[Frontiers in Bioscience 17, 1891-1903, January 1, 2012]

Acidic bone matrix proteins and their roles in calcification

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1. ABSTRACT

Mammalian bones are composed of calcium phosphate crystals in a protein matrix. The major form of the calcium phosphate is hydroxyapatite. The most abundant matrix protein in bone is type I collagen. Collagen contributes to the mechanical properties of bone and is necessary for calcification of the tissue. In addition to collagen, several acidic proteins are present as minor components. Osteocalcin is a gamma-carboxyglutamic acid-containing protein of bone, which has an affinity to hydroxyapatite and can prevent crystal growth. Bone sialoprotein (BSP) and osteopontin are acidic glycophosphoproteins of bone. These proteins have RGD cell-attachment sequences and consecutive sequences of acidic amino acids. The poly glutamic acid sequences of BSP act as possible nucleation sites for hydroxyapatite crystals. Dentin phosphoprotein is the major non-collagenous protein of dentin. This protein has (Asp-Ser-Ser) repeat sequences, in which most of the Ser residues are phosphorylated. Some of these acidic matrix proteins are immobilized on the collagen fibrils and induce nucleation of hydroxyapatite crystals. They can also modulate crystal shape by adsorption on a specific face of the crystals.

2. INTRODUCTION

Mammalian bones and teeth (dentin) are composite tissues made up of inorganic crystals and proteins (1). On a weight basis, these tissues are composed of approximately 70% inorganic components, 20% proteins and 10% water. These components are well organized in the tissues. The inorganic crystals are deposited on the matrix proteins, contributing to the hardness of the tissues and resisting compressional forces. The major inorganic component of the tissues is hydroxyapatite, a thermodynamically stable phase of calcium phosphate. Hydroxyapatite is a hexagonal crystal having the composition of $Ca_{10}(PO_4)_6OH_2$. In these tissues the mineral crystals are not the stoichiometric hydroxyapatite; instead some of the phosphate ions are substituted by carbonate ions.

The major protein component of the matrix is type I collagen. Collagen molecules self-assemble into collagen fibers, which resist tensile forces and give bones resilience. Within the collagen fiber, molecules form a 1/4-staggered array. The gap between adjacent molecules is called the hole zone. The collagen fibers are important as the matrix for calcification. Osteoblasts, the bone

Table 1. Acidic matrix proteins in bone and dentin

Proteins	Localization	characteristics
osteocalcin(BGP)	bone, dentin	gamma-carboxy glutamic acid, inhibition of hydroxyapatite crystal growth
matrix Gla protein (MGP)	bone, dentin, blood vessel, cartilage	gamma-carboxy glutamic acid, inhibition of calcification in soft tissues
bone sialoprotein (BSP)	bone, dentin	RGD sequence, sialate-containing oligosaccharides, nucleation of hydroxyapatite,
osteopontin	bone, dentin, basement membrane	RGD sequence, sialate-containing oligosaccharides, inhibition of calcification *in vitro*, attachment of osteoclasts
DMP1	bone, dentin	phosphorylation, response to mechanical stress
dentin phosphoprotein (DPP)	dentin	high level of phosphorylation, DSS repeat sequences, nucleation of hydroxyapatite

forming cells, secrete collagen and then deposit calcium phosphates on the collagen fibers. The hole zone is known to be the site of initial nucleation of bone mineral (2). If the normal staggered array of the molecules is disturbed, normal calcification of the matrix is impaired. During the later stage of calcification the crystals are distributed within and around the collagen fibrils. However bone collagen is essentially the same type I collagen as that present in noncalcified tissues except for minor differences in the type of crosslinking. Therefore minor proteins other than collagen should be considered as essential factors for calcification.

In addition to collagen, several acidic glycoproteins are present in the bone matrix (Table 1) (1). These proteins are rich in acidic amino acids, and some of the proteins are highly phosphorylated. Among them are Gla proteins (gamma-carboxy glutamic acid-containing proteins), SIBLING family proteins (small integrin-binding ligand N-linked glycoproteins) and proteoglycans. These proteins can bind many calcium ions and accelerate nucleation of calcium phosphates. They have affinity for hydroxyapatite crystals. Some of the proteins have affinity for insoluble collagen matrix. Here we present a review of these acidic matrix proteins and discuss their possible roles in the mechanism of tissue calcification.

