On the Possible Relation between Morphology and Precursors of the Crystallities in Calcified Tissues

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Crystal structure data on hydroxyapatite, octocalcium phosphate and brushite have been used in order to predict their crystal morphology on the basis of the Hartman-Perdok theory. The predicted forms are pencil-like for hydroxyapatite, board-like for octocalcium phosphate and flattened needle-like for brushite. Although the biominerals in bone, dentine and tooth enamel have an apatite structure, their form is not pencil-like. This may partially be due to the fact that precursor phases are nucleated first in these tissues during mineralization and that these precursor phases are transformed later by topotactical reactions into compounds with apatite structure or that they serve as nuclei for ongrowth of apatite. The form of the mineral particles in mature bone and dentin is board-like which indicates that octocalcium phosphate might be their precursor phase. However, the form of the crystals in mature enamel is flattened needle-like which indicates that brushite is their precursor phase. It is argued that a possible difference in the nature of the precursor phase may be due primarily to differences in the cellular activities of the odontoblasts and osteoblasts as compared to those of the ameloblasts, and secondarily to matrix effects. In both cases, however, the main effect of the matrix seems to be that it acts as a mechanical barrier leading to a limited form and size and, of course, a certain orientation of the crystals, rather than as an agent for heterogeneous nucleation. The validity of the present considerations depends as yet on the assumption that the ions and molecules occurring in body fluids do not dominate the habit of calcium phosphate crystals.

Introduction

It is known since a long time [1, 2] that the crystal structure of the mineral in bone, dentin and enamel is apatite. However, the form of the crystalline particles in these calcified tissues is far from the mostly rod-like form reported for apatite minerals. These minerals are most frequently characterized by a hexagonal cross-section and a flat top face, sometimes rounded off by some less important faces on the edges [3].

The discrepancy between the morphology observed from electron microscopy and that expected on the basis of the symmetry of the space group might be explained by the fact that in calcified tissues other calcium phosphates occur as precursor phases. Such a precursor might be transformed into apatite later on by topotactical reactions. Brown [4] has proposed octocalcium phosphate as a possible precursor in this sense. The precursor might also serve as a nucleus for ongrowth of apatite later on. In this sense Francis and Webb [5] have proposed brushite as a possible precursor.

In case of a precursor phase which is transformed into apatite or which serves as a nucleus for ongrowth of apatite, the form of the final crystallites should still resemble that of the original phases, being either octocalcium phosphate-like or brushite-like. Recently, the forms expected for such crystals were derived from several theories and compared with experimental studies on these compounds when synthesized *in vitro* [15–18]. This paper is intended

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to compare these predicted forms as reviewed in the section "Procedure" with the crystallite forms reported for calcified tissues as reviewed in the section "Experimental Results". This enables a "Discussion" of the physiological conditions controlling the calcified tissues during their mineralization.

Procedure

Several theoretical considerations have been used to predict the morphological importance of a crystal face hkl in the form and shape of mineral particles. According to the classical law of Bravais-Friedel [6] this morphological importance decreases with decreasing interplanar spacing d_{hkl}. Donnay and Harker [7] extended this theory by taking into account screw axes and glide planes. A later extension by Donnay and Donnay [8] takes also into account pseudosymmetric features.

Hartman and Perdok [9–12] introduced the concept of periodic bond chains and so were able to predict the relative growth rates of different crystallographic forms hkl ([12], see also [13, 14]). Terpstra *et al.* [15–17] applied these theories to predict the form of apatite and octocalcium phosphate crystals, whereas Simon and Bienfait [18] predicted the form of gypsum crystals. As the structure of gypsum and that of brushite are identical, the prediction for the form of gypsum crystals holds also for brushite. The predicted forms are given schematically in Fig. 1. Brushite crystals should have the form of a flat needle, octocalcium phosphate crystals should look like a short board, whereas apatite crystals should look like a short hexagonal pencil when the periodic bond chains in the crystals would be dominant in determining the crystal shape. These theoretical predictions are compared with experimental results for the mineral particles in the calcified tissues as reported in the literature in the next section.

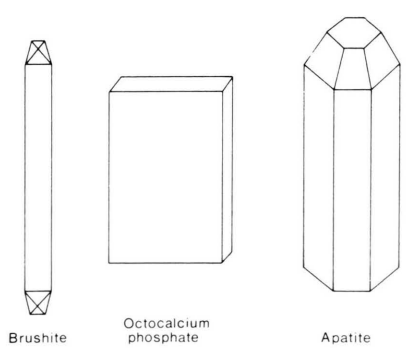

Fig. 1. Shape of some calcium phosphate crystals as predicted from their crystal structure according to the periodic bond chain theory.

