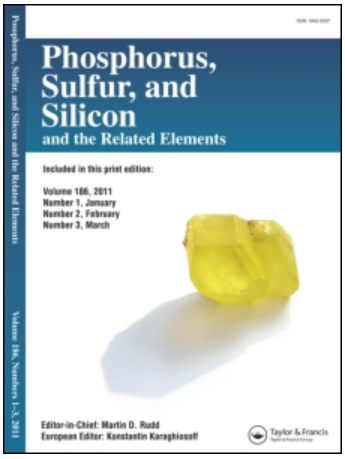

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Formation of Calcium Phosphates in Developing Enamel as Model of Biomineralization

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FORMATION OF CALCIUM PHOSPHATES IN DEVELOPING ENAMEL AS MODEL OF BIOMINERALIZATION

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Abstract The stoichiometry and solubility of tooth enamel mineral at various developmental stages were determined on the basis of the model taking into account most putative ionic species occupying lattice positions. The enamel mineral, in common with other biominerals, is most adequately described as (Mg, Na)-containing calcium carbonatoapatites. An important finding was that changes in the stoichiometry and solubility of the enamel mineral take place with tissue development, suggesting that the nature and properties of the biomineral are modified markedly by possible changes in the microenvironments where the precipitation occurs.

INTRODUCTION

A central issue in biomineralization is the process(es) by which biological systems exert a precise control over the nature and properties (size, morphology, structural defects, and solubility) of the crystals in hard tissues. Concerning the mechanism of calcium phosphate formation *in vivo*, our working hypothesis is that the precipitation of calcium phosphate crystals are determined primarily by the degree of supersaturation *(DS)* in fluid environment surrounding the forming crystals, although the presence of various regulators (e.g., matrix proteins, Mg^{2+}, and F^-) may substantially modify the situation. In the case of a calcium phosphate, the DS can be expressed as the ratio of the ionic activity product *(IP)* of the ionic lattice constituents (reflecting a given stoichiometry) in solution to the solubility product (K_s) of the solid, i.e., $DS = IP/K_s$. Thus, our experimental strategies are: (1) to determine the stoichiometries and solubility product constants of biominerals formed in various hard tissues; (2) to assess the driving force for precipitation of the crystals in mineralizing fluids; and (3) to investigate temporal and spacial modifications of mineralization kinetics caused by various native regulators.

Tooth enamel formation provides a unique model for investigation of biomineralization mechanisms, since the mineralization process is not intervened by any remodeling of the formed tissue, as in the case of bone mineralization. In

the present study using enamel minerals at various stages of amelogenesis, we aim at investigating changes in the stoichiometry and solubility of enamel mineral with tissue development. In order to gain a complete picture about the driving force for the precipitation of enamel mineral, in common with other biominerals, the solubility product constant of the mineral should be determined on the basis of the stoichiometry taking into consideration all putative ionic species occupying lattice positions. Thus attention was given to assess separately the labile (free or exchangeable on the surface) and stable (incorporated into lattice position) pools of major ionic constituents.

MATERIALS AND METHODS

Preparation of Enamel Samples

Enamel samples used were obtained from porcine enamel at various developmental stages, namely the early secretory (S1), late secretory (S2), early maturing (M1), and mature (M2) enamel. All enamel tissues were dissected from the labial side of the permanent incisors of 6-month-old slaughtered piglets. Parts of these pooled samples were deproteinated by plasma ashing at about 60°C. The specific surface areas of the deproteinated S2, M1, and M2 samples (N_2 adsorption) were 62.2, 58.9, and 9.8 m^2/g, respectively.

Physical and Chemical Analyses

The nature of the mineral phase was also examined by x-ray diffraction and Fourier transform infrared (FTIR) spectroscopy. The solid samples were also analyzed with respect to the total contents of Ca and Mg (atomic absorption, AA), Na and K (in the emission mode by AA), phosphorus (colorimetry), carbonate (Conway's microdiffusion), and acid phosphate (Gee and Deitz procedure after pyrolysis at 400-500°C). The same samples were also used for assessment of the labile pools of ionic species, namely carbonate (removal of surface CO_3 species in an activated oxygen atmosphere[1]), acid phosphate (deprotonation or exchange with PO_4^{3-} in alkaline solutions[2]), magnesium (determination of adsorption onto crystal surfaces[3]), and sodium (successive extractions with aqueous solvents[2]).

Solubility Measurements

Each of the deproteinated samples was equilibrated in solutions containing 3 mM phosphate, 0.9 mM $Mg(NO_3)_2$, and 100 mM KNO_3 at 25°C. A N_2-CO_2 gas mixture was used to maintain Pco_2 = 1.8 %. Equilibrium conditions were assumed when the solution composition did not change significantly for a period of 3-4 days which usually took from 20-25 days. Detail of the equilibration procedures was reported previously.[4] The solubility product constants of the enamel mineral, on the basis of a stoichiometry model (see below), were calculated from individual solution composition at equilibrium. To compare the solubility products of various samples having

different stoichiometries, the K_{EN} value was expressed as the mean activity, $(IP)^{1/t}$, where t is the sum of the stoichiometric coefficients.