3. OSTEOCALCIN (BGP)

Osteocalcin (bone Gla protein/BGP) was the first bone matrix protein to be characterized in detail. It is a small protein having a molecular mass of 6 kD and is specific to bone and dentin. It is synthesized and secreted by differentiated osteoblasts and odontoblasts. It has 3 gamma-carboxy glutamic acid (Gla) residues, which are synthesized post-translationally and require vitamin K for their synthesis (3). It is also rich in acidic amino acids. Gamma-carboxy glutamic acid has 2 adjacent carboxyl groups in the side chain and has moderate affinity to calcium ions. Side-chains of this amino acid coordinate to Ca ions in the malonate chelation mode (4). Decarboxylation of the residue abolishes the protein's calcium-binding ability. Osteocalcin binds strongly to hydroxyapatite, but not to amorphous calcium phosphate (5), with adsorption occurring preferentially on the (100) face of the crystal (6).

In calcium-free conditions, osteocalcin is mostly in a random coil conformation. However in the presence of calcium, it adopts an alpha-helical conformation as shown by spectroscopic studies (7, 8). An X-ray crystallographic study supports the estimated structure (9). The calcium-bound protein has 3 alpha-helices. Three gamma-carboxy glutamic acid residues lie on the same face of the 1st alpha-helix (Figure 1). These residues coordinate with 5 calcium ions together with an Asp residue and constitute a binding site for the (100) face of a hydroxyapatite crystal (9).

Osteocalcin inhibits the crystal growth of calcium phosphate *in vitro* (5). Protein adsorbed on the crystal may prevent the access of free ions to the growing surface. In osteocalcin-deficient mice, bone mass was increased, without any change in the level of calcification (10). This result indicates that the *in vivo* function of this protein is related to control of matrix synthesis. As this protein is synthesized by fully-differentiated osteoblasts, it is used as a marker of osteoblastic activity. Recently, a hormone-like function of this protein has also been found in the field of energy metabolism.

Another member of this group of proteins is matrix Gla-protein (MGP) (11). This protein is larger than osteocalcin and has 5 gamma-carboxy glutamic acid residues. It is also less soluble than osteocalcin. Although this protein was first isolated from bone, it has a wide distribution in the body including blood vessels and cartilage. The results observed in MGP-KO mice indicate that the function of this protein is to inhibit calcification of soft tissues such as arteries, growth plate cartilage and tracheal cartilage (12). This inhibitory activity is dependent on the gamma-carboxy glutamic acid residues (13).

4. BONE SIALOPROTEIN (BSP)

Bone sialoprotein (BSP/IBSP) is one of the acidic glycoproteins in bone and dentin (14). It is synthesized concurrently with or just before calcification. It has a molecular mass of 75 kD and half of this weight is due to sugar chains (15). It contains many sialic acid-containing oligosaccharides and some phosphorylated and sulfated amino acids. The RGD (Arg-Gly-Asp) cell attachment sequence is present near the C-terminal end (16, 17). This sequence is recognized by integrin $alpha_V beta_3$ and mediates attachment of fibroblasts and osteoblasts. Other characteristic sequences are polyglutamic acid sequences present within the N-terminal half of the molecule (Figure 2), which consist of successive arrays of 8–10 glutamic acid residues.

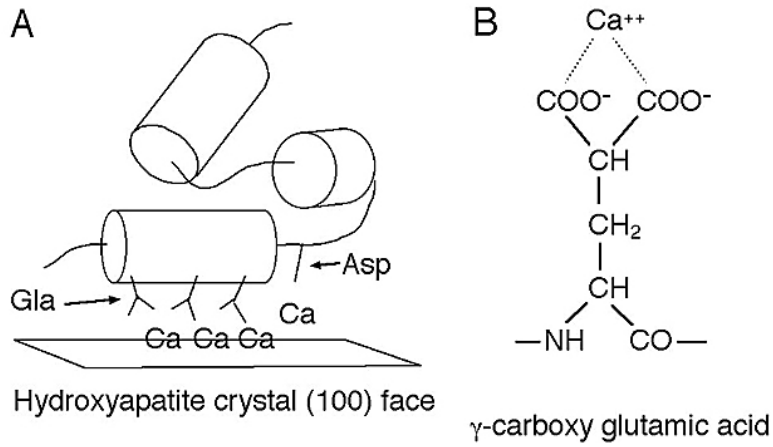

Figure 1. Osteocalcin (BGP) and gamma-carboxyglutamic acid. Osteocalcin is a small noncollagenous protein in bone and dentin which contains gamma-carboxyglutamic acids. A: In the presence of calcium ions the protein contains 3 alpha-helical segments. Three gamma-carboxyglutamic acid (Gla) residues are located in the same face of the helix. The 3 Gla residues constitute a binding site for the (100) face of a hydroxyapatite crystal. B: A Gla residue is synthesized by carboxylation of the side chain of glutamic acid. Vitamin K is required for this reaction.

