

may result that will produce two different iron sites. In view of the small difference of the two ΔE_Q values, viz., $\Delta E_Q^I = 2.41 \text{ mm s}^{-1}$ and $\Delta E_Q^{II} = 1.99 \text{ mm s}^{-1}$ at 115.1 K, and the identical temperature dependence of the quadrupole splitting, both iron(II) atoms will have presumably the same 5A_1 ground state. In contrast to several related transitions,^{27,30,34} an orbital ground-state reversal may thus be ruled out for $[\text{Fe}(\text{bi})_3](\text{ClO}_4)_2$. In the low-spin 1A_1 phase, the inequivalence of the

two iron sites is not resolved due to the much smaller value of $\Delta E_Q(^1A_1)$, which is insensitive to lattice distortions.

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Contribution from the Laboratory of Biological Structure, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205

Lattice Defects in Nonstoichiometric Calcium Hydroxylapatites. A Chemical Approach

JOHN L. MEYER* and BRUCE O. FOWLER

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A number of defect hydroxylapatites were prepared with Ca/P molar ratios varying from 1.40 to 1.62. These samples were analyzed for their hydroxide contents by a titration method after chemically determining their calcium, phosphate, acid phosphate, and carbonate contents. The results were compared with various general compositional formulas previously used to describe the structure and composition of the defect hydroxylapatites. Hydroxide contents between 0 and 1.13 per theoretical unit cell of hydroxylapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, were obtained depending upon experimental conditions. The general compositional formula that best represented the experimental data was $\text{Ca}_{10-X-Y}(\text{HPO}_4)_X(\text{PO}_4)_{6-X}(\text{OH})_{2-X-2Y}$, which describes two types of calcium vacancies in the defect lattice. The first, or X type, is coupled to the loss of a hydroxide and the addition of a hydrogen ion to the lattice and seems to be dependent upon the conditions of preparation of the precipitates. The second calcium vacancy (Y type) is electrically compensated by two vacant hydroxide positions and seems to be insensitive to solution environment with an average of about 0.5 calcium vacancy per unit cell. Other models that assume only X-type vacancies or that require one or more hydroxides for lattice stability seem to be in error.

Introduction

Hydroxylapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) serves as a model structural component for all the calcium-containing apatites of biological interest.¹ The inorganic apatitic materials present in biological hard tissue, with the possible exception of dental enamel, have carbonate-corrected calcium/phosphate molar ratios less than the theoretical 1.67, and a number of theories have been advanced to explain this discrepancy. One suggestion was that the defect hydroxylapatites (DHA) or nonstoichiometric hydroxylapatites contained the theoretical amount of hydroxide and that the low calcium phosphate ratios were due entirely to calcium ion defects with electroneutrality being maintained by the presence of two protons in the lattice for each missing calcium.²⁻⁴ This concept results in the general formula I in Table I for the DHAs. Winand et al.^{5,6} later proposed the alternative formula II, which suggests that both calcium and hydroxide defects can occur. Hydrogen ion enters the lattice in this formulation also but must necessarily be coupled with the removal of a calcium and a hydroxide ion from the lattice. Kuhl and Nebergall⁷ proposed the more general formula III, which maintains electroneutrality by a combination of (a) a one-proton addition for each calcium and hydroxide defect and (b) the absence of two hydroxides per calcium ion defect. Kuhl and Nebergall⁷ also allowed for the uptake of carbonate by the apatite lattice by suggesting formula IV, which substitutes CO_3^{2-} for HPO_4^{2-} . Berry,⁸ using

Table I. General Compositional Formulas for Defect Calcium Hydroxylapatites

no.	formula	remarks	ref
I	$\text{Ca}_{10-X}(\text{HPO}_4)_X(\text{PO}_4)_{6-X}(\text{OH})_2$	$0 \leq X \leq \sim 2$	2-4
II	$\text{Ca}_{10-X}(\text{HPO}_4)_X(\text{PO}_4)_{6-X}(\text{OH})_{2-X}$	$0 \leq X \leq 2$	5, 6
III	$\text{Ca}_{10-X-Y}(\text{HPO}_4)_X(\text{PO}_4)_{6-X}(\text{OH})_{2-X-2Y}$	$0 \leq X \leq 2$ $Y \leq 1 - X/2$	7
IV	$\text{Ca}_{10-X-Y}(\text{HPO}_4)_X(\text{CO}_3)_Y(\text{PO}_4)_{6-X}(\text{OH})_{2-X-2Y}$	$0 \leq X \leq 2$ $Y \leq 1 - X/2$	7
V	$\text{Ca}_{10-X}(\text{HPO}_4)_X(\text{PO}_4)_{6-X}(\text{OH})_{2-X}$	$\text{Ca/P} \geq 1.5$ $0 \leq X \leq 1$	8, 10
VI	$\text{Ca}_9-X(\text{HPO}_4)_{1+2X}(\text{PO}_4)_{5-2X}(\text{OH})_2$	$\text{Ca/P} < 1.5$ $0 \leq X \leq 1$	9, 11