Experimental Results

Transmission electron microscopy having a resolution of 0.5 to 10 nm is probably the most reliable method to determine the size and shape of the crystallites in calcified tissues. With this method Robinson [19] found an average crystallite size in human bone of $50 \times 25 \times 10$ nm. In a later study Robinson and Watson [20] reported $40 \times 20 \times 5$ nm. Several investigators reported rod-like in stead of plate-like crystals [21]. However, Steve-Bocciarelli [22] showed by using a tilting specimen stage that rod-like images were actually edge views of plate-like structures. He found further a thickness of 4 nm for the plates and on some plates an angle of 90° was observable, indicating the form of a short board. Jackson *et al.* [23] found a thickness of about 5 nm for the crystals in rabbit, ox and human bone, whereas the *c*-axial lengths were about 35 nm. In conclusion, the typical dimensions of the crystallites of bone mineral are probably about $35 \times 20 \times 5$ nm. Hence, their form looks like a short board with the *c*-axis of the apatite structure being parallel to the length of the board. This indicates that octocalcium phosphate might be the precursor phase in bone, whereby the apatite phase may be formed by topotactical reaction of the OCP precursor.

For dentin Johansen and Parks [24] reported a length of up to 100 nm and a thickness of 3 ± 1 nm. Voegel and Frank [25] reported a width of 36 ± 2 nm and a thickness of 10 ± 3 nm. Daculsi *et al.* [26] found a width of 30 ± 4 nm and a thickness of 3 ± 0.5 nm. In conclusion, the typical shape of dentin crystals is probably about $100 \times 35 \times 5$ nm. Their crystallographic *c*-axis is parallel to their length. This also indicates that octocalcium phosphate might be the precursor phase in dentin.

Kerebel *et al.* [27] studied the thickness and width of the crystallites in human enamel. In the mature stage the thickness is 26 ± 2 nm and the width 68 ± 13 nm. The first crystallites observable during mineralization have a thickness of not more than 1.5 nm and a width of 15 nm. Later, Daculsi *et al.* [28] showed that crystals in mature enamel are at

least 100 μm long and that they obtain their length by intergrowth of units with smaller lengths. Hence, the average size of the crystals in mature enamel is about $100{,}000 \times 70 \times 25$ nm. This form is that of a flat needle with the crystallographic *c*-axis parallel to its length and thus indicates that brushite might be the precursor in tooth enamel.

Discussion

For bone there is also a lot of chemical evidence that octocalcium phosphate is the precursor phase. According to Driessens and Verbeeck [29] this precursor is continuously formed during bone turnover. However, it is not stable in bone extracellular fluid, but it is transformed within about one month into a mixture of the following three phases: a small amount of a whitlockite with the formula

$$Ca_9\,Mg\,(HPO_4)\,(PO_4)_6 \tag{1}$$

probably being amorphous to X-ray diffraction, a sodium and carbonate containing apatite with the formula

$$Ca_{8.5}\,Na_{1.5}\,(PO_4)_{4.5}\,(CO_3)_{2.5} \tag{2}$$

and a calcium deficient, heavily carbonated apatite with the formula

$$Ca_9\,(PO_4)_{4.5}\,(CO_3)_{1.5}\,(OH)_{1.5} \tag{3}$$

which are all known from *in vitro* syntheses. The two apatites may occur as domains in the same apatitic crystals. Each of these three phases has a solubility much lower than that of octocalcium phosphate [30] but new octocalcium phosphate is continuously formed due to bone turnover.

In dentin no turnover occurs, but if also in this case octocalcium phosphate is the precursor, it should be expected that this is transformed into a mixture of the same three phases (1) through (3). This has indeed been found [31].

Indirect chemical evidence for the fact that the precursor in enamel is different from octocalcium phosphate may be derived from the fact that the phase composition of the mineral of mature enamel is quite different from that of the mineral in bone and dentin [31–33]. Although there is an apatite of formula (2) in tooth enamel, the other phases are a dolomite with the formula

$$Ca\,Mg\,(CO_3)_2 \tag{4}$$

(in an amount not detectable by physical means, see ref. [33]) a calcium deficient apatite of the formula

$$Ca_9\,(HPO_4)\,(PO_4)_5\,(OH) \tag{5}$$

and the well known hydroxyapatite

$$Ca_{10}\,(PO_4)_6\,(OH)_2 \tag{6}$$

Again the three apatites can occur as domains in the same apatitic crystals.