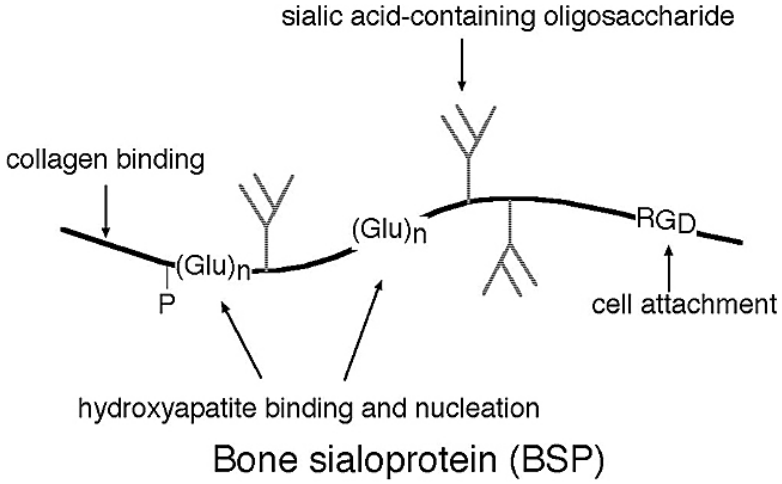

Figure 2. Bone sialoprotein (BSP). BSP is an acidic glycoprotein in bone. BSP has sialic acid-containing oligosaccharide chains. An RGD integrin-binding sequence is present near the C-terminus. Poly glutamic acid sequences ($(Glu)_n$) are present and are thought to constitute sites for nucleation of hydroxyapatite as well as binding sites for hydroxyapatite. A collagen-binding site is present at the N-terminus.

This protein can induce nucleation of hydroxyapatite crystals *in vitro* (18), with the polyglutamic acid sequences being responsible for this effect (19). A study using recombinant BSP protein indicates that at least 8 contiguous glutamic acid residues are required for crystal nucleation (20). This site is not in an alpha-helical conformation. However posttranslational modification is also important for this activity, with phosphorylation of a serine residue adjacent to the polyglutamic acid sequence enhancing the nucleating activity of the protein (21). Molecular-dynamics simulations indicate that this site can interact with the (100) face of hydroxyapatite crystals (21). In another simulation, nucleation of amorphous calcium phosphate has been suggested (22).

Another interesting property of this protein is its affinity to collagen (23, 24). BSP can bind to collagen fibrils, especially to their hole zones. This localization is interesting considering that the hole zone is the site of early mineral deposition. BSP can also affect collagen fibrillogenesis *in vitro*. The collagen-binding site is the N-terminal region (residues 19–46) (25) and the interaction is supposed to be mostly hydrophobic (25, 26). Only native triple-helical collagen can interact with BSP and the binding site on collagen does not involve telopeptide (26). Upon binding to collagen, BSP exhibits an increase in its nucleation potency for hydroxyapatite (26). This protein is also a substrate for tissue

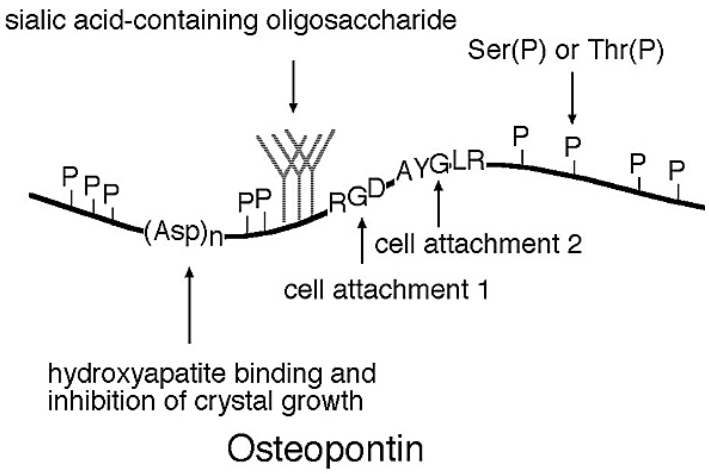

Figure 3. Osteopontin. Osteopontin is a phosphoprotein in bone. It also contains an RGD integrin-binding sequence. This site is involved in attachment of osteoclasts to bone matrix. A second cell attachment site is present adjacent to this site. Instead of the $(Glu)_n$ sequences present in BSP, it contains a poly aspartic acid sequence ($(Asp)_n$). This sequence may be responsible for the inhibition of hydroxyapatite crystal growth by this protein.

transglutaminase and forms protein aggregates when crosslinked by this enzyme (27). This crosslinking may affect its potency for calcification.

