Evidence for Intermediate Metastable States during Equilibration of Bone and Dental Tissues

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Experimental data were collected on the solubility equilibrium of the mineral of bone, tooth enamel and calcium phosphate renal stones. Evaluation in the form of potential diagrams of $Ca(OH)_2$ versus H_3PO_4 shows that Ca/P ratios of 1, 4/3, 10/7, 3/2 and 5/3, related to phases like brushite, octocalcium phosphate, whitlockite, defective apatite and hydroxyapatite respectively can be important. These facts allow the interpretation that many of these calcium phosphates are present simultaneously in biominerals or that they are formed during the equilibration.

Introduction

In a previous paper [1] it was shown that variations in the composition of bone and tooth mineral are consistent with the model that the constituents are a mixed microcrystalline apatite (AP) – octocalciumphosphate (OCP) like phase and an amorphous or submicrocrystalline calcium phosphate (ACP) like phase whereby these phases can occur in different proportions. The occurrence of several in stead of one calcium phosphate phase and the incorporation of several foreign ions in these phases are probably related with the fact that the internal medium is always heavily supersaturated with respect to pure hydroxyapatite whereas yet this compound does not form under physiological conditions. For the apatite phase it was shown [1] that the incorporation of Na^+ and CO_3^{2-} ions into the crystal structure is the main cause for shifts in the solubility product to higher values as compared to pure hydroxyapatite. For the OCP and ACP phase other ions are thought to play this role. If both the effect of crystal size and that of incorporation of foreign ions are taken into consideration, it can be presumed that calcified tissue minerals are in momentary physico-chemical equilibrium with the body fluids bathing them [2].

Reversely, if calcified tissue minerals are equilibrated in aqueous solutions the final composition of the aqueous solution may indicate which of their calcium phosphates predominantly controls the solubility behaviour. One of the purposes of this paper is to show how this can be checked. From the point of solubility behaviour it has been made clear [3] already that the presence of Na^+ and CO_3^{2-} ions in an equilibrated calcium phosphate system is sufficient to cause supersaturation with respect to hydroxyapatite and even to explain the simultaneous presence of apatite (AP), brushite (BR) and/or OCP in such a system. However, in general only one of these phases controls at a given moment the solubility behaviour of such a mixture of solid phases in a certain environment.

Recently, we found that these mixtures of calcium phosphate phases are always supersaturated with pure hydroxyapatite, but undersaturated with the calcium phosphate phase (either BR or OCP or tricalciumphosphate (TCP) whitlockite?) which controls their solubility behaviour [4]. Expressed in terms of the negative logarithm of the ionic product of hydroxyapatite pI_{OHA} versus pH the solubility areas controlled by the different calcium phosphates are illustrated in Fig. 1.

In area A, B, C and D the calcium phosphates BR, OCP, TCP and AP respectively can be controlling the solubility behaviour often, although there is some overlap between the areas depending on the history of the calcium phosphate samples. Also there is possible a certain shift of these areas parallel to the shifts of the borderlines, indicated as a function of the Ca/P ratio in the aqueous solution in Fig. 2 for

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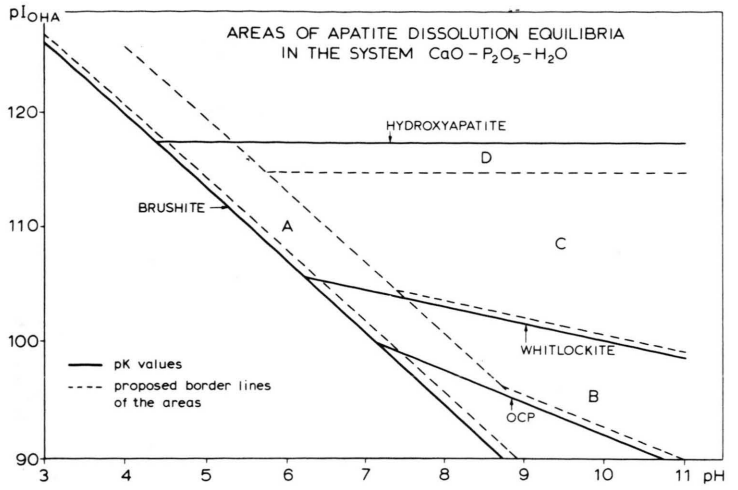

Fig. 1. Areas of metastable solid/solution equilibria found in the system $CaO-P_2O_5-H_2O$ as a function of pH and the negative logarithm of the ionic product for hydroxyapatite $pI_{OHA} = 10\ pCa + 6\ pPO_4 + 2\ pOH$. Solid lines represent the solubility product constants for the indicated calcium phosphates. The approximate borders for the areas of metastable equilibrium are given by dashed lines: A, brushite like phase; B, OCP like phase; C, whitlockite phase and D, apatite like phase.

