Fibers and extracellular matrix of hard tissues

Collagen and non-collagen proteins in hard tissues

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February, 2016
Radiograph of teeth –
remarkable harmony of organic and inorganic components
Possible consequence of disharmony
(Example: Oligodontia in a patient with hypohidrotic ectodermal dysplasia, HED)
Tooth development – harmony in regulatory and structure proteins

Epithelium

Oral ectoderm Otx2/Pitx2

Dental lamina Lef-1

BMP FGF

BMP FGF Shh Wnt

Dlx1 & 2, Msx1 & 2

Dental papilla

Condensed dental mesenchyme Msx1, Pax9 Gli1, -2, -3 Lef1

Enamel knot p21, Msx-2

BMP FGF Shh Wnt

Activin βA

Gli2 & 3

BMP FGF Activin

Mesenchyme

Odontogenic mesenchyme Lhx6, -7, Barx1 Msx1, -2 Dlx1, -2, Pax9
Inorganic components of bone

- Inorganic components:
  - Water (about 20%)
  - Minerals (45%)
    - Hydroxylapatite and other Ca-phosphate and Ca-carbonate salts (stores easily mobilized)
    - Trace elements (Zn, Cu, Sn, Mg, F)
Extracellular matrix of hard tissues

- **Inorganic components of bone**
  - Water (approx. 5-30%)
  - Minerals (approx. 45%, variable depending on the tissue)
    - Hydroxylapatite and other Ca-phosphate and Ca-carbonate salts (stores easily mobilized from bone)
    - Trace elements (Zn, Cu, Sn, Mg, F)

- **Organic components**
  - Structural proteins – collagenous and non-collagenous proteins (traces-30%, variable depending on the tissue)
  - Bioactive regulatory peptides - (very small quantity)
The extracellular matrix of hard tissues:

both the enamel, and the dentin and acellular cementum actually corresponds to cell free extracellular matrix.
• Most important protein components of bone and dentin:

Collagens

Non-collagen proteins in hard tissues
   Glucoproteins
   Proteoglycans
   Gla proteins
   Blood plasma proteins
   Phosphoproteins

• Most important components of enamel

amelogenin and enamelin
Collagen – three polypeptide chains forming a rope.
Types and distribution of collagen

<table>
<thead>
<tr>
<th>Type</th>
<th>Polypeptide composition</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$[\alpha_1(I)]_2 \alpha_2(I)$</td>
<td>Skin, bone, tendon, cornea, blood vessels</td>
</tr>
<tr>
<td>II</td>
<td>$[\alpha_1(II)]_3$</td>
<td>Cartilage, intervertebral disk</td>
</tr>
<tr>
<td>III</td>
<td>$[\alpha_1(III)]_3$</td>
<td>Fetal skin, blood vessels</td>
</tr>
<tr>
<td>IV</td>
<td>$[\alpha_1(IV)]_2 \alpha_2(IV)$</td>
<td>Basement membrane</td>
</tr>
<tr>
<td>V</td>
<td>$[\alpha_1(V)]_2 \alpha_2(V)$</td>
<td>Placenta, skin</td>
</tr>
</tbody>
</table>
Structure of procollagen

Figure 2.2 The structure of the procollagen molecule showing the triple-helical central collagenous domain and the amino- and carboxy-terminal propeptides which are cleaved during the processing of procollagen to collagen.
Collagen structure and fibril formation
Specific cellular reactions in the biosynthesis of collagen
Overview of collagen biosynthesis

Polypeptide synthesis

→ Post-translational modifications

→ Procollagen triple helical cable

→ Secretion

→ Removal of extension peptides

→ Tropocollagen

→ Aggregation into microfibril

→ Cross-linking

→ Collagen fiber

Within RER and Golgi of fibroblast cell

Within extracellular spaces of connective tissue

Fig. 1. Overview of the biosynthesis of collagen.
Hydroxylation during collagen biosynthesis

Fig. 2. Formation of hydroxyproline and hydroxylysine.
Stages in collagen synthesis - rope formation
Conversion of lysine to allysine by lysyl oxidase

Fig. 5. Conversion of lysine to allysine by lysyl oxidase.
Stages in collagen synthesis - microfibril formation
Enzymatic cleavage of collagen by mammalian collagenases
## Collagen digesting enzymes