The significance of BSP in osteogenesis was shown in an experiment with BSP-KO mice (28). In these mice, bone growth and mineralization is impaired, especially in young mice, and both bone formation and resorption are reduced. BSP may also be involved in bone turnover in addition to calcification. However PTH enhances bone turnover in both wild and BSP-KO mice, suggesting the presence of some compensatory mechanisms (29).

5. OSTEOPONTIN AND DMP1

Osteopontin (SPP1) is a glycosylated phosphoprotein in bone (30-32). It has a molecular mass of ~66 kD, is rich in phosphorylated amino acids (P-Ser and P-Thr) and sialic acid-containing oligosaccharides. The RGD cell-attachment sequence is present at the center of the molecule (33). This site is responsible for interaction with integrin $alpha_v beta_3$. A second cell-attachment sequence, which is responsible for the interaction with integrin $alpha_9 beta_1$, is present adjacent to this site. A thrombin cleavage site is located at the end of the 2nd site. Sites for interaction with CD44 (hyaluronate receptor) are also present. A poly aspartic-acid sequence, instead of the poly glutamic-acid sequence of BSP, is present near the N-terminus (Figure 3). The level of phosphorylation depends on the cell type and affects the adhesive function of this protein (34).

Osteopontin has affinity for hydroxyapatite crystals and inhibits formation and growth of the crystals (35, 36). This inhibitory activity is in contrast to the nucleating activity of BSP. The difference may be attributable to the presence of a poly aspartic-acid sequence in this protein in contrast to the poly glutamic-acid sequence in BSP (36). The inhibitory activity depends on amino acid phosphorylation. Non-phosphorylated osteopontin has no effect on hydroxyapatite formation (37), while in contrast highly phosphorylated milk osteopontin promotes its formation (37).

An important function of osteopontin is the anchoring of the bone-resorbing cells, osteoclasts, to the bone matrix (38). Osteoclasts form sealed spaces on the bone surface and secrete acid into the spaces to dissolve hydroxyapatite crystals. Anchoring of these cells is necessary for their function. Osteopontin at the surface of bone matrix is recognized by integrins of osteoclasts and supports their attachment. Although osteopontin-null mice demonstrate no apparent abnormality in bone formation, the response to ovariectomy and low mechanical stress is reduced in these mice (39, 40). This protein may be significant in responding to these special conditions. Osteopontin is also a substrate for tissue transglutaminase (27, 41), and crosslinking by this enzyme increases its binding to collagen (42). Osteopontin is present in many noncalcified tissues including kidney, blood vessels, and epithelial cells of the urinary tract, and is involved in multiple functions, including prevention of urinary stones (43) and in the immunological response (44). Osteopontin is secreted by T cells and attracts macrophages.

DMP1 (dentin matrix protein 1) is a phosphoprotein of bone and dentin originally cloned from a rat incisor cDNA library (45). This protein is composed of 472 residues and has homology to osteopontin. An RGD cell-attachment site is present in the middle of the molecule and multiple Asp, Glu-rich sequences are present throughout the molecule. DMP1 is processed by proteolysis and is present as 37 kD N-terminal and 57 kD C-terminal fragments in tissues (46). Both of the peptides are highly phosphorylated. A chondroitin-4-sulfate chain is attached to the N-terminal fragment (47).

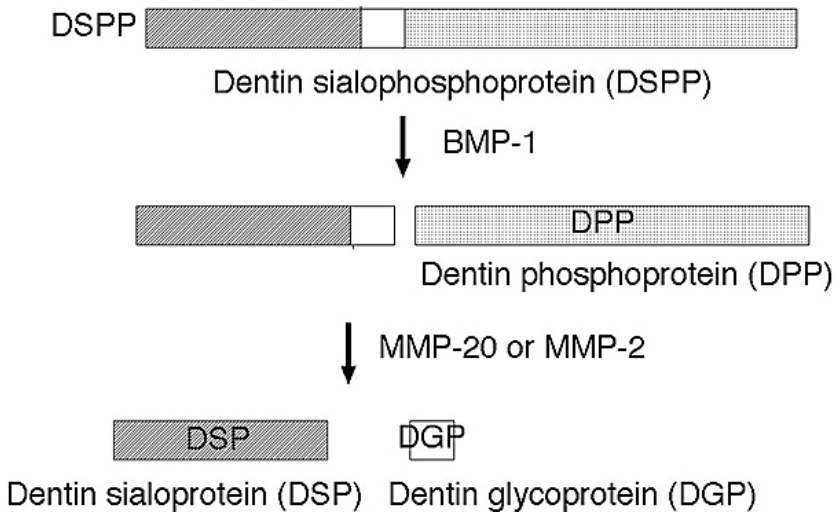

Figure 4. Processing of dentin sialophosphoprotein (DSPP) (57). DSPP is the precursor of dentin phosphoprotein (DPP), a phosphoprotein unique to dentin. DSPP is processed by proteases (BMP-1, MMP-20, MMP-2) into N-terminal dentin sialoprotein (DSP), intermediate dentin glycoprotein (DGP) and C-terminal DPP. DPP is adsorbed on hydroxyapatite crystals and is deposited in dentin matrix.