thermal and spectroscopic measurements, confirmed the general formula proposed by Winand et al.^{5,6} for apatites in the Ca/P molar range 1.50-1.67 but with the added constraint that $0 \leq X \leq 1$ (formula V). Berry⁹ proposed that a different formulation was required for the Ca/P molar range 1.40-1.50 (formula VI) that substitutes two hydrogen ions for each calcium as required by formula I to a lattice that contains half the theoretical hydroxide. It should be noted that Berry's^{8,9} proposed structural formulas require that all DHAs contain at least 50% of the theoretical amount of lattice hydroxide. Joris and Amberg^{10,11} later agreed with the two formulations proposed by Berry^{8,9} on the basis of their independent measurements of the spectroscopic and catalytic properties of a number of DHAs. An alternate proposal that has received considerable attention was that the calcium defect was due to interlayered mixtures of the structurally similar octacalcium phosphate (OCP) and HA.^{12,13} A general chemical formula for this system would be identical with formula II. Each of

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the studies referred to above based their proposed compositional formulas on incomplete or indirect chemical, thermal, and/or spectroscopic measurements. In no case was hydroxide, the most controversial unit of the apatite lattice, directly measured.

It would seem possible to estimate lattice hydroxide by means of the areas of the hydroxide stretching, librational, and translational bands in infrared¹⁴ and Raman¹⁵ spectra. A previous infrared study,¹¹ utilizing only the hydroxide librational band for hydroxide quantitation in DHAs, was inconclusive. Another infrared investigation¹⁶ indicated no or very low hydroxide in pH 7.4 DHA preparations and some hydroxide in pH 10 preparations, but no quantitation was made. An additional infrared and chemical study^{17,18} proposed that considerable hydroxide is present in pH 7.4 preparations from chemical data but that these hydroxide ions are spectrally undetected because their infrared hydroxide bands are "smeared out" by surface-related effects. A Raman study of hydroxide quantitation in stoichiometric hydroxylfluoro- and hydroxylchloroapatites has been carried out,¹⁵ but apparently none has been made on the DHAs. A preliminary report¹⁹ has shown, however, that the infrared hydroxide stretching band can be used to semiquantitate lattice hydroxide if proper standards and procedures are used. This will be expanded upon along with Raman hydroxide measurements in a subsequent publication.

The first chemical method²⁰ devised to directly measure hydroxide in apatitic solids became the subject of considerable controversy^{21,22} since it failed to distinguish between hydroxide, carbonate, or water. The latter two are often present in large quantities in samples of biological origin. An improved chemical method²³ recently has been developed, however, for the specific determination of hydroxide in calcium phosphate precipitates that has sufficient accuracy to quantitate the hydroxide content of calcium defect apatites. This method was used in the investigation reported here in an attempt to determine which of the structure-compositional models discussed above best describes the composition of DHAs. Calcium, phosphate, acid phosphate, carbonate, and hydroxide analyses were performed on the series of solution-precipitated calcium phosphates of variable Ca/P ratios used in this investigation.

Experimental Section

Preparation of Samples. Nonstoichiometric amorphous and crystalline calcium phosphate samples were prepared from precipitation reactions performed at constant pHs of 7.00, 7.40, 8.25, 9.75, and 10.00. So that the presence of extraneous ions in the precipitates could be minimized, all initial reaction solutions, with the exception of the pH 9.75 experiments, were prepared by combining phosphoric acid (1 M) and calcium hydroxide (0.01 M) solutions to attain the desired pH. The phosphoric acid solution was prepared by diluting reagent grade concentrated acid, and the base solution was prepared by filtering (Millipore filtration, 0.22 μ m pore size) a saturated solution of reagent grade calcium hydroxide. The filtration step removed all precipitated calcium carbonate and resulted in a calcium hydroxide solution relatively low in carbonate content (ca. 7.7 μ M based on carbonate ion in solution in equilibrium with solid calcium carbonate²⁴ and as

