The Dynamics of Bone Mineral in Some Vertebrates

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In the literature octocalcium phosphate as well as brushite have been proposed as the precursor phase for bone mineral in the higher vertebrates. In this study it is shown that the situation is more complex. A sodium and carbonate containing apatite (NCCA) and a magnesium whittlockite (MWH) are present from the beginning of mineralization together with octocalcium phosphate. Upon further bone development the NCCA and the MWH phase are deposited in amounts proportional to the amount of mineral deposited. However, the octocalcium phosphate is transformed slowly in a heavily carbonated defective hydroxyapatite. From the available experimental data it can be derived that the time of this transformation is smaller than four weeks under in vivo conditions.

Introduction

In addition to the main components calcium and phosphate the mineral in bone contains also considerable amounts of the minor components carbonate, sodium and magnesium. On the basis of experimental observations and theoretical predictions the occurrence of different mineral phases in bone is more likely than that of a single apatite solid solution [1]. In a previous paper [2] it was proposed that the sodium content of bone mineral is representative for the amount of an apatite phase with the stoichiometry

$$\text{Ca}_8\text{Na}_{1.5}(\text{PO}_4)_{4.5}(\text{CO}_3)_{1.5}\text{CO}_3.$$

(1)

This compound is known from in vitro synthesis [3]. Further, it was proposed [2] that the magnesium content is representative for the amount of a whitlockite phase with the stoichiometry

$$\text{Ca}_9\text{Mg}(\text{HPO}_4)(\text{PO}_4)_{6}.$$

(2)

This compound is known as a mineral [4]. On the basis of these assumptions and using the chemical composition of over 100 bone samples reported in the literature, it could be shown [2, 5—7] that the inorganic phase of mature bone of vertebrates is composed of compounds (1) and (2) in addition to an apatite “rest” phase with the composition

$$\text{Ca}_9(\text{PO}_4)_{4.5}(\text{CO}_3)_{1.5}(\text{OH})_{1.5}.$$

(3)

This apatite is also known from in vitro synthesis [7]. Variations in the Ca, P, Na, Mg and CO$_3$ content of bone mineral occurring in the samples of mature bone considered could be explained with this three phase model by assuming variations in the relative amounts of the phases (1), (2) and (3).

However, the situation in young and developing bone might be quite different. Brown [8] proposed that the precursor of mature bone mineral is octocalcium phosphate (model 1) with the stoichiometry

$$\text{Ca}_8(\text{HPO}_4)_{2}(\text{PO}_4)_{4}.\text{H}_2\text{O}.$$

(4)

On the other hand Francis and Webb [9] and also Glimcher et al. [10] proposed brushite with the stoichiometry

$$\text{CaHPO}_4.2\text{H}_2\text{O}.$$

(5)

as the precursor phase (model 2).

Support for these hypotheses was drawn from the following observations: the Ca/P ratio of bone mineral increases, whereas the HPO$_4$ content decreases with the age of the bone [11]. The purpose of this paper is to investigate whether octocalcium phosphate (model 1) or brushite (model 2) is likely to be the precursor of bone mineral, or that another, more
complex mechanism must be proposed for the dynamics of the mineral in developing bone.

Methods

Burnell et al. [13] analyzed rat diaphyseal bone of different ages for Ca, Na, Mg, P and CO_3. From their data the amount of the phases (1) and (2) was calculated and by subtraction the Ca/P ratio as well as the CO_3/Ca ratio of the “rest” of the mineral was found. In Fig. 1 these ratios are plotted versus each other.

Pellegrino and Biltz [14] analyzed avian embryonic bone of different ages for Ca, P, HPO_4 and CO_3. The Na and Mg content of the mineral (mmol/g ash) appears not to vary with age [12, 13]. Taking into account the Na and Mg content of avian bone as reported by Klement [16], the amounts of phases (1) and (2) could be calculated. Correcting the experimentally determined HPO_4 and CO_3 contents for the presence of these phases, results in the HPO_4 and CO_3 content of the “rest” of the mineral. Fig. 2 gives a plot of these quantities versus each other.