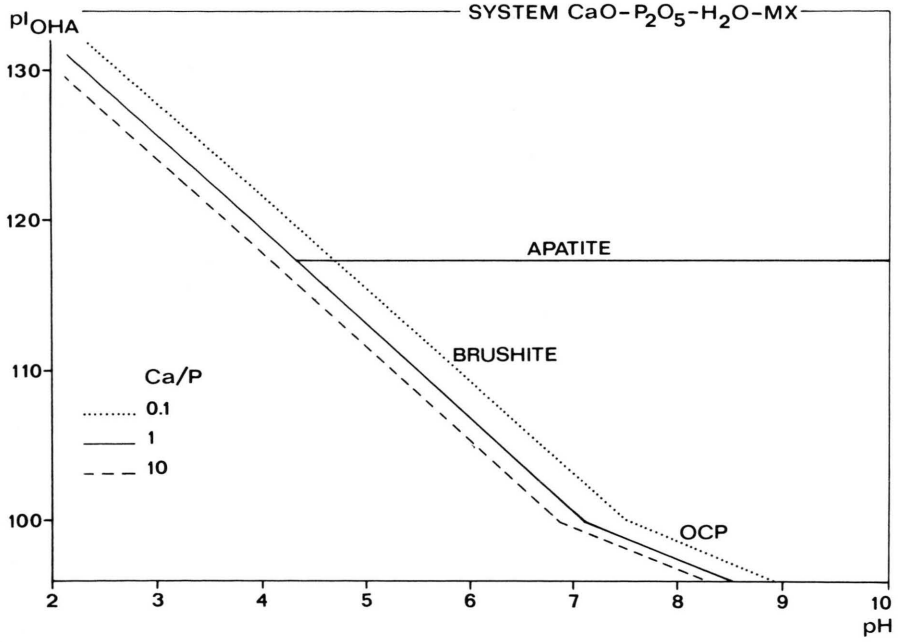

Fig. 2. Representation of the solubility products of apatite, brushite and octocalciumphosphate (OCP) in the quaternary system $CaO-P_2O_5-H_2O-MX$ as a function of Ca/P ratio in the aqueous solution, of pH and of the negative logarithm of the ionic product of hydroxyapatite pI_{OHA}.

the system $CaO-P_2O_5-H_2O-MX$. Here MX is defined as an indifferent electrolyte necessary to attain the indicated Ca/P ratio in the aqueous solution [3]. The possible meaning of Figs. 1 and 2 for the physiology of bone and tooth mineral will also be discussed in this paper.

Interpretation of solubility data

The most direct check on which calcium phosphate controls the solubility behaviour of a mixture of calcium phosphates as can occur in biominerals is formed from a chemical potential diagram [4]. Suppose that a calcium phosphate with general composition

$$Ca_x(PO_4)(H^+)_y(OH^-)_z \cdot n\ H_2O \qquad (1)$$

comes into equilibrium with an aqueous phase through:

$$Ca_x(PO_4)(H^+)_y(OH^-)_z \cdot n\ H_2O + m\ H_2O \leftrightarrows x\ Ca(OH)_2 + H_3PO_4 \qquad (2)$$

where $y = 3 - 2x + z$ and $m = 2x - z - n$. The thermodynamic potentials of H_2O, $Ca(OH)_2$ and H_3PO_4 in solution can be determined experimentally in this system:

$$\mu_{H_2O} = \mu^0_{H_2O} + RT \ln (H^+)(OH^-) \quad (3)$$

$$\mu_{Ca(OH)_2} = \mu^0_{Ca(OH)_2} + RT \ln (Ca^{2+})(OH^-)^2 \quad (4)$$

$$\mu_{H_3PO_4} = \mu^0_{H_3PO_4} + RT \ln (H^+)^3 (PO_4^{3-}). \quad (5)$$

The brackets represent the molar activity in solutions of the species which they enclose. If G_2 is the free energy of the calcium phosphate represented by Eqn (1) the following relation holds:

$$\mu_{Ca(OH)_2} = -1/x\, \mu_{H_3PO_4} + 1/x\, (G_2 + m\, \mu_{H_2O}). \quad (6)$$

Substitution of Eqns (4) and (5) into (6) and rearrangement gives

$$\log (Ca^{2+})(OH^-)^2 = -1/x \log (H^+)^3 (PO_4^{3-}) + K \quad (7)$$

where K is constant as long as G_2 and x are constant and where x represents the Ca/P ratio of the calcium phosphate. So, for brushite $x = 1$, for hydroxyapatite $x = 5/3$, etc.