the soluble ion pair, CaCO_3^{25}). The pH 9.75 preparation was made by combining equal volumes of reagent grade calcium nitrate (0.02 M) and potassium dihydrogen phosphate (0.013 M) and adjusting the pH with concentrated ammonium hydroxide. Since a pH drop accompanies the maturation of the calcium phosphate precipitates in unbuffered systems, constant pH was maintained, after the initial precipitation step, by adding base solution controlled by a pH stat (Metrohm, Combitorator 3-D). A solution of potassium hydroxide was used for pH control for the pH 7.00 and 8.25 experiments whereas an ammonium hydroxide solution was used for the pH 7.40, 9.75, and 10.00 experiments. The choice of reagents was influenced by the experience of this laboratory that potassium, ammonium, and nitrate ions are not incorporated into crystalline calcium phosphate lattices. The pH electrode (Sargent-Welch S-30072) was standardized by buffers prepared according to NBS specifications. All precipitation reactions were conducted at 25 ± 0.1 °C in a nitrogen atmosphere. All solutions were prepared from deionized, distilled, carbonate-free water.

Samples (~100 mg) were removed from the precipitation experiments at preselected time intervals that corresponded to the existence of previously characterized calcium phosphate phases^{26,27} and that were thought to represent the formation of solid materials containing varying hydroxide contents. Aliquots of the solution slurries were filtered through 47 mm diameter, 0.22- μ m Millipore filters, aspirated briefly to remove excess solvent and washed several times with deionized distilled water. The samples were then frozen in containers immersed in liquid nitrogen, lyophilized, and stored in a desiccator.

Stoichiometric HA samples were prepared by both hydrothermal and solution precipitation methods. The S-1 and S-2 samples were prepared by heating a gravimetric mixture of $10\text{CaO} \cdot 3\text{P}_2\text{O}_5$ in steam at 1.5 atm at 900 and 1100 °C, respectively; preparative details are given elsewhere.²⁸ Sample S-3 was prepared by dropwise addition over a period of 16 h of 0.45 L of 0.12 M calcium nitrate and 0.45 L of 0.066 M diammonium phosphate solutions both 1 M in ammonium nitrate to 2.5 L of boiling 1 M ammonium nitrate solution maintained at pH ~9 by means of an ammonia atmosphere. Calcium was kept in excess throughout the precipitation by offset delivery. Sample S-4 is a portion of sample S-3 that was heated at 900 °C in 1.5 atm of steam for 24 h in apparatus described in ref 28. Sample S-5 was prepared by dropwise addition of 1.6 L of 0.0250 M diammonium phosphate solution to 1.4 L of 0.0476 M calcium nitrate both at 25 °C and pH 12. The pH was adjusted with concentrated ammonium hydroxide. The apatite slurry was then boiled in an ammonia atmosphere (pH 10.2) for 88 h. The precipitates, S-3 and S-5, were washed five times by resuspension in boiling water, centrifuged, and dried at 60 °C under vacuum for 72 h. All of the S-series samples were identified as single phases of well-crystallized HA by X-ray diffraction and infrared analyses. Further preparative details and physical characterization of these essentially stoichiometric solution-prepared HAs will be given elsewhere.

Analytical Methods. The procedure for determining the hydroxide content of calcium phosphate precipitates has been described in detail.²³ Briefly, ~20 mg of precipitate was dissolved in a slight excess of standard 0.1 M hydrochloric acid and diluted to 25 mL. Two aliquots of this solution were titrated at 25 ± 0.1 °C to a previously calculated pH end point with standard 0.1 M potassium hydroxide. The calculation of the end point, the neutralization of the first proton of phosphoric acid, was based on both acid-base and calcium phosphate ion-pair equilibria. Calcium and phosphate analyses were performed on separate aliquots of the same solution used for the hydroxide titration. Calcium was determined by atomic absorption with a Perkin-Elmer Model 603 spectrophotometer. Phosphate analyses were performed by the method of Murphy and Riley.²⁹ Carbonate and acid phosphate contents were determined on separate sample portions by the method of Hirschman and Sobel³⁰ and a modified method of Gee and Deitz,³¹ respectively.