DMP1 affects *in vitro* calcification. The phosphorylated full-length protein inhibits calcification (48), but in contrast, the C-terminal fragment and the non-phosphorylated protein both enhance nucleation of hydroxyapatite (48). In the presence of collagen, the non-phosphorylated protein as well as the native protein enhance nucleation (49). The N-terminal fragment inhibits nucleation and stabilizes amorphous calcium phosphate in this condition. The significance of this protein in calcification is demonstrated by experiments with DMP1-deficient mice (50), in which calcification of dentin is significantly reduced. DMP1 is also expressed in osteocytes and is involved in the response to mechanical stress (51). Another function of this protein is related to chondrogenesis (52).

6. DENTIN PHOSPHOPROTEIN (DPP)

Dentin phosphoprotein (phosphophoryn, DMP2) is a highly phosphorylated protein unique to dentin (53, 54). It is the most abundant noncollagenous protein in dentin matrix. The amino acid composition of this protein is characterized by its extremely high content of phosphoserine and aspartic acid, with most of the serine residues being phosphorylated. This protein is synthesized as a multicomponent precursor, dentin sialophosphoprotein (DSPP) (55). DSPP is then cleaved into 3 fragments: N-terminal dentin sialoprotein (DSP), intermediate dentin glycoprotein (DGP) and C-terminal dentin phosphoprotein (DPP) (Figure 4) (56). This processing is performed by MMP-2 and MMP-20 (57) or possibly by autolysis (58).

DSP contains sequences rich in acidic amino acids (aspartic acid and glutamic acid). It also contains sialic acid-containing N- and O-linked oligosaccharides and chondroitin sulfate chains (59). DGP also contains N- and O-linked oligosaccharides (56). The N-terminal short segment of DPP (except for rat DPP) contains an RGD cell-attachment sequence. Most of the DPP sequence is occupied by so-called (DSS) repeat sequences $(Asp\text{-}Ser(P)\text{-}Ser(P))_n$ (55). These sequences may have a unique structure with a defined ridge of phosphate and carboxyl groups (60). Another motif found in this protein is an Asn-Ser(P)-Ser(P) motif. Variation in the length of the DSS repeat sequences is high among species, and even within a species, polymorphism of the length is observed (61). This polymorphism is one of the unusual characters of DPP.

DPP can bind a large number of calcium ions with modest affinity (Figure 5) (62-64). It adopts an unordered conformation in calcium-free conditions, but changes its conformation upon calcium binding (62). It forms complexes with calcium ions and precipitates in the presence of this ion (62, 65). The complex produced has been observed to consist of high molecular weight particles about 25 nm in diameter (66, 67). Protein conformation also depends on pH, assuming a folded conformation at acidic pH (68). DPP has affinity to hydroxyapatite crystals and binding depends on the phosphate groups (69). The binding is preferentially on the (100) face of the crystals (6), with the adsorbed protein adopting an extended conformation on the surface of the crystals (70). Morphologically, the adsorbed protein seems to form microspheres on the crystals (71).

DPP also has affinity to collagen (72), especially to the "e" band within the hole zone of collagen fibrils (73). By binding to collagen it retards the rate of fibrillogenesis (74), and the collagen fibrils formed in the presence of DPP are thicker than in its absence (75). The binding site is in the C-terminal segment (alpha1CB6) of the collagen molecule (76). After initial noncovalent association, the