Accordingly, when a double-logarithmic plot of the activities

$$a_{Ca(OH)_2} = (Ca^{2+})(OH^-)^2$$

and

$$a_{H_3PO_4} = (H^+)^3 (PO_4^{3-})$$

obtained from solubility studies is made, a straight line (or pieces of straight lines) is (are) expected with a slope reflecting the Ca/P ratio of the calcium phosphate that controls the solubility behaviour. A number of literature data on the solubility of biological minerals [5–17] was evaluated in this way. The results are summarized in Table I.

Results

Table I shows that the solubility of the mineral of surviving human bone is probably governed by a brushite-like phase with Ca/P = 1. However, dead bone reacts differently. The solubility of bone of calfs, children and rats is probably controlled by an OCP like phase with Ca/P = 4/3, whereas that of human adults tends to be controlled by an apatite with Ca/P = 5/3.

The data for human tooth enamel are scattered over a wide range. There seems to be variation from Ca/P = 1 to 1.5. However, by plotting these data in the form of a histogram as given in Fig. 3, it is observed that the distribution is bimodal with peaks at Ca/P = 0.96 ± 0.04 and Ca/P = 1.42 ± 0.08. The first number is related apparently to a brushite-like phase, but the second number could – within the limits of error – be related with either OCP or whitlockite or defective apatite at Ca/P = 4/3, 10/7 and 3/2 respectively [3].

Finally, the data about renal calculi [15–17] indicate that from the side of the calculi and in the

Table I. Ca/P ratio's of calcium phosphates controlling the solubility behaviour of biological minerals, as derived from chemical potential diagrams.

Mineral	Reference	Equilibration medium	Ca/P	pH range
human surviving bone	[5]	natural	0.80 ± 0.28	6.8 – 7.8
calf bone	[6]	synthetic ultrafiltrate	1.35 ± 0.09	6.2 – 7.8
child bone	[7]		1.35 ± 0.08	6.6 – 7.4
adult bone	[7, 8]		1.62 ± 0.08	6.6 – 7.4
rat bone	[7]		1.23 ± 0.09	6.6 – 7.4
human tooth enamel	[9]	saliva + lactic acid	0.95 ± 0.02	4.2 – 7.2
human tooth enamel	[10]	acetic acid buffer	0.78 ± 0.23	3.8 – 6.0
human tooth enamel	[11]	saliva (series 10)	0.86 ± 0.05	4.5 – 6.3
human tooth enamel	[11]	saliva (serie 1)	0.97 ± 0.10	6.5 – 8.3
human tooth enamel	[12]	acetic acid buffer	1.12 ± 0.16	4.5 – 6.2
human tooth enamel	[13]	$NaCl + KHCO_3$	1.34 ± 0.12	5.4 – 7.3
human tooth enamel	[13]	$KCl + NaCl$, $CaCl_2Na_2HPO_4$	1.01 ± 0.56	7.2 – 8.8
human tooth enamel	[13]	KCl, NaCl, Na_2HPO_4	1.51 ± 0.16	7.0 – 8.5
human tooth enamel	[14]	H_3PO_4	1.42 ± 0.08	4.5 – 7.6
apatitic renal calculi	[15]	TRIS buffer	1.32 ± 0.05	7.0 – 8.2
commercial apatite	[16, 17]	urine	1.01 ± 0.20	5.3 – 8.0