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Table II. Effect of Pyrolytic Atmospheres on Pyrophosphate Production in Defect Calcium Hydroxylapatites

Ca/P molar ratio	pH	soln maturation time, h	550 °C for 24 h										predicted ^d % P ₂ O ₇	
			unheated		vacuum ^a				air, ^b aged		tube, ^c aged			
			surface area aged, m ² /g	% CO ₃ fresh aged	fresh, ^e % P ₂ O ₇ ^g	aged ^f		% P ₂ O ₇	% CO ₃	% P ₂ O ₇	% CO ₃ ^h			
1.40	7.00	1.5	163	0.19	0.20	18.5	25.7 ± 0.4		26.8 ± 0.8	0.23	27.8 ± 0.5	(≤0.1)	27.0 ± 2.1	
1.44	7.40	2.2	133		0.23		24.1 ± 0.4		0.20	24.0 ± 0.0	0.20	24.7 ± 0.8	(≤0.1)	22.8 ± 2.1
1.45	8.25	1.5	149	0.69	1.04	7.5	17.8 ± 0.5		0.81	18.7 ± 0.1	0.47	22.7 ± 1.4	(≤0.2)	21.8 ± 2.1
1.54	8.25	24.0	163	0.83	0.98	3.3	7.8 ± 1.3		0.90	11.0 ± 0.9	0.24	11.1 ± 1.3	(≤0.2)	12.4 ± 2.1
1.58	10.00	19.0	142	1.53	2.04	1.6	6.5 ± 0.3		1.50	7.7 ± 0.6	0.92	7.8 ± 0.3	(≤0.6)	8.3 ± 2.1

^a Pressure 10⁻⁴ atm. ^b Pressure 1 atm, ~50% relative humidity. ^c Water vapor pressure ~2-3 atm (see text). ^d Predicted percent of pyrophosphate and error (±0.02 in Ca/P ratio) according to formula II (Table I). ^e Fresh: lyophilized precipitate age in desiccator of ~3 months. ^f Aged: lyophilized precipitate age in desiccator of ~36 months. ^g Percent of total P in pyrophosphate form. ^h CO₃ values in parentheses were estimated from infrared spectra.

The Gee and Deitz method³¹ was modified by employing pyrolytic conditions of 550 °C under vacuum for 24 h. Four DHAs were reanalyzed for pyrophosphate under the same pyrolytic conditions after desiccator storage over calcium sulfate for about 36 months. A comparison of pyrophosphate production, residual carbonate, and apatite hydroxide changes by infrared in five DHAs of varying carbonate contents pyrolyzed at 550 °C for 24 h under vacuum and air and in sealed platinum tubes was made. The different pyrolytic atmospheres were used to determine the effect of increasing water vapor pressure on pyrophosphate production. The sealed platinum tubes contained nitrogen gas and the DHAs. The total pressure in the tubes at 550 °C was estimated at 5-6 atm, and the partial water-vapor pressure arising from the DHA water component was estimated at 2-3 atm from both original water in the samples (~10%) and weight loss after pyrolysis.

The DHA water component was deduced as the difference in sample weight and total weight of lattice ions. All analytical procedures were performed two to four times. Weighings were made with a Mettler microbalance to an accuracy of ±1 μg. Pipet delivery and volumetric errors were less than 0.1% as determined by frequent calibration. Surface areas were determined by a dynamic nitrogen gas absorption system (Quantasorb-Quantachrome Corp.).

Results

The working equation for chemical quantitation of hydroxide, T_{OH} , in a calcium phosphate precipitate is given with all T_x terms in moles of component x:

$$T_{OH} = T_A + T_{HPO_4} + T_{HCO_3} - T_B - 2T_P - 2T_{CO_3}$$

T_A is the amount of excess acid used to dissolve the precipitate and neutralize all hydroxide, phosphate, and carbonate in the system. Additional hydrogen ion is introduced if the precipitates contain HPO_4^{2-} ; thus, T_{HPO_4} must be added to the total acid balance. The amount of hydroxide, T_{OH} , that was originally present in the precipitate and neutralized by the excess hydrochloric acid is represented by the difference in the total acid added to the system, the amount of base required to back-titrate to the first phosphoric acid end point, T_B , and the amount of hydrogen ion known to exist at the end point in the form of $H_2PO_4^-$ or twice the total phosphate content of the sample, $2T_P$. If CO_3^{2-} ion is present in the samples, it will also consume twice its molar content of acid in the neutralization step; hence, the term $2T_{CO_3}$ must be subtracted from the total acid. The generally negligible term T_{HCO_3} is included in the equation for completeness and represents the quantity of H_2CO_3 neutralized in the back-titration.