RESULTS AND DISCUSSION

Nature of enamel mineral

Studies combining chemical analyses, FTIR, and x-ray diffraction showed that (1) all enamel minerals are most adequately described as calcium carbonatoapatites; (2) the initial enamel mineral was rich in acid phosphate and carbonate; and (3) the contents of carbonate and acid phosphate decreased with the advancement of mineralization. These results are consistent with the formation of precursors, such as octacalcium phosphate, at the onset of enamel mineralization, followed by its transformation to apatitic phase. The carbonate of the early secretory enamel was mainly substituted for phosphate groups (B-type) in the apatite lattice. With developmental advancement, an increase in the CO_3 occupying OH sites (A-type) in the crystalline lattice became apparent. It is interesting to note that the evolutionary process from elasmobranch enameloid (comprising francolite) to mammalian enamel is accompanied by an increase of carbonate substitution in the crystals.[5]

State of Ionic Species in Porcine Enamel

Table shows the results of chemical analysis of the total contents of Mg, Na, CO_3, and HPO_4 in developing enamel samples and their fractions corresponding to the labile pool. A remarkable finding was that significant fractions of all ionic species were in labile forms in the early secretory enamel and that the corresponding fractions decreased consistently with the developmental advancement, except for the accumulation of labile Mg in the M1 sample. This unique feature of Mg incorporation into developing enamel was explained by the competitive adsorption reaction of Mg and Ca ions onto enamel apatites.[6]

Table Total Contents (wt %) of ionic species and its percentage (parenthesis) in the labile pool

Sample	Magnesium wt% (%)	Sodium wt% (%)	Carbonate wt% (%)	HPO_4 % P (%)
S1	0.22 (52)	0.99 (46)	3.6 (22)	15.6 (60)
S2	0.28 (38)	1.16 (37)	3.8 (15)	10.8 (45)
M1	0.33 (56)	1.04 (36)	2.8 (17)	8.3 (35)
M2	0.18 (14)	0.68 (26)	2.8 (trace)	5.3 (5)

Stoichiometry of Enamel Apatites

With the attained information about the total contents and state of the major lattice constituents, we attempted to describe the stoichiometry and solubility of enamel minerals

on the basis of the model, $(Ca)_{5-x}(Mg)_q(Na)_u(HPO_4)_v(CO_3)_w(PO_4)_{3-y}(OH)_{1-z}$, where $y = x - q - u$. The stoichiometric coefficients, x, q, u, v, w, y, and z, were obtained on the basis of the determined chemical composition and the electrical neutrality condition for the solid, $2x - 2q - u + 2v + 2w - 3y - z = 0$. Most appropriate stoichiometries for porcine enamel mineral at various developmental stages are:

S1 $(Ca)^{3.950}(Mg)^{0.038}(Na)^{0.201}(HPO_4)^{0.405}(CO_3)^{0.359}(PO_4)^{2.193}(OH)^{0.070}$

S2 $(Ca)^{4.077}(Mg)^{0.051}(Na)^{0.228}(HPO_4)^{0.286}(CO_3)^{0.374}(PO_4)^{2.360}(OH)^{0.084}$

M1 $(Ca)^{4.266}(Mg)^{0.039}(Na)^{0.183}(HPO_4)^{0.226}(CO_3)^{0.323}(PO_4)^{2.491}(OH)^{0.222}$

M2 $(Ca)^{4.526}(Mg)^{0.036}(Na)^{0.131}(HPO_4)^{0.151}(CO_3)^{0.230}(PO_4)^{2.695}(OH)^{0.408}$

Solubility of Enamel Apatites at Controlled Pco_2

The results of solubility measurements showed that the youngest enamel mineral (S1), $K_{EN} = 3.28x10^{-6}$, was the most soluble; and b) the solubility of enamel mineral decreased with advancing developmental stages: the K_{EN} values for S2, M1, and M2 were $2.20x10^{-6}$, $1.56x10^{-6}$, and $1.23x10^{-6}$, respectively. Previously, we reported[7] that the ionic composition of the enamel fluid separated from porcine secretory enamel was: pH, 7.26; (Ca^{2+}), 0.053 mM; [total P], 3.9 mM; [Mg], 0.83 mM; [Na], 140 mM; [K], 21 mM; [Cl], 150 mM; [F], $5x10^{-3}$ mM; and Pco_2 = 1.8 %. Interestingly enough, it was ascertained that the K_{EN} value ($3.29x10^{-6}$) calculated on the basis of *in vivo* (enamel fluid) composition was close to that obtained *in vitro* for the most soluble sample (S1). This observation support the contention that the nature and properties of the formed mineral are determined primarily by the microenvironments where the precipitation occurs.

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