

The mineral in bone, poorly crystalline apatite, provides animals with structural support and has tremendous mechanical strength.[3,5] It is structurally very similar to the mineral hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, but vibrational data obtained from bone samples indicate that CO_3^{2-} and HPO_4^{2-} substitute for OH^- as well as for PO_4^{3-} .[3,5,6] Mg^{2+} and Na^+ are sometimes seen as substitutes for Ca^{2+}.[3] The apatite crystals are extremely small, approximately 4 nm x 40 nm x 300 nm in size, form in spaces between collagen chains, and have their c-axes aligned with the long axis of the collagen molecules (figure 1).[7]

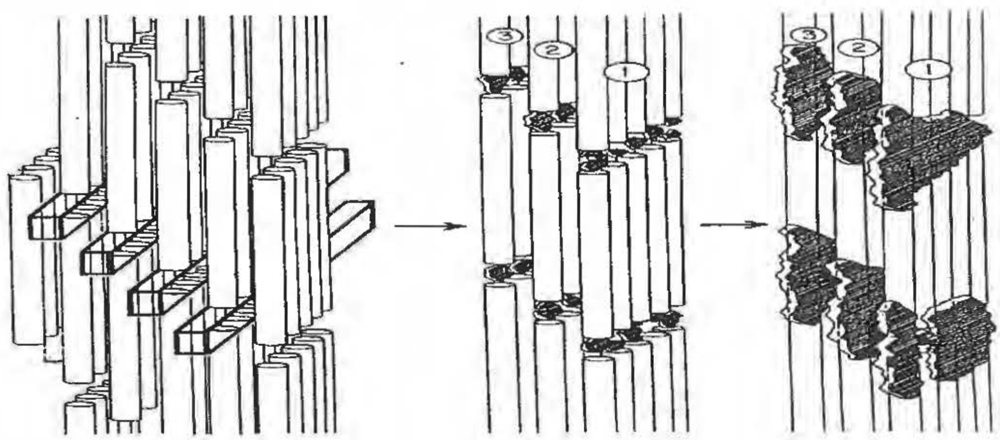

Figure 1

Current research shows that control of mineral formation and growth is due to the presence of phosphorylated proteins in the organic matrix.[8-12] Two of the most prominent phosphorylated proteins in the bone matrix are bone sialoprotein (BSP, figure 2a) and osteopontin (OPN, figure 2b). They are both sulfated and highly acidic glycoproteins. BSP contains repeat glutamic acid sequences, OPN contains repeat aspartic acid sequences, and each protein contains an RGD (Arg-Gly-Asp) cell binding sequence. Researchers have been attempting for many years to ascertain the exact function of these two proteins. Their efforts have included studies to determine the role of phosphate and carboxylate groups with respect to facilitation of hydroxyapatite nucleation or the inhibition thereof, as well as how these functional groups affect cell binding.[8-13] BSP is believed to facilitate nucleation, while the role of OPN is still unclear. Studies show that OPN does inhibit nucleation in certain environments. Recent studies have also examined the actual phosphorylated sites.[14,15] However, because of the complex nature of both the organic and inorganic phases of bone, the roles of these proteins remain uncertain.[2,8-13]

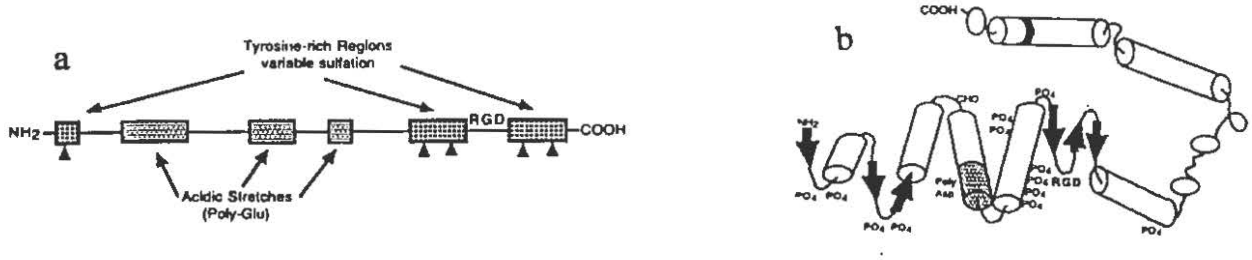

Figure 2

Understanding bone chemistry could facilitate discovery of bone regeneration technology or development of more biocompatible synthetic implant material. Some of the problems associated with current implants are rejection by the body, wear, and corrosion.[1] Hydroxyapatite is biocompatible and can bond to bone, therefore hydroxyapatite can be used as a bone graft or the coating of a metal implant. However, its mechanical properties are fairly poor so it is best used only to repair small bone defects.[1,16] There is promise of incorporating natural bone mineral into implants and grafts due to the successful removal of the organic matrix.[17] To be able to regenerate bone, the growth of the mineral must be regulated and the implant needs to interact with cells and other tissue. Phosphorylated proteins may provide a viable means for this to occur.[14,16]

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