An error analysis of the experimental data used in the equation is important since T_{OH} represents a difference in quantities of comparable magnitude. This is easily seen since the dissolution and neutralization of pure HA (unit cell formula $Ca_{10}(PO_4)_6(OH)_2$) requires 9 times more acid to completely neutralize the phosphate than that required for the hydroxide; consequently, any error in either the titration or the phosphate measurement is magnified approximately 9 times in the computed hydroxide content. The relative errors

associated with the determinations of T_P , T_A , and T_B were found to be ±0.3, 0.2, and 0.3%, respectively. The errors involved in the estimation of T_{HPO_4} and T_{CO_3} in the solid materials were larger and were estimated to be ±3%. The contribution of these larger errors to the total error was generally not important, since their concentrations in the solid calcium phosphates used in this study were relatively small. A propagation of error analysis³² of the above uncertainties previously suggested²³ that the absolute error for the percent of hydroxide in the type of samples reported here was of the order of ±5% or ±0.10 hydroxide per unit cell of HA.

Table II gives data on (a) the effect of different pyrolytic atmospheres (vacuum, air, and sealed-tube atmospheres) on pyrophosphate production, (b) pyrophosphate analyses under the same vacuum conditions for fresh (~3 months) and aged (~36 months) DHAs, and (c) carbonate analyses before and after pyrolysis. A large increase in pyrophosphate with increased DHA age was observed. Overall, pyrolysis in air caused slightly higher pyrophosphate production than pyrolysis in vacuum; this agrees with previous vacuum and air pyrolytic data.³³ Pyrolysis in vacuum reduced the original carbonate contents of the DHAs containing ~1-2% carbonate by about 25% or less, whereas pyrolysis in air reduced the carbonate by 50% or more and tube pyrolysis even more. Overall, the DHAs that progressively lost more carbonate on pyrolysis also showed progressively higher pyrophosphate contents; the low (~0.2%) carbonate-containing DHAs showed smaller differences in pyrophosphate contents under the different pyrolytic atmospheres. Infrared spectra were obtained on all DHAs in Table II. Spectra of the fresh and aged DHAs of Ca/P ratios, 1.40 and 1.45, that had large increases in percent of pyrophosphate on aging, 18.5-25.7 and 7.5-17.8, respectively, also concomitantly had considerable increases in structural hydroxide as deduced from normalized infrared hydroxide stretching band areas at 3570 cm⁻¹. This infrared hydroxide increase was not attributed to an appreciable increase in crystal size by surface area comparisons between the aged (~150-160 m²/g) and the fresh DHA surface areas that were estimated to be about 10-20% larger.²⁶ Infrared spectra of the pyrolyzed aged DHAs indicated progressively increased HA hydroxide contents in the vacuum-air-tube sequence. This hydroxide increase also correlated with the pyrophosphate increase in the same sequence, although hydroxide contents were indicated by infrared analyses to be higher than those deduced from increased pyrophosphate contents with the assumption that the additional OH⁻ was generated by HOH splitting to form additional apatite OH⁻ and HPO_4^{2-} to form more $P_2O_7^{4-}$. The thermally effected increase in crystal size and crystallinity

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Table III. Chemical Composition of Synthetic Amorphous Calcium Phosphates and Stoichiometric Calcium Hydroxylapatites

Ca/P (molar ratio)	sample ^a	Ca ^b	PO ₄ ^b	HPO ₄ ^b	CO ₃ ^b	OH ^b	H ₂ O ^b
1.44	pH 8.25, 0.5 h	8.67	5.54	0.46	0.06	-0.09	8.02
1.51	pH 10.00, 1.0 h	9.08	5.93	0.07	0.12	0.14	8.68
"Amorphous"							
1.65	S-1	9.92	6.00	0.00	0.00	1.95	0.14
1.67	S-2	10.03	6.00	0.00	0.00	2.06	0.33
1.64	S-3	9.87	5.96	0.04	0.02	1.89	1.51
1.65	S-4	9.89	6.00	0.00	0.02	1.84	0.27
1.66	S-5	9.93	5.94	0.06	0.10	1.90	3.40
"Stoichiometric"							

^a Preparative details are in the Experimental Section. ^b Numbers correspond to the number of species in a hypothetical unit cell of hydroxylapatite containing six phosphate ions. Charges have been omitted.