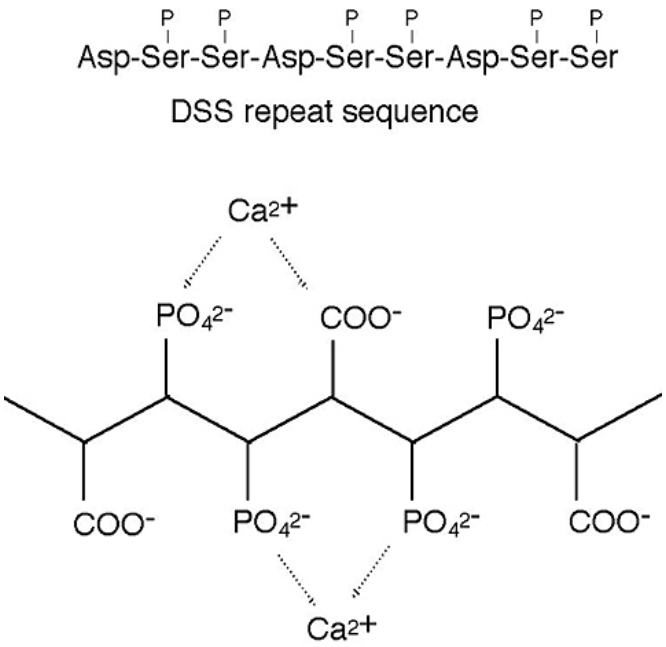

Figure 5. Characteristic sequences in dentin phosphoprotein. Most of the DPP molecule is composed of repetitive sequences, DSS repeats (above). A unit of this sequence consists of 1 aspartic acid and 2 phosphoserines. The DSS repeat sequences are rich in negative charges and provide binding sites for many calcium ions (below) and for hydroxyapatite crystals.

protein becomes covalently immobilized to the collagen (53, 76-79), especially to the C-terminal region (76). Formation of this covalent complex may be significant for collagen-based calcification. DPP is subjected to nonenzymatic degradation by beta-elimination of phosphoserine residues (80). It is also a substrate of transglutaminase and is crosslinked to form a high molecular weight complex by this enzyme (81).

The most interesting property of DPP is its ability to induce nucleation of hydroxyapatite *in vitro* (82). The effects are bidirectional, with the protein also having an inhibitory effect on crystal growth of the mineral (83). At low concentrations the inductive effect is predominant, but at high concentrations the inhibitory effect predominates (84). The protein forms a ternary complex with calcium and phosphate ions (63), and the resulting complex may transform into the nucleus of a crystal. Protein adsorbed on the surface of the crystals, on the other hand, may prevent crystal growth.

The role of this protein in calcification is demonstrated by the discovery of mutations of its gene in dentinogenesis imperfecta, a genetic disease of dentin (85, 86). The mutation results in a low level of calcification of dentin. The results have been confirmed by the DSPP gene-KO experiment (87). DSPP-null mice display hypomineralization of dentin, a widened predentin zone and an enlarged pulp chamber. These results indicate that DPP is necessary for normal calcification and development of dentin. This protein is also involved in cellular activities other than calcification. It activates the Smad pathway in mesenchymal stem cells (88), and suppresses inorganic phosphate-induced apoptosis of odontoblast-like cells (89). The expression of the DSPP gene is controlled by the transcription factor Runx 2 (90, 91). Although the function of DSP is not clear, it may be related to the early stage of dentin calcification.

DPP is a member of the SIBLING family of proteins (small integrin-binding ligand N-linked glycoproteins). This family contains DSPP, DMP1, BSP, MEPE (matrix extracellular phosphoglycoprotein), and osteopontin (92). These proteins share common features, although they do not have distinct sequence homology. They are phosphorylated glycoproteins rich in acidic amino acids. Most of them have an RGD cell-attachment sequence. They have affinity to hydroxyapatite crystals. The genes encoding these proteins have similar exon-intron structures. The genes are present as a gene cluster at the 4q22 site of human chromosome 4 (Figure 6). The SIBLING family belongs to the larger gene family, the SCPP (secretory calcium-binding phosphoprotein) family (93). In addition to the SIBLING family, the SCPP family contains caseins, salivary proteins (proline-rich proteins and statherin), and enamel proteins (amelogenin, enamelin and ameloblastin/sheathlin). These genes may have an evolutionary relationship (94). The ancestor of this gene family is possibly SPARCL1 (SPARC like protein 1), an extracellular matrix protein in nerve tissues.

7. CALCIFICATION

Biological calcification proceeds in two steps, involving nucleation followed by crystal growth, as does crystallization in general. Nucleation is the rate-limiting step; once nuclei are formed, their growth proceeds easily. There are two modes of nucleation, homogeneous

