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TRANSFORMATION OF AMORPHOUS CALCIUM PHOSPHATE HYDROXYAPATITE IN THE PRESENCE OF SOME IONS

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ABSTRACT. Studies have been carried out on effects of the ions Mg^{2+}, Ba^{2+}, Pb^{2+}, Sr^{2+}, Zn^{2+}, Cd^{2+}, and F^- on the transformation of amorphous calcium phosphate (ACP), into hydroxyapatite (HAP), with the aim of determining how these ions influence nucleation of HAP crystal, crystallinity index and the mean sizes of the resultant HAP crystals. The ions delayed nucleation and reduced crystal growth to different extents. Mg^{2+}, Zn^{2+}, Ba^{2+} and F^- delayed nucleation only, while Cd^{2+}, $Pb^{2+}$and Sr^{2+} reduced crystal growth and the means size of HAP as well as delaying nucleation of HAP crystals.

INTRODUCTION

Cations and anions separately or in combination act as inhibitors to ACP-HAP transformation [1-3]. Ions such as Mg^{2+}, Sr^{2+}, Ba^{2+} and F^- have been found to inhibit calcification *in vitro*. The inhibitory action of these ions is due to the inhibition of apatite nucleation and crystal growth. However, due to simultaneous presence of many ions in biological apatite, it is difficult to assess the individual effect of these ions on the apatite structure. The inhibitory action of Mg^{2+} ions on the formation of apatite probably involves interference with the formation of HAP critical nuclei, by entering the pre-nuclei structures and creating a structural mismatch [2]. In contrast, it has been proposed that the crystallisation of HAP at low supersaturation in presence of Sr^{2+} ion is accompanied by the incorporation of this ion in an ideal manner [4]. Furthermore, it may compete with Ca^{2+} ions and prevent the formation of appropriate clusters and nuclei and slow down crystal growth, due to their larger size. It has also been shown that pH values above 9, Mg^{2+} substitutes for Ca^{2+} in HAP to give solid solutions in the complete range. Generally the replacement of calcium with other cations in HAP induces changes in the apatite lattice parameters which may be related to the ionic radius of the cation compared to that of calcium [4-7]. It has also been shown that F^- ion retards the formation of ACP which precedes the hydrolysis of octacalcium phosphate (OCP), to HAP and inhibits or even eliminates the formation of OCP-like precursor phases [2]. Nucleation and crystal growth of the apatitic material may also slow down in the presence of F^- ions [1].

Though foreign ions in calcifying solutions inhibit transformation of ACP into HAP, and explanation proposed for such inhibition, the effect of these ions on the crystallinity index and approximate mean sizes of the resultant HAP crystals have not received much attention. In this study we have followed the transformation of ACP into HAP in the presence of various ions (at pH 9 and 25 °C) by measuring the crystallinity index and approximate meas sizes form X-ray diffraction patterns of the products at known reaction times. Metal analysis was also carried out on selected samples.

EXPERIMENTAL

Procedure. ACP was precipitated by mixing pre-buffered 0.04 M $Ca(NO_3)_2.4H_2O$ solution (0.4 L) (0. 15 M Tris-HCl, pH 9.0 ± 0.05 at 298°) with 0.036 M $(NH_4)_2$ HPO_4 solution (0.5 L) and stirring vigorously [3]. In separate experiments, 0.001 M (or 2.5% relative to the molar concentration of Ca^{2+} each of Mg^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} respectively were substituted for Ca^{2+} ions in the mediating solution. 0.001 M of F^- ion was added in a separate experiment at a pH of 9.00 and keeping the initial number of ions in the solution constant.

X-ray analysis. The X-ray powder diffraction patterns of the dried precipitates were taken using a Philips powder diffractometer (PW 1730) with Nickel-filtered Copper K radiation, $\lambda = 0.154$ nm was employed. The scattering region 24 - 36^0 2θ was principally surveyed; this portion of the diffraction pattern included the main hydroxy apatite profile.

At a given time, the extent of reaction (i.e the fraction of ACP converted), is equivalent to the weight fraction of the hydroxy apatite present in the slurry [6]. In the transformation of ACP to HAP, the integrated intensity of and X-ray diffraction peak characteristic of the crystalline material obtained in the presence of inhibitors, were compared to the intensity of samples obtained without inhibitors. Because of extensive overlaps, only the 002 peak at about 25.8^0 2θ was judged to be sufficiently resolved for line-breath measurement. The width at one-half the maximum height of this peak, $\beta_{1/2}$ was measured in degrees 2θ. The quantity, $1/\beta 1/2$ which is proportional to the mean crystallite size and/or degree of perfection in the direction along the c-axis of the crystals was taken as the measure of the crystallinity index [7]. The mean size, D, of the apatite crystals at different sampling intervals were estimated approximately from the corresponding $\beta_{1/2}$ by use of Scherrer's equation [7]: $D = 57.3K'/\beta_{1/2}Cos\theta$, where K' is the crystal shape factor with an approximate value of 0.9, $\beta_{1/2}$ is the wavelength of the x-radiaiton employed and is one-half of the scattering angle.