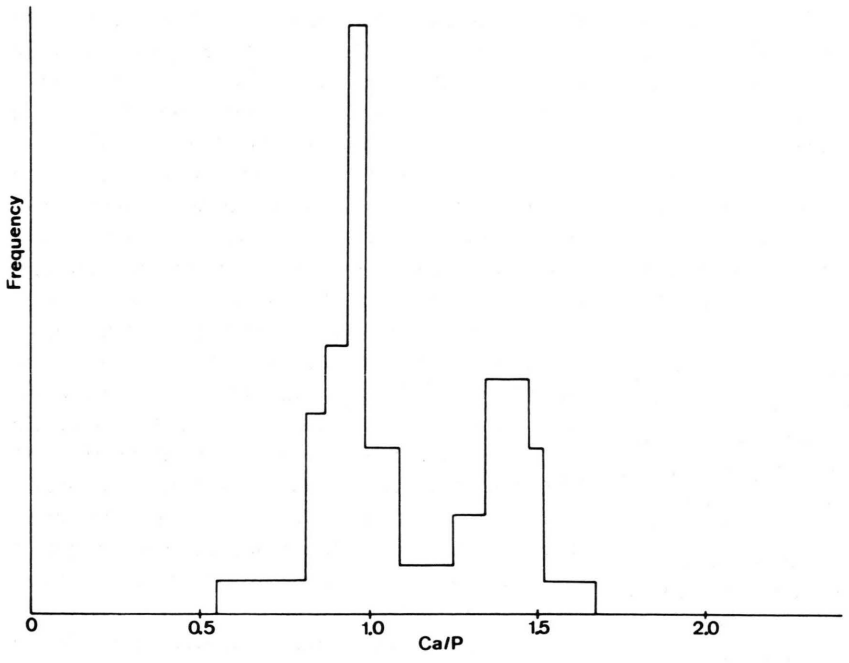

Fig. 3. Histogram of the Ca/P ratio's apparent from potential diagrams of solubility studies on tooth enamel mineral.

high pH range OCP seems to be the governing phase, whereas from the urine side a brushite-like phase is controlling, especially in the lower pH range.

Discussion

Solubility data form only part of the evidence for or against the occurrence of more than one calcium phosphate phase in calcified tissues and pathological calcifications. The results of studies to determine the crystal structure directly should be compared with those derived from the solubility data.

As far as the mineral of tooth enamel is concerned, mineral phases other than apatite are thought to be present to only a small percentage of the total enamel mass, if at all. [18]. OCP accounts for less than 2% of fully developed enamel mineral from the structural point of view [19] despite its possible importance as an intermediate phase in the formation of enamel apatite [20]. ACP has not been observed in enamel [18]. Enamel crystallites are an order of magnitude larger than apatite-like particles found in other mineralized vertebrate tissues like bone and dentin so that their identification as an apatite phase can be made without any technical difficulty.

Yet, according to the present study the solubility behaviour of this mineral shows up apparent Ca/P ratios ranging from 1 to 1.5. This can be explained by assuming that certain surface layers like those of OCP or brushite do not only control the growth of biological apatites [20, 21], but also the dissolution of those crystals. This would also explain the fact that these compounds are mostly not detectable as separate solid phases. A necessary condition though, is a good epitaxy of these surface layers on to certain crystal faces of the apatite. This has been established for the OCP [20] and the brushite [21] structure. On the other hand, these layers must be fairly thick on the crystals of tooth enamel if they are thought to explain the presence of HPO_4^{2-} ions up to an amount of 5% by weight [22] in sound human and bovine enamel. In carious enamel the HPO_4^{2-} content can be even as high as 15% [22] but the presence of calcium phosphate phases other than apatite has been reported only scarcely [23]. On the other hand etching of enamel leads to the formation of brushite crystals [24].

Further evidence for our hypothesis that certain surface layers control the dissolution behaviour of enamel mineral can be found in direct experimental studies. Linge and Nancollas [25] found that at moderate temperatures a chemical surface reaction

has a substantial rate control of the dissolution process of tooth enamel and is dominant as the reaction proceeds towards equilibrium. Gray [26] concludes from acidic solubility studies on enamel that "the hydroxylapatite of the enamel dissolves or reacts irreversibly, but as the products-calcium and phosphate-accumulate, another calcium phosphate phase, such as dicalcium phosphate dihydrate, forms on the enamel surface. Under these acid conditions, the formation of $CaHPO_4 \cdot 2H_2O$ is a reversible reaction that can come to an equilibrium".

In this respect two points must be considered. First, with tooth enamel the pH range in which a brushite-like phase controls the equilibrium extends certainly above pH 4.1, the singular point where hydroxyapatite, brushite and saturated aqueous solution coexist in the ternary system $CaO-P_2O_5-H_2O$ [27]. Second, the aqueous solutions equilibrated with tooth enamel above pH 4.1 are undersaturated with brushite and supersaturated with hydroxyapatite. They behave very similar to the synthetic apatites for which the models of metastable equilibria are derived [4].