Table IV. Chemical Composition of Defect Calcium Hydroxylapatites

Ca/P (molar ratio)	pH	maturation time, h	Ca ^a	PO ₄ ^a	HPO ₄ ^a	CO ₃ ^a	OH ^a	H ₂ O ^a
1.40 ^b	7.00	1.5	8.42	4.89	1.11	0.03	0.10	6.78
1.41 ^b	7.40	0.5	8.46	4.88	1.12	0.02	0.05	7.14
1.43	7.40	2.5	8.58	4.96	1.04	0.01	0.41	5.60
1.45 ^b	8.25	1.5	8.67	5.55	0.45	0.12	-0.03	7.92
1.47	7.40	6.0	8.83	5.21	0.79	0.03	0.64	5.64
1.49	7.00	17.0	8.93	5.29	0.71	0.02	0.81	5.31
1.50	8.25	4.0	8.99	5.65	0.35	0.16	0.49	6.87
1.50	7.40	25.0	9.03	5.40	0.60	0.03	0.75	5.51
1.52	7.00	94.0	9.14	5.39	0.61	0.01	1.11	5.22
1.54	8.25	24.0	9.22	5.80	0.20	0.15	0.67	5.78
1.57	8.25	67.0	9.39	5.87	0.13	0.16	0.90	5.69
1.58	10.00	19.0	9.46	5.91	0.09	0.28	0.86	6.30
1.58	9.75	24.0	9.48	5.90	0.10	0.14	0.62	6.49
1.58	9.75	48.0	9.50	5.87	0.13	0.13	0.86	6.17
1.59	9.75	72.0	9.52	5.78	0.13	0.15	0.69	6.45
1.62	10.00	90.0	9.75	5.97	0.03	0.21	1.13	5.17

^a Numbers correspond to the number of species in a hypothetical unit cell of hydroxylapatite containing six phosphate ions. Charges have been omitted. ^b Thermodynamic evidence suggests octacalcium phosphate may be present in these samples.

increase undoubtedly contributed to the infrared hydroxide-band area and intensity increases. The DHA carbonate component in the unheated and all pyrolyzed samples were essentially all in phosphate positions as based on the CO₃²⁻ infrared band positions at about 1455 and 1415 cm⁻¹.³⁴ Pyrophosphate in all DHAs pyrolyzed under vacuum or air appeared to be amorphous or cryptocrystalline because of the broad unresolved infrared pyrophosphate bands, whereas the tube-pyrolyzed samples, containing about 15% or more pyrophosphate, had distinct bands of β-Ca₂P₂O₇ plus additional bands at 745 and 518 cm⁻¹, which were not attributed to α or γ forms of Ca₂P₂O₇. These additional bands may arise from triphosphate, but definite identification was not made.

Data are presented in Table III for two types of calcium phosphate solid phases representing the two extremes in hydroxide content. The two samples of "amorphous" calcium phosphate (ACP) contained a negligible amount of hydroxide ion, as expected, although they differed considerably in their acid phosphate contents and Ca/P ratios. The "stoichiometric" hydroxylapatites, however, contained within experimental error the predicted amounts of calcium and hydroxide ions for HA with only small amounts of carbonate and acid phosphate as impurities.

The "unit cell" compositions, based on six phosphate ions per unit cell, for all crystalline DHAs analyzed in this investigation are given in Table IV. A wide variety of samples were prepared with Ca/P molar ratios varying from 1.40 to 1.62 and hydroxide contents ranging from 0 to 1.13 per theoretical unit cell. Acid phosphate and carbonate contents also

differed considerably, depending on the preparative conditions and the maturity of the precipitates, and varied between 0.03 to 1.11 and 0.01 to 0.28 ions per unit cell, respectively. The results of Table IV show that compositional variation in the DHAs was largely dependent on both pH and maturity of the precipitates.

Table V compares the chemically determined hydroxide contents of the precipitates, listed in order of increasing Ca/P ratio, with those predicted by the six compositional formulas in Table I. The best agreement between chemically determined and predicted hydroxide contents was obtained by formulas III and IV proposed by Kuhl and Nebergall.⁷ Because of this agreement, an attempt was made to determine, from the chemical evidence, which type of vacancies were present in the DHA structures. Two types of calcium vacancies were considered: X type, or the loss of a calcium ion coupled with the loss of a hydroxide and the addition of a hydrogen ion, and Y type, or a missing lattice calcium ion coupled with vacancies in two hydroxide positions. The numbers of both types of vacancies were calculated from the acid phosphate and hydroxide content data of Table IV, and the results are presented in Table V. The X-type vacancy per unit cell was estimated from the acid phosphate content of the precipitate and varied from 0.03 to 1.11. The Y-type vacancy was computed as half the difference of total hydroxide and X-type vacancy. This type of calcium ion defect appears to be insensitive to the conditions used for the preparations and variations in composition because all 16 DHAs had an average value of 0.45 ± 0.17 SD.