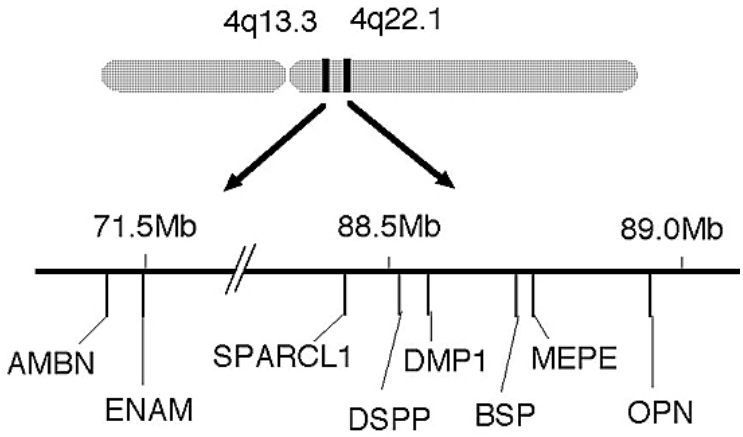

Figure 6. SIBLING-family genes. The SIBLING family is a family of acidic glycoproteins present in bone and dentin. The genes of this family are present as a gene cluster at the 4q22 site of human chromosome 4. SPARCL1: SPARC-like protein 1, DSPP: dentin sialophosphoprotein, DMP1: dentin matrix protein 1, BSP; bone sialoprotein, MEPE: matrix extracellular phosphoglycoprotein, OPN: osteopontin. Two genes of enamel matrix proteins are also present near this gene cluster; AMBN: ameloblastin, ENAM: enamelin.

nucleation and heterogeneous nucleation. In the former mode, nucleation occurs throughout the system. In the latter mode nucleation is induced only by nucleation-sites in a matrix. Nucleation occurs against an activation energy barrier. Clusters of the solid phase will continue to grow only if the energy required to form the new interface is overcome by the energy released by the phase transition. The activation energy can be reduced by lowering the interfacial energy. In the case of biological calcification, the lowering of the energy is provided by the inorganic-organic interaction with matrix macromolecules (95).

The interfacial energy can be reduced at the surface of a solid phase substrate. The energy at a solid-solid interface is generally lower than that at a solid-liquid interface. Collagen fibrils may therefore provide a semisolid surface for nucleation. High molecular weight aggregates of the dentin phosphoprotein-calcium complex are another example of a semisolid material (67). However the phase interface is not enough for efficient nucleation. Clusters of negative charges provide binding sites for calcium ions at the surface. These negative charges should be arranged in an array in order to provide suitable nucleation sites for hydroxyapatite. These charges can be provided by acidic matrix proteins such as SIBLING family proteins, some of which are immobilized on the surface of collagen fibrils by noncovalent or covalent interactions (72). Calcium ions accumulated on the acidic groups of the acidic matrix proteins can attract phosphate ions. The protein-calcium-phosphate ternary complex thus formed may lead to formation of crystal nuclei (Figure 7). The interaction of the acidic proteins with collagen fibrils is necessary to yield a configuration capable of orienting the acidic groups in the optimal way for nucleation (96).

Although the presence of clusters of acidic groups is important for their function, structural requirements may not be so strict for the acidic matrix proteins. Most of these proteins have an unordered conformation, and they can be classified as intrinsically disordered proteins (97). Proteins in this class can fit any unstructured surfaces. This flexible property is beneficial for recognition of clusters of inorganic ions. In the case of dentin phosphoprotein (DPP), even the length of the molecule is not determined (61). This variation in the length of the molecule allows further flexibility without altering its function, the induction of crystal nucleation. Charge density rather than molecular length may be critical for this function.

The acidic matrix proteins have affinity to hydroxyapatite crystals and inhibit their growth. This inhibitory effect is prominent when the acidic proteins are in a free form and not in association with collagens. Proteins adsorbed on the surface of the crystal can prevent access of free ions to the surface and/or prevent the propagation of the growing edge of the crystal. Most of the acidic proteins are adsorbed on a specific face of the crystal. For example, osteocalcin and DPP are preferentially adsorbed on the (100) face. This specific adsorption results in the inhibition of growth perpendicular to the adsorbed face. Consequently the area of the adsorbed face will be increased in comparison with the other faces. By this mechanism the proteins can control the crystal shape (Figure 8) (98). Outside the calcified tissues, the inhibitory mechanism functions to prevent unwanted calcifications, such as kidney stones and salivary stones. The disordered structure of the proteins may help recognition of the crystal surface. However osteocalcin has a helical conformation, and recognition of the crystal surface by this protein can be compared to the recognition of ice crystals by antifreeze proteins.