Driessens et al. [12] analyzed rat femora of different ages for Ca, Na, Mg and P. From their analytical figures the amounts of the phases (1) and (2) could be calculated and by subtraction the Ca/P ratio of the “rest” of the mineral was found. A plot of this ratio as a function of age is given in Fig. 3 together with the data derived from the results of Burnell et al. [13] and Pellegrino and Biltz [14].

Results

Sodium and magnesium are present right after the beginning of mineralization in bone mineral [12, 13], whereas their amount in mmol/g ash is practically constant. In Fig. 1 the theoretical relations between the Ca/P and the CO_3/Ca ratio of the rest phase in bone is represented by the full lines, on the assumption that either model 1 or model 2 applies. A comparison with the data derived from the study of Burnell et al. [13] shows that it is not possible to discriminate between these models.

When the HPO_4 and CO_3 content of the “rest” phase of the bone mineral is calculated on the basis of model (1) and model (2) the relations represented by the full lines in Fig. 2 are obtained. It is clear that the data derived from the experiments of Pellegrino and Biltz [14] closely follow the prediction on the

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Fig. 1. Ca/P molar ratio of the “rest” of the mineral in rat diaphyseal bone versus its CO_3/Ca molar ratio as derived from the data of Burnell et al. [13]. The lines represent the theoretical relations according to model 1 and 2 respectively.

Fig. 2. HPO_4 content (in mmol/g ash) of the “rest” of the mineral in avian embryonic bone as a function of its CO_3 content in mmol/g ash. As derived from the data of Pellegrino and Biltz [14]. The lines represent the theoretical relations according to model 1 and 2 respectively.
The Ca/P molar ratio of the "rest" of bone mineral as a function of age is compared for avian and rat bone. For rat bone the agreement between the data of references [12] and [13] is acceptable in view of the large standard deviations (mainly related to biological variations). Moreover, the evolution of the Ca/P ratio of the rest phase seems to be the same for avian and rat bone, as the data of Fig. 3 must be corrected for bone apposition.

Discussion

The results represented in Figs. 1 through 3 can be interpreted by the following model for the dynamics of bone mineral. Phase (4) is present from the beginning of mineralization and phase (1) and (2) right after the beginning. Upon further development of the bone the phases (1) and (2) become deposited in amounts nearly proportional to the total amount of mineral deposited. However, phase (4) is slowly transformed into phase (3).

The time of transformation of phase (4) into phase (3) is smaller than four weeks according to the data of Fig. 3. It may be species independent. This is supported by the fact that it concerns a physicochemical process occurring in bone fluid, which probably has about the same pH and carbonate and phosphate concentration in all higher vertebrates. If the transformation is indeed species independent then it may offer the possibility for determining the turn-over of mature bone samples by an accurate determination of the Ca, P, Na, Mg, HPO$_4$ and CO$_3$ content and by calculation of their positions in plots like that of Figs. 1 and 2 [12]. This hypothesis will be tested in a subsequent study.

The fact that it is observed in this study that model 1 applies instead of model 2, could have been expected on purely physico-chemical grounds [15]: at pH 7.4 octocalcium phosphate is slightly more stable than brushite and on its turn phase (3) is slightly more stable than octocalcium phosphate, whereas phase (1), (2) and (3) are all in equilibrium with the body fluids under normal physiological conditions. For local mineralization of a bone or an osteon compartment, the local bone fluid must be supersaturated during a certain period with phases (1), (2) and (3) and, apparently, the supersaturation goes so far that even octocalcium phosphate can precipitate, but not brushite, at least not around pH 7.4. From the relative stability of calcium phosphates as a function of pH [15] it can be estimated that brushite should be formed as a bone mineral precursor rather than octocalcium phosphate, only if the local pH of the bone or osteon compartment would be lower than 7.0 during mineralization. That brushite is observed in certain studies of bone samples [10] is, therefore, expected to be an exception rather than a rule for the higher vertebrates.