Discussion

The spontaneous precipitation of calcium phosphate at near physiological conditions involves the participation of at least

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Table V. Comparison of Analyzed Hydroxide Contents to Those Predicted by Various Formulas for Defect Calcium Hydroxylapatites and the Calculated Number of X- and Y-type Vacancies per Unit Cell

Ca/P (molar ratio)	anal. OH	I ^a	II ^a	III ^a	IV ^a	V ^a	VI ^a	X type ^b	Y type ^b
1.40	0.10	2.00	0.89	-0.05	-0.11		1.00	1.11	0.40
1.41	0.05	2.00	0.88	0.04	-0.01		1.00	1.12	0.42
1.43	0.41	2.00	0.96	0.19	0.17		1.00	1.04	0.28
1.45	-0.03	2.00	1.55	-0.20	-0.43		1.00	0.45	0.78
1.47	0.64	2.00	1.21	0.46	0.39		1.00	0.79	0.28
1.49	0.81	2.00	1.29	0.58	0.54		1.00	0.71	0.24
1.50	0.49	2.00	1.65	0.32	0.00	1.65	1.00	0.35	0.58
1.50	0.75	2.00	1.40	0.66	0.60	1.40	1.00	0.60	0.32
1.52	1.11	2.00	1.39	0.90	0.88	1.39		0.61	0.14
1.54	0.67	2.00	1.80	0.65	0.35	1.80		0.20	0.56
1.57	0.90	2.00	1.87	0.92	0.57	1.87		0.13	0.48
1.58	0.86	2.00	1.91	1.43	0.83	1.91		0.09	0.52
1.58	0.62	2.00	1.90	1.05	0.76	1.90		0.10	0.64
1.58	0.86	2.00	1.87	1.13	0.84	1.87		0.13	0.50
1.59	0.69	2.00	1.87	1.16	0.84	1.87		0.13	0.59
1.62	1.13	2.00	1.97	1.52	1.05	1.97		0.03	0.42

^a Roman numerals correspond to general compositional formulas in Table I. ^b Number of vacancies per unit cell as predicted by compositional formula III in Table I.

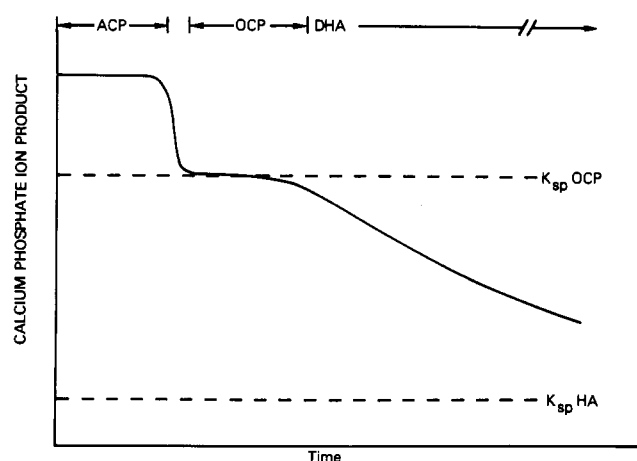


Figure 1. Phase transitions that occur upon the spontaneous precipitation of calcium phosphate in aqueous solutions. Amorphous calcium phosphate (ACP) is the first-formed phase, which transforms after a reproducible time period into a crystalline phase with the thermodynamic solubility of octacalcium phosphate (OCP). After a predictable length of time OCP transforms into defect hydroxylapatite (DHA), which gradually grows and matures into a crystalline phase with the thermodynamic properties of hydroxylapatite (HA). Dashed lines indicate the approximate calcium phosphate ion product for the solubility products, K_{sp} , of OCP and HA for an arbitrary set of conditions in this schematized drawing.

three distinct phases as suggested by previous work.^{26,27} These phases and phase changes are schematized in Figure 1. An amorphous form, ACP, is first formed and is so characterized by its diffraction and spectroscopic properties. It has been shown, however, to have a structural unit with a rather well-defined chemical composition,³⁵ and a consistent thermodynamic solubility product can be assigned.²⁶ The kinetic stability of ACP is greatly dependent upon solution conditions. At constant temperature, the most important condition is pH; with increasing pH, ACP stability increases substantially. Two samples of ACP, isolated at pH 8.25 and 10.00, were analyzed by the methods used in this study for comparison purposes and, as expected, contained no hydroxide ion (Table III).