RESULTS AND DISCUSSION

The various effect of the ion Mg^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, and F^- on the crystallinity and mean sizes of HAP crystals are given in Table 1. Metal ions apart from sometimes substituting for Ca^{2+} in apatites, also affect the transformation of ACP into HAP and the dimensions of the resultant crystals. Mg^{2+} a type II inhibitor [3] was found to inhibit ACP to HAP transformation. Nancollas [2] described the inhibitory role of Mg^{2+} in the formation of HAP critical nuclei by entering the prenuclei structures and creating a structural mismatch. At the concentration used in this study (0.001 M), Mg^{2+} ion inhibited the nucleation of HAP for 2 h, but did not inhibit crystal growth. The mean size of the apatite crystals obtained in presence of Mg^{2+} ions are comparable to the control (Table 1).

Although it has been reported that Sr^{2+} ion cannot easily enter the apatite lattice [5], they similarly compete with Ca^{2+} and prevent the formation of the appropriate nuclei and slow down crystal growth. The presence of Sr^{2+} in the crystallizing solution was found to reduce not only the crystal growth, but also the mean size of the apatite crystals (Table 1). In the crystallization of hydroxyapatite from aqueous solution, Ca-Sr-hydroxyapatite crystals were much smaller than HAP crystals obtained [4]. The reason being that, the presence of Sr^{2+} results in the reaction of Ca^{2+} ionic vacancies in the lattice and insignificant incorporation of the Sr^{2+} ions.

Zn^{2+} ion which has been found to substitute for Ca^{2+} in HAP only to a little extent [5], was found to delay the nucleation of HAP crystals for about 2 h. Zn^{2+}, like Mg^{2+}, did not reduce

Table 1. Crystallinity index, $1/\beta_{½}$ and mean size, D of apatite crystals obtained in presence of some ions at 25^0 and pH 9.

Ions	Time (min)	1/β ½	D (Å)	Ions	Time (min)	1/β ½	D (Å)
Mg^{2+}	10	*	*	Pb^{2+}	10	*	*
	30	*	*		30	*	*
	60	*	*		60	*	*
	120	*	*		120	1.25	97.15
	240	1.25	97.17		240	1.25	97.19
	480	1.25	97.17		480	1.25	97.21
	540	1.25	97.16		540	1.40	102.95
Sr^{2+}	10	*	*	Ba^{2+}	10	*	*
	30	*	*		30	*	*
	60	*	*		60	*	*
	120	0.83	66.2		120	0.91	79.4
	240	1.20	95.7		240	1.20	95.7
	480	1.18	93.4		480	1.22	96.8
	540	1.14	90.2		540	1.22	96.8
Zn^{2+}	10	*	*	F$^-$	10	*	*
	30	*	*		30	*	*
	60	*	*		60	1.0	79.4
	120	1.01	80.2		120	1.04	82.7
	240	1.11	88.2		240	1.18	93.4
	480	1.19	94.5		480	1.16	92.3
	540	1.22	96.8		540	1.18	93.4
Cd^{2+}	10	*	*				
	30	*	*				
	60	1.00	79.4				
	120	1.12	89.2				
	240	1.11	88.2				
	480	1.14	90.2				
	540	1.14	90.2				

*Precipitates were essentially amorphous.

significantly the mean size of the apatite crystals formed. Cd^{2+} (ionic radius 0.97 Å) *is similar to* Ca^{2+} in size (ionic radius 0.99 Å), and is therefore expected to substitute for Ca^{2+} in HAP at the low concentration used. Cd^{2+} was found to delay the nucleation of apatite crystals for about 30 min, and reduced the crystals. This reduction in size of the apatite crystals is in agreement with the smaller ionic radius of Cd^{2+} with respect to that of Ca^{2+}. However, this difference in ionic radii appears too small to justify that observed reduction in size. Instead the reduction may be attributed to the nature of the cation-oxygen interactions [6].

Pb^{2+} was found to inhibit the nucleation process, but enhanced crystal growth. The replacement of lead for calcium in the apatite structure induces an enlargement of the lattice constants in agreement with the larger ionic radius of lead (1.30 Å) with respect to that of calcium. However, this cannot be justified on the basis of the difference in ionic radius and nature of the cation-oxygen interaction alone, but may be accounted for more completely by the structural differences caused by the inhomogeneous lead distribution in the two non-equivalent cation sites of the HAP structure [7].

Ba^{2+} substitutes to a small extent for Ca^{2+} in HAP in aqueous medium [9]. Ba^{2+} like Sr^{2+} inhibited nucleation process for sometimes, but did not reduce the crystal growth, thus giving crystals similar to those obtained for the control. It appears that under the experimental condition used in this reaction, Ba^{2+} did not substitute for Ca^{2+} in HAP.

The F^- ion was found to inhibit ACP-HAP transformation by reducing the growth rate of the apatetic crystals, and delaying the nucleation of HAP crystals. Fluorapatite (FAP) is less soluble compared to hydroxyapatite and thus incorporation of F^- within the lattice would be expected to result in a more rapid precipitation of the apatite with a lower final calcium and phosphate concentrations. However studies of the ionic nuclei ratios in the precipitating phase was reported to be suggestive that this phase is not FAP, but rather a fluoridated hydroxyapatite [2]. The F- ion was also reported to retard the formation of ACP [2], but this was not observed under the conditions used in this experiment. The effect of F^- on the size of the resultant HAP crystals was not very significant.

