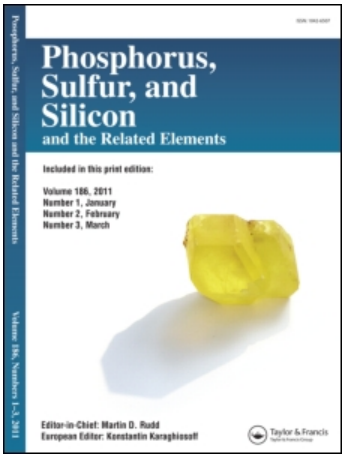

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Phosphoproteins and Biomineralization

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Phosphoproteins play multiple roles in regulating biomineralization. This paper reviews the data implicating bone and dentin phosphorylated proteins in this process, and provides data suggesting which protein domains may be important in the interaction with apatite mineral crystals.

Keywords: biomineralization; phosphoproteins; osteopontin; bone sialoprotein; phosphophoryn; dentin sialoprotein

INTRODUCTION

Phosphorylated proteins were first associated with the mineralization process by Weinstock and Leblond[1], who demonstrated phosphate uptake into the non-mineralized dentin matrix preceded mineral deposition. It was initially believed that the collagen, which was known to provide the template for the deposition of mineral in these tissues, might be the phosphorylated protein controlling mineralization. Today at least 8 other (Table 1). This paper presents the evidence implicating the non-collagenous phosphoproteins in mineralization of bones and teeth.

Because body fluids are undersaturated with respect to apatite (the mineral phase in bones and teeth) and apatite deposition does not occur in all collagenous tissues, the formation of the first apatite crystals (nucleation) is dependent on non-collagenous species. Regulation depends on both collagen and non-collagenous proteins.

To prove a protein is important for the mineralization process, either as a nucleator, supplier of ions, or regulator of growth, several types of studies are essential. First, it must be shown that the protein is present at the mineralization front, and preferably that its composition, concentration, or conformation is altered as mineralization commences. Second, the protein should be shown to affect mineralization in solution in the absence of cells. Third, it should also affect mineralization in cell culture systems. Evidence of altered mineralization occurring in the case of naturally occurring or engineered mutations in the protein provide the ultimate confirmation. As summarized in Table 1, this type of data exists for many of the phosphorylated bone and dentin matrix proteins.

PHOSPHOPROTEIN LOCALIZATION

The localization of several of the phosphorylated matrix proteins to sites of mineralization was established using radiolabelling[1], immunohistochemistry and immunogold labelling[2], *in situ* histochemistry[3], and northern analysis[4,5]. Bone sialoprotein (BSP)[3,5] and dentin

phosphoproteins[4,6] appear just prior to mineralization while osteopontin and osteonectin appear later. The presence of kinases and phosphatases which can add and remove protein phosphates in these tissues[7] suggests a mechanism for regulating mineralization. Similarly in mineralizing cell culture systems the expression of the kinases and BSP, OPN, ON and the dentin phosphophoryns paralleled mineralization[5,6,8-10]. Furthermore, in culture, blocking phosphoprotein formation by inhibiting the matrix protein kinases was shown to retard mineralization[11,12].

TABLE 1 PHOSPHORYLATED PROTEINS* OF BONE (B) AND DENTIN (D)

	Source	Location[a]	Solution Effect[b]	Cell Culture Effect[c]	Animal Model[d]
Collagen (type I)	B,D		N	✓	✓
Osteopontin (OPN)	B,D		I	✓	✓
Osteonectin (ON)	B,D		N,I		
Bone Sialoprotein (BSP)	B	+	N,I	✓	✓
Dentin Sialoprotein (DSP)	D		N,I		
Phosphophoryn(s) (PPN)	D	+	N,I	✓	✓
Dentin Matrix Protein 1(DMP1)	D	+	?		✓
Bone acidic glycoprotein 75 (BAG 75)	B		?		

*The presence and proportion of phosphate in these proteins varies with species, tissue site, and method of isolation. [a]localized at mineralization front: [b]nucleator(N), inhibitor(I), or unknown(?); [c]Mineral changes noted in cell culture; [d]Mineral changes noted in genetic or naturally occurring model.

PHOSPHOPROTIENS AND *IN VITRO* APATITE FORMATION

Solution studies of the effects of isolated phosphoproteins using different systems[13-17] have shown that BSP, DSP and phosphoryn can act both as "nucleators", and "inhibitors" of apatite growth, while OPN to date has only been shown to be an inhibitor. The presence of phosphate, but not necessarily carboxyl groups on these proteins seems to be essential for apatite nucleation. Many of these phosphoproteins exist *in situ* associated with other macromolecules, or as higher MW complexes[18,19], the *in vitro* effects of these complexes are not yet established.

In the system developed in my laboratory for analyzing the effects of proteins available in limited amounts on apatite formation and growth[16] the protein (100μl) being studied is sandwiched between layers of gelatinin 6cm long tubes, and remains fixed there over the 3-5 day duration of the experiment. Calcium and phosphate ions enter the gel from opposite sides.

At 3.5 days the CaxP product (5.5mM²) is sufficiently high that apatite can form in the absence of a nucleator. Comparing the Ca and P concentrations in the visible precipitant band at this time, to protein-free controls, reveals whether mineralization is accelerated or retarded. Comparing the yield in the presence of seed crystals, or at 5 days demonstrates whether the protein inhibits growth. Figure 1 summarizes results in this system which demonstrate the efficacy of 25 μg/ml of a variety of phosphorylated and dephosphorylated bone and dentin matrix proteins.

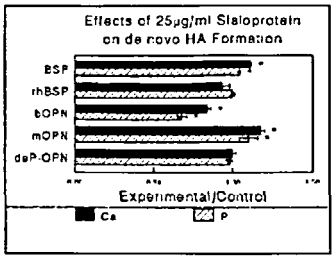

FIGURE 1 Comparative effects of native and recombinant BSP, bovine (b) OPN, milk (m OPN) and dephosphorylated (deP-OPN) on de novo apatite formation. Mean ± SD *p≤ relative to control.

To determine the domains of these proteins that interact with apatite crystals and nucleii, we and others have dephosphorylated the proteins, cleaved them enzymatically, and studied model peptides. Such studies have demonstrated the proteins bind to the 100 face of large apatite crystals, and that the binding is via phosphate groups and poly-aspartate and poly-glutamate domains[17,20,21]. Computer modeling shows optimal binding of oligo Pse-Asp and Pse-Pse-Asp to the 001 face[21].

MINERAL FORMED *IN SITU* IN THE PRESENCE OF MUTANT PROTEINS

Additional verification that phosphoproteins influence mineralization comes from studies of naturally occurring and genetically engineered mutations of these proteins. For example, the impaired mineralization and decreased mechanical strength in the hypophosphatemic (hyp/hyp) mouse[22] may be related to the decreased phosphorylation of OPN and BSP in these animals[23]. Two distinct forms of dentinogenesis imperfecta have been associated with abnormalities in dentin phosphoproteins. Animals deficient in BSP, OPN and ON have been engineered using embryonic stem cell technologies. These knockout mice are viable and their bone mineral properties are currently under investigation. Preliminary data suggest that while there is no radiographic or histologic phenotype in the OPN knockout, their bones may be more mineralized than their wildtype controls. Since the functions of the proteins involved in a critical process such as biomineralization may be redundant, detailed examinations[24] will be required to provide *in situ* validation of the postulated functions of the bone and dentin phosphoproteins.

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