<table>
<thead>
<tr>
<th>Collagen form</th>
<th>Enzyme</th>
<th>Tissue location</th>
</tr>
</thead>
</table>
| 1. Insoluble polymeric collagen fibres | Depolymerases (including two specific proteases)  
Granulocyte elastase  
Granulocyte collagenase | Extracellular  
Neutral pH |
| 2. Native collagen fibres and molecules | Type-specific collagenases  
Granulocyte elastase  
Macrophage collagenase | Extracellular  
Neutral pH |
| 3. Large collagen peptides            | Numerous gelatinases  
Collagenolytic cathepsin  
Non-specific tissue proteases/peptidases | Intracellular acid pH    |
| 4. Final degradation                 | Endo- and exopeptidases  
PZ-peptidases | Extracellular fluid  
Intracellular |
Osteogenesis imperfecta  (OI)

OI can be caused by mutation in either the COL1A1 gene (120150) or the COL1A2 gene (120160),

with other words, by mutation of any of the two α chains in collagen type I
Non-collagen proteins in hard tissues

- Glycoproteins
- Proteoglycans
- Gla proteins
- Blood plasma proteins
- Phosphoproteins
- In enamel: amelogenin and enamelin
<table>
<thead>
<tr>
<th>Protein</th>
<th>Known function</th>
<th>Regulation of production by osteoblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin</td>
<td>Inhibits mineralization, recruits bone-cell precursors</td>
<td>1,25-(OH)(_2)D(_3), PTH, glucocorticoids</td>
</tr>
<tr>
<td>Osteonectin</td>
<td>Facilitates type I collagen mineralization, suppresses rate of hydroxyapatite crystal growth, modulates cell attachment and detachment</td>
<td>Glucocorticoids, TGF-β, IGF-1</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Cell-binding activity, osteoclast-anchoring activity, mineral-binding activity</td>
<td>1,25-(OH)(_2)D(_3), TGF-β, retinoic acid, glucocorticoids, PTH</td>
</tr>
<tr>
<td>Bone sialoprotein</td>
<td>Cell-binding activity</td>
<td>Glucocorticoids, 1,25-(OH)(_2)D(_3)</td>
</tr>
<tr>
<td>Bone proteoglycan (biglycan)</td>
<td>Function unclear</td>
<td>Not well characterized</td>
</tr>
<tr>
<td>Bone proteoglycan II (decorin)</td>
<td>Binds to collagen fibers, regulates fiber growth, binds/presents growth factors in matrix</td>
<td>Not well characterized</td>
</tr>
<tr>
<td>Thrombospondin</td>
<td>Binds and organizes matrix, cell attachment</td>
<td>TGF-β</td>
</tr>
<tr>
<td>Matrix gla-protein</td>
<td>Prevents growth plate mineralization</td>
<td>Retinoic acid, 1,25-(OH)(_2)D(_3)</td>
</tr>
<tr>
<td>Latent TGF-β1 binding protein-1</td>
<td>Storage of latent TGF-β1</td>
<td>1,25-(OH)(_2)D(_3)</td>
</tr>
</tbody>
</table>
Interactions between hydroxyapatite crystals and ionic substances

Figure 3.2 Diagram illustrating the reactive major constituent ions of the hydroxyapatite mineral (OH-Apatite) of calcified bone matrix. Ionic substances in the crystal environment have a high potential for interaction with the mineral.
Disorder scores of amino acid sequences of proteins participating in biomineralization
Disorder frequency of amino acid chains of proteins participating in various biological functions

![Box plot showing disorder frequency for different functional categories]

- Human all
- Secreted
- Ossification
- Biomineral tissue dev.
- Transcription

Significant p-values:
- p = 0.0004
- p = 0.0025
Suggested role of Intrinsicly Disordered Proteins (IDPs) in biomineralization

- Integrin binding
- Hyperglycosylation
- Collagen binding
- Induction of crystal growth in the presence of collagen matrix

IDPs in biomineralization

- High Serine content
- Extended conformation
- Binding to HAP crystal with induced folding on a specific crystal face