Chemical analysis of ACP-HAP precipitates showed an initial increase in the Ca/P molar ratio of the control, and a lowering at about 2 h, after which a constant value (1.3) was maintained. In the absence of such ions as F^- and CO_3^{2-}, HAP is the thermodynamically stable and product of calcium phosphate precipitations occur in neutral or basic pH. The data obtained in this study (Tables 2 - 5) show that the crystals are apatite-like in their general structural features. The first crystals to form in the spontaneously precipitating systems deviated markedly from HAP in composition and structural details as exemplified by low Ca/P molar ratios, X-ray diffraction patterns and distorted mean sizes. These primary crystal are unstable and steadily change upon solution ageing to compositions and structures nearly resembling HAP. The rate at which these chemical and structural changes decrease with time suggests a maturation behaviour similar to that observed in other sparingly soluble inorganic precipitates, known as Ostwald ripening [10, 11]. Eanes and Meyer [12], observed similar changes in the growth curve of HAP under physiological condition. They reported that these phase changes and the final apatitic stage of the maturation process was marked mostly by changes in the chemistry of the solids. Furthermore, the significant increase in the Ca/P ratio of the solid obtained at 1 h occurred, but was accompanied by very little decrease in calcium solution. This finding indicates that the increase

Table 2. Concentration of Ca^{2+}, PO_4^{3-} and Ca/P molar ratios for ACP-HAP precipitates obtained at different times.

Time (min)	$Ca^{2+}(10^{-5}M)$	P (10^{-5}M)	Ca/P Ratio
10	2.38	2.90	0.82
30	2.92	2.94	0.98
60	2.38	2.20	1.08
120	2.22	2.20	1.00
240	2.88	2.22	1.30
480	2.59	2.00	1.30

Table 3. Concentration of Ca^{2+}, Mg^{2+}, PO_4^{3-} and Ca-Mg/P molar ratios for ACP-HAP precipitates obtained in presence of Mg^{2+}.

Time(min)	Ca^{2+} (10^{-5} M)	Mg^{2+}(10^{-6} M)	P (10^{-5} M)	Ca-Mg/P
10	3.75	3.90	3.14	1.24
30	1.81	1.93	2.98	0.65
60	3.81	4.00	2.91	1.34
120	3.81	3.98	3.92	1.02
240	3.12	2.26	3.87	0.84
480	3.25	3.41	2.48	1.40
540	2.72	2.84	2.03	1.40

Table 4. Concentration of Ca^{2+}, Zn^{2+}, PO_4^{3-} and Ca-Zn/P molar ratios for ACP-HAP precipitates obtained in presence of Zn^{2+}.

Time (min)	Ca^{2+}(10^{-5} M)	Zn^{2+} (10^{-7} M)	P (10^{-5} M)	Ca-Zn/P
10	2.31	2.40	3.15	0.77
30	2.31	2.41	3.02	0.81
60	2.50	2.60	2.91	0.89
120	2.22	3.14	3.90	0.81
240	3.44	3.57	3.90	0.92
480	3.62	3.76	2.50	1.51
540	2.99	3.11	2.04	1.50

Table 5. Concentration of Ca^{2+}, Cd^{2+}, PO_4^{3-} and Ca-Cd/P molar ratios for ACP-HAP precipitates obtained in presence of Cd^{2+}.

Time (min)	Ca^{2+} (10^{-5} M)	Cd^{2+} (10^{-6}M)	P (10^{-5} M)	Ca-Cd/P
10	3.88	1.38	4.70	0.85
30	2.69	1.08	3.32	0.84
60	2.59	1.03	3.17	0.84
120	3.94	1.46	4.72	0.87
240	2.00	0.91	2.42	0.86
480	2.19	0.97	2.62	0.87

in crystal mass during this time span from newly deposited material was negligible and consequently could not account for the observed increase in Ca/P ratio. Instead, the significant amount of the earliest apatite formed by hydrolysis change into more stoichiometric material through continuous dissolution/growth process such as Ostwald ripening [12].

The behaviour of the precipitating phase differ in the presence of the different metal ions, that is Mg^{2+}, Zn^{2+}, and Cd^{2+}. The Ca-Mg/P molar ratio shows an initial decrease and then an increase before it finally attains a stable composition of 1.4. This increase in Ca-Mg/P ratio indicates maturation of the apatite crystals and this consistent with results obtained from X-ray analysis of the precipitated discussed earlier. The Ca-Zn/P molar ratio also shows a general increase, giving a value of 1.5, nearing the expected value for apatite. The Ca-Cd/P molar ratio deviated markedly from the expected value for apatites, which is approximately 1.67. This result is also consistent with the inhibitory role Cd^{2+} plays on the crystal growth of HAP [6]. The low Ca-Cd/P ratio may be as a result of the contribution from the other precipitating of phases of calcium phosphate system unable to convert the apatite in presence of Cd^{2+} ions.

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