With the mineral of bone, the situation is quite different. The occurrence of ACP has been shown convincingly [28]. However, the degree of crystallinity and the nature of the crystals is still uncertain. According to some authors apatite is the only crystalline component of bone mineral. Thereby, an apparent lower degree of crystallinity may be either due to a certain ACP content alone [29] or, in addition, to the so-called paracrystallinity of the apatite [30]. So, Lénart, Bidlo and Pinter [31, 32] have shown the presence of brushite $CaHPO_4 \cdot 2H_2O$ and monetite $CaHPO_4$ in some cases in addition to a carbonated apatite by X-ray diffraction, whereas Münzenberg and Gebhardt suggest that the presence of brushite and OCP can be proven in many bone samples. In a pathological case they found monetite as well [33–35].

The theory that bone apatite forms from ACP, which is transformed firstly in OCP and then in apatite [1, 2, 29, 36] receives widespread acceptance. Certain pathologies like renal osteodystrophy are even connoted with disturbances in this presumed mechanism of transformations [37]. However, Münzenberg and Gebhardt [34] were able to show that at least part of the bone apatite is not formed through OCP as an intermediate. Therefore, they proposed that for another part brushite is the precursor of bone apatite and also that bone apatite may be formed directly from ACP. This proposed mechanism is thus identical to the transitions observed *in vitro* in the ternary system $CaO-P_2O_5-H_2O$ [3].

The only change in the bone mineral with age which has been clearly observed, is the increase in the crystalline part and the decrease in ACP content [38]. But even at higher ages a considerable amount of ACP is still present. It is, therefore, reasonable to assume that – despite the possible mechanism of transitions mentioned above – bone mineral can contain all of the calcium phosphate phases observed in bone mineral samples during the whole life span. This interpretation is supported by the fact that bone is subject to continuous turn-over involving the bone mineral. There is no doubt, however, that except for the beginning of the mineralization of calcified tissues the main inorganic component has an apatite structure.

Yet, in the solubility studies analyzed according to the method of a potential diagram (Table I) hydroxyapatite with $Ca/P = 5/3$ may only be governing the solubility behaviour in dead adult human bone. In dead child, rat and calf bone the governing phase is probably OCP. In surviving human bone where the bone cells are still alive, the governing phase is brushite with $Ca/P = 1$, as indicated also by Neuman [39]. Interpretation of these data in relation to the physiology of bone is difficult as part of the solubilities have been measured far beyond the physiological pH range. But a closer inspection of the solubility data [5–8, 39] reveals that in the physiological pH range they approach closely the area where brushite, OCP and carbonated apatite can coexist in equilibrium with an aqueous solution (see Fig. 4, ref. [33]). Therfore, it may be more or less incidental which of the calcium phosphates present in bone mineral appears to govern the solubility behaviour in a solubility study. All of these phases are probably close to physico-chemical equilibrium with the bathing bone fluid. This contains ions like CO_3^{2-} and Na^+ which can affect the apatite solubility [3] and Mg^{2+} which can affect the ACP solubility. The believe in a moment-to-moment equilibrium between bone mineral and bone ECF has been expressed often [40] especially in relation with the success of the "Ca x P" product in explaining the occurrence of rickets [41] and also in relation with the calcification of bone [42, 43]. But the applicability of a

simple solubility product constant has been rejected [44] rightly in view of the present findings and interpretation.

The calcium phosphates in human renal and urinary calculi show mostly an X-ray diffraction pattern very similar to that of bone mineral which is ascribed to apatite. Nevertheless, the apparent solubility behaviour in the higher pH range [15] is that of OCP with Ca/P = 4/3. Especially in the lower pH range urine samples show an equilibrium with commercial apatite similar to that of brushite [16, 17]. Apparently, the renal phosphate and calcium excretion are such that they fall within the area bordered by the apatite, the brushite and the OCP lines in Fig. 2.

As the structure of the urine calcium phosphate stones is mainly that of apatite, we may conclude that the situation is very similar to that of bone mineral, in which Na^+ and CO_3^{2-} ions induce increased solubility as compared to pure hydroxyapatite [2, 3].

Finally, we would like to stress that further experiments on the effect of foreign ions such as Na^+, CO_3^{2-} and Mg^{2+} on the solubility and relative stability of calcium phosphates are necessary to render the presented study a more firm physico-chemical basis.

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