8. SUMMARY AND PERSPECTIVE

Acidic matrix proteins in bone and dentin (osteocalcin, BSP, osteopontin, DMP1, dentin phosphoprotein) are calcium-binding proteins, which can

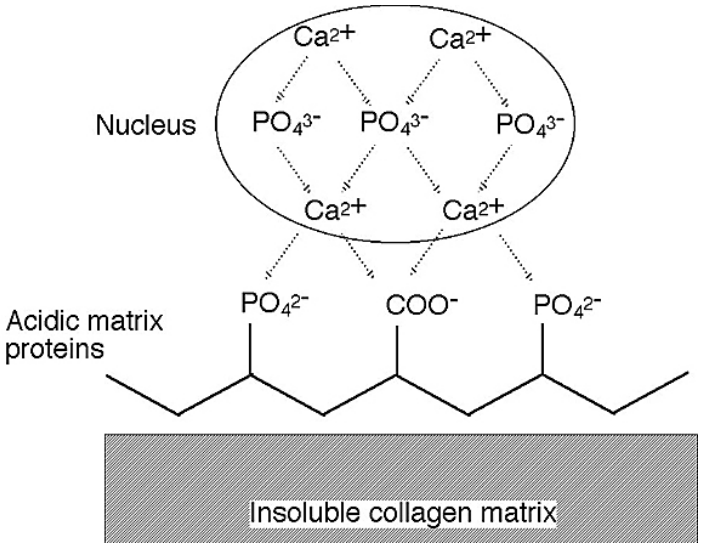

Figure 7. Nucleation of hydroxyapatite by acidic matrix proteins immobilized on insoluble collagen matrix. Some acidic matrix proteins, e.g. dentin phosphoprotein, have an affinity to collagen. The surface of the insoluble collagen matrix provides loci to reduce interfacial energy for nucleation. Calcium ions are bound to the acidic groups of the acidic proteins, and inorganic phosphates are attracted by the calcium ions. The ionic complex thus formed may constitute a crystal nucleus.

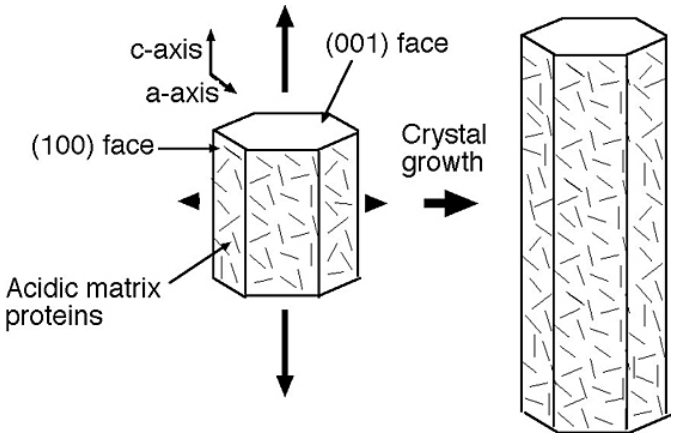

Figure 8. Control of crystal shape by the acidic matrix proteins. Some acidic matrix proteins, e.g. dentin phosphoprotein, have affinity to a specific face (e.g. the (100) face) of hydroxyapatite crystals. These proteins are potent inhibitors of crystal growth. Their specific adsorption results in the inhibition of growth perpendicular to the adsorbed face. For example, if the (100) face is covered by proteins, growth in the direction of the a-axis will be inhibited. As a result the crystal will preferentially grow in the direction of the c-axis.

affect tissue calcification. These proteins contain clusters of acidic amino acids, including phosphorylated amino acids, which constitute binding sites for calcium ions and hydroxyapatite crystals. Most of these proteins have an intrinsically disordered conformation. They activate nucleation of hydroxyapatite when immobilized on insoluble substrates. However they can also inhibit crystal growth when in a free state. In addition to their function in calcification, some of these proteins can support cell attachment and are involved in cell growth and differentiation.

Sites of organic-inorganic interaction are not restricted to proteins. Micelles of phospholipids, for example, also constitute such interaction sites. However a general principle is likely to be present in the interaction between these acidic molecules and inorganic ions. In future we will achieve a better understanding of the detailed mechanism of the interactions between the acidic matrix proteins and inorganic crystals, but a novel tool will be required to analyze the interactions. Such knowledge on the organic-inorganic interactions between the acidic proteins and calcium ions will help us to develop a method for artificial calcification in the regeneration of bones and

teeth, treatment of unwanted calcifications, or control of crystal formation in the field of inorganic engineering.

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Abbreviations: BGP: bone Gla protein; BSP: bone sialoprotein; DMP1: dentin matrix protein 1; DSP: dentin sialoprotein; DPP: dentin phosphoprotein; DSPP: dentin sialophosphoprotein; DGP: dentin glycoprotein; MEPE: matrix extracellular phosphoglycoprotein; SIBLING: small integrin-binding ligand N-linked glycoproteins; SCPP: secretory calcium-binding phosphoprotein

Key Words: Calcification, Extracellular matrix, Bone, Dentin, Phosphoprotein, Review

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