Hyperphosphorylation

- High Asp/Glu content
- High negative charge
- Binding high amount of calcium

Inhibition of crystal growth in the absence of collagen matrix
Sialic acid, a major constituent of sialoproteins

(a)

(b)

Figure 3.3 Formulae of the most common sialic acid, N-acetyllneuraminic acid, by the conventions of Fischer (a) and Haworth (b). Neuraminic acid is substituted at the amino group by an acyl residue, the most common being an acetyl group, CH_3CO^-, as shown here but often by a glycolyl residue, HOCH_2CO^-.
**Composition of bovine bone sialoprotein**

**Table 3.1 Composition of bovine bone sialoprotein**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>(% w/w)</th>
<th>(moles/23 000 g)</th>
<th>Constituent</th>
<th>(% w/w)</th>
<th>(moles/23 000 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialic acid</td>
<td>20.7</td>
<td>15.3</td>
<td>Aspartic acid</td>
<td>8.80</td>
<td>15.2</td>
</tr>
<tr>
<td>Galactose</td>
<td>7.9</td>
<td>10.0</td>
<td>Threonine</td>
<td>5.02</td>
<td>9.7</td>
</tr>
<tr>
<td>Mannose</td>
<td>2.4</td>
<td>3.0</td>
<td>Serine</td>
<td>3.20</td>
<td>7.0</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>4.4</td>
<td>5.6</td>
<td>Glutamic acid</td>
<td>12.80</td>
<td>20.0</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>4.4</td>
<td>5.6</td>
<td>Proline</td>
<td>2.15</td>
<td>4.3</td>
</tr>
<tr>
<td>Fucose</td>
<td>0.7</td>
<td>1.0</td>
<td>Glycine</td>
<td>3.49</td>
<td>10.7</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.9</td>
<td>2.2</td>
<td>Alanine</td>
<td>1.36</td>
<td>3.5</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.2</td>
<td>4.3</td>
<td>Valine</td>
<td>1.32</td>
<td>2.6</td>
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<tr>
<td>Lysine</td>
<td>1.59</td>
<td>2.5</td>
<td>Isoleucine</td>
<td>1.25</td>
<td>2.2</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.74</td>
<td>1.1</td>
<td>Leucine</td>
<td>1.43</td>
<td>2.5</td>
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<tr>
<td>Arginine</td>
<td>0.76</td>
<td>1.0</td>
<td>Tyrosine</td>
<td>1.58</td>
<td>2.0</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>0.81</td>
<td>1.1</td>
<td>Phenylalanine</td>
<td>0.72</td>
<td>1.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Tryptophan</td>
<td>0.89</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ammonia</td>
<td>1.07</td>
<td>14.5</td>
</tr>
</tbody>
</table>

**Most important proteins of the group: osteopontin, bone sialoprotein**
The most important glucose-aminoglycans
Structure of proteoglycans

'Most important proteoglycans in mineralizing tissues:
- decorin, biglycan
Formation of $\gamma$-carboxyglutamyl residues

Figure 3.4 Vitamin K-dependent carboxylation of a glutamic acid in peptide linkage (glutamyl residue) to form a $\gamma$-carboxyglutamyl residue. (From Esnouf, M. P. (1984) Biochemistry of blood coagulation. In Anticoagulants and Myocardial Infarction: A Reappraisal, edited by T. W. Meade, p. 29. Chichester, John Wiley & Sons Ltd. with permission)
Structural basis of interactions between mineral phase and Gla protein osteocalcin (BGP)

Most important ones in mineralizing tissues:

- osteocalcin, matrix Gla protein
Blood plasma proteins

- Albumin

- $\alpha_2$HS-glycoprotein
  synthesized by the liver
  concentrated in dentin
  unknown role
The most important amino acids in hard tissue phosphoproteins

Most important members of the group
- Osteopontin, osteonectin and SPARC in bone
- Phosphophorine in dentin