An explanation for the difference in the nature of the precursor in dentin and tooth enamel may be derived as follows from data on cellular activities of the odontoblasts and ameloblasts during mineralization. From tissue culture technics it is known [34] that carbonic anhydrase and alkaline phosphatase which increase the local inorganic phosphate concentrations, are active both in dentin organ and in enamel organ. However, there seems to be active calcium transport mainly in the secretory ameloblasts and not in the odontoblasts. This means that the pulpaorgan provides continuously inorganic phosphate ions to the mineralizing dentin, whereas by the enamel organ apart from the inorganic phosphate ion transport also calcium will be transported into the mineralizing enamel.

The physicochemical consequences of these cellular actions can be visualized as follows. The solubility isotherms of the relevant compounds are given in a plot of log I_{OHA} *versus* pH in Fig. 2. In this plot log I_{OHA} is the logarithm of the ionic activity product for hydroxyapatite OHA. (The isotherm for dentin mineral applies to the phases (1) through (3).) The position of the body fluids in such a plot is given by point A [35]. Let us consider first dentino-genesis and let us assume that odontoblast activity leads to an increase of the inorganic phosphate concentration in the local dentinal fluid by 50%. Then the position of the dentinal fluid is given by point B. Hence, the odontoblasts activity makes the dentinal fluid supersaturated with OCP, but it is still undersaturated with brushite. Hence, OCP can be the precursor in dentin.

On the other hand, the ameloblast activity increases not only the inorganic phosphate concentration in the enamel fluid, but also the ionized calcium concentration. If we may assume that both increases amount to about 50%, then enamel fluid reaches position C in Fig. 2. Hence, enamel fluid will become not only supersaturated with OCP, but even with brushite. Therefore, brushite can be the precursor in enamel.

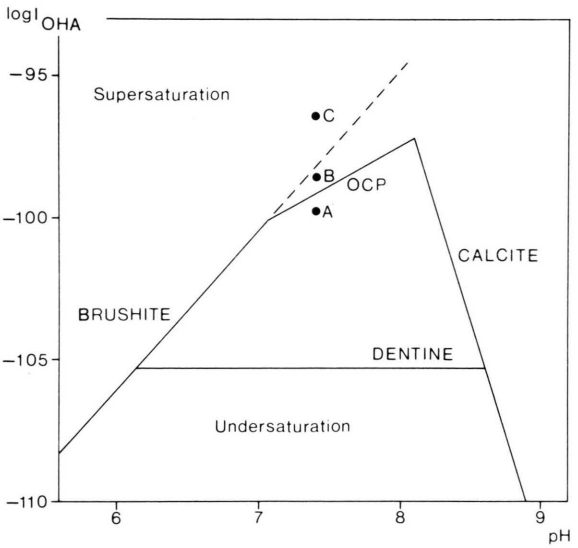

Fig. 2. Isotherms of brushite, octocalcium phosphate OCP, calcite and dentin mineral in a plot of the logarithm of the ionic activity product of hydroxyapatite log I_{OHA} *versus* pH. Point A is the position of normal body fluids under physiological conditions, point B the presumed position of the fluids in mineralizing bone and dentin and point C the presumed position of the fluid in the mineralizing enamel organ.

An important question with respect to biominerals is the function of the organic matrix in their formation. The physicochemical meaning of the above data about OCP being a precursor in bone and dentin suggests that collagen probably acts primarily as a mechanical barrier leading to a limited form and size (and thus of a particular orientation) of the crystals [36] rather than as an agent promoting heterogeneous nucleation [37–42]. The function of enamel matrix is probably more complex as, due to the fact that only brushite nuclei are formed, this matrix must at least inhibit the formation of OCP nuclei from the enamel fluid which is supersaturated both with OCP and with brushite. Certainly, the enamel matrix allows for the formation of the needle-like brushite crystals [43–45] in probably the same way as collagen allows for OCP nucleation, *i.e.* it acts primarily as a mechanical barrier leading to a limited form and size of the crystals [43] rather than as an agent promoting the heterogeneous nucleation of brushite. In any way, the most predominant role of the matrix seems to be that of a spatial constriction and orientation.

Some caution about the conclusions of this paper is justified. It is known that the specific adsorption of solute molecules [18] or of foreign ions like Mg^{2+}, CO_3^{2-}, etc. as they occur in body fluids may change the form of the crystals to a shape different, from what may be expected from periodic bond chain theory. What all the ions occurring in body fluids do to the habit of calcium phosphate crystals is unknown at the moment. Further, it must be realized that the crystals in calcified tissues are mostly very small so that their faceting is much less precise than that predicted from the periodic bond chain theory. For this reason comparison of theory and practice about crystal forms on the basis of faces with secondary importance is not possible.

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