Figure 3.9 Chemical structures of (a) phosphoserine, (b) phosphothreonin
Dentin phosphoprotein (DPP), a highly acidic protein, is the major noncollagen component of dentin, being solely expressed by the ectomesenchymal derived odontoblast cells of the tooth. Takagi and Sasaki (1988) suggested that a deficiency of this protein is a causative factor in dentinogenesis imperfecta (DGI1; 125490). MacDougall et al. (1997) demonstrated that 2 major noncollagenous dentin matrix proteins, dentin sialoprotein (DSP) and dentin phosphoprotein (also known as phosphophoryn) are encoded by a single gene termed dentin sialophosphoprotein (DSPP).
Involvement of hard tissue proteins in mineral formation

Figure 3.11 Diagram illustrating the possible involvement of phosphophoryns in the nucleation of mineral formation and the growth and structure of mineralized dentine. Od, odontoblast; PD, predentine; MF, mineralization front; D, dentine; PPr, phosphophoryns; Sp, secreted proteins including collagen type I and non-collagenous proteins; C, collagen; BPPr, mineral-bound phosphophoryn
Some possible functions of proteins of hard tissue matrices affecting mineralization
Integrin binding

Hyperglycosylation

Collagen binding

High Serine content

IDPs in biomineralization

Extended conformation

Hyperphosphorylation

High Asp/Glu content

High negative charge

Binding high amount of calcium

Binding to HAP crystal with induced folding on a specific crystal face

Inhibition of crystal growth in the absence of collagen matrix

Induction of crystal growth in the presence of collagen matrix
Enamel, the outermost covering of the teeth and the hardest tissue in the body, contains both enamelin (606585) and amelogenin. Amelogenins are highly conserved proteins secreted by ameloblasts, and constitute 90% of the enamel organic matrix. As the proteins are digested and removed, the mineral crystals grow in well-organized prism patterns.

By Southern blot analysis, Lagerstrom et al. (1991) demonstrated a deletion extending over 5 kb of the amelogenin gene (300391.0001) in males with the hypomineralization form of amelogenesis imperfecta. Carrier females were heterozygous for the molecular defect which appeared to include at least 2 exons of the gene. The extent of the deletion was verified by polymerase chain reaction (PCR) analysis.
Current concept of the role of amelogenins in the mineralization of enamel

Fig 3-6 Current concept of the role of amelogenins in the mineralization of enamel. The hydrophobic amelogenins form globular aggregates (nanospheres) on secretion into the extracellular space. The nanospheres form lattices that regulate the spacing and the orientation of the C-axis of the newly forming enamel crystallites. (Adapted from Fincham et al51 with permission from Elsevier Science.)
Proteins of enamel involved in AI

Amelogenin: (product of AMELX and AMELY genes located on the X and Y chromosomes) is the most abundant protein in developing enamel [26, 27]. While its exact role in enamel formation is not fully understood, it is thought to be crucial for regulating the size and shape of the mineralizing enamel crystallites. Multiple human mutations in the AMELX gene are associated with different AI types. There are no known AMELY mutations. A transgenic mouse lacking expression of this gene has only a very thin covering of enamel that lacks a prismatic structure [28].

Ameloblastin: (product of AMBS gene located on chromosome 4) is another enamel associated protein that appears to be the second most abundant enamel matrix protein [29]. The function of this protein is not completely known but it may regulated ameloblast differentiation and formation. It is considered a likely candidate for being associated with some AI types.

Enamelin: (product of ENAM gene located on chromosome 4) is secreted by ameloblasts in relatively low amounts. It is speculated that this protein could interact with amelogenin or other enamel matrix proteins and be important in determining growth of the length of enamel crystallites. Three different mutations ENAM gene mutations are associated with different AI types.

Enamelysin: (MMP20 gene located on chromosome 11) is a proteinase that cleaves amelogenin and is thought to be the major proteinase involved in processing the enamel matrix proteins [32, 33]. The enamelysin knockout mouse has a reduced enamel thickness and the enamel lacks a prismatic structure.

Kalikrein 4: (KLK4 gene located on chromosome 19) is a proteinase that is secreted predominantly during the maturation stage of enamel development [34]. This aggressive proteinase could be responsible for processing any proteins not cleaved by enamelysin.
Summary

• Most important protein components of bone and dentin:

  Collagens

  Non-collagen proteins in hard tissues
    Glucoproteins
    Proteoglycans
    Gla proteins
    Blood plasma proteins
    Phosphoproteins

• Most important components of enamel

  amelogenin and enamelin