Although ACP spontaneously transforms into crystalline apatitic material, an intermediate calcium phosphate phase appears to be involved in the pH range 7–9.²⁷ This intermediate phase is characterized by a thermodynamic solubility product consistent with that of OCP. The kinetic stability of

this phase is also greatly dependent on the pH of the system with an increase in pH decreasing its lifetime in solution. In fact, at pH greater than 9 the amorphous-crystalline transition occurs without an apparent intermediate phase. Because of the similarity of this intermediate calcium phosphate phase to OCP, minimal hydroxide was expected in samples isolated during this secondary transition. Three samples of the OCP-like phases were prepared at pH 7.00, 7.40, and 8.25. Although their overall chemical compositions were similar to those of materials containing hydroxide isolated at later stages, these OCP-like phases were unique in that they contained no appreciable amounts of hydroxide.

If the precipitates are left in equilibrium with the solution phase for sufficient time, DHAs are formed and the calcium phosphate ion product in solution asymptotically approaches the solubility product of HA. It is doubtful, however, that pure thermodynamic HA is ever obtained, at least at near physiological conditions, because rather vigorous conditions must be employed to obtain stoichiometric HA. The most generally successful methods involve precipitation at elevated pH and temperature for extended time periods or rely completely on hydrothermal techniques. Several of these stoichiometric HA preparations were analyzed, for comparison purposes, by the methods used to study the DHAs and, as indicated in Table III, all contained, within experimental error, the predicted unit cell content of hydroxide ion for pure HA.

Previous X-ray diffraction analyses^{26,36} showed that precipitates collected at time intervals and solution chemistries corresponding to the OCP and DHA regions in Figure 1 were apatitic in structure, although poor resolution and weak intensities in X-ray patterns due to thinness in crystals collected in the OCP region did not allow a clear distinction between OCP structure and more HA-like structure. Moreover, one of the X-ray diffraction lines previously observed for these apatitic samples had a d spacing larger than that of HA.³⁶ The spacings of this apparent d_{100} apatite line were largest for samples of lower Ca/P collected in the OCP region, and this d spacing progressively decreased with apatite maturation and increasing Ca/P and approached, but never reached, that of stoichiometric HA. Brown et al.³⁷ have made innovative calculations that showed this apparent d_{100} apatite diffraction line and its larger spacing, previously used to suggest a -axis increases in the apatitic pH 7.40 preparations with decreasing Ca/P ratios,³⁶ both could arise from diffraction properties of

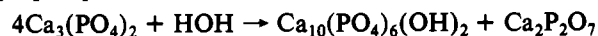
(36) Eanes, E. D.; Meyer, J. L. *Calcif. Tissue Res.* 1977, 23, 259.

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interlayered OCP-HA mixtures rather than from apatite with an expanded a axis. Brown et al.³⁷ attributed this apparent apatite diffraction line to an OCP-HA combination line that appears when OCP-HA individual interlayers are a few unit cells thick and the combination line shifts, with decreasing Ca/P ratios, to increasing proportions of OCP in the interlayered mixtures. Comparisons of the calculated and experimental X-ray values showed³⁷ a reasonably good correlation to indicate OCP-HA interlayered structure in the pH 7.40 preparations; however, the HPO_4^{2-} values for these samples were about half those expected with the assumption that these samples were OCP-HA mixtures.³⁶ No X-ray diffraction analyses were carried out on the samples in Table IV; however, 13 of the 16 samples were collected in the DHA region and were expected by comparison with the previous X-ray data³⁶ to be apatitic in structure; 5 of these 13 DHA samples were prepared at high pH (9.75 and 10.00), which reduced the possibility of OCP as a precursor phase or interlayered OCP-HA mixtures in these samples;²⁷ 3 were collected in the OCP region and as indicated in Table IV and above may have contained some OCP.

Pyrophosphate contents in excess of those predicted according to HPO_4^{2-} originally present have been indicated to occur in the pyrolytic reactions of OCP.³⁸ The excess HPO_4^{2-} was derived from H^+ liberated from HOH splitting to furnish OH^- in HA formation, and the excess HPO_4^{2-} was enhanced on pyrolysis in a water vapor atmosphere.³⁸ The extent to which this secondary type of reaction occurs causes uncertainty in the pyrolytic assay for HPO_4^{2-} in apatitic calcium phosphates. Pyrolysis under vacuum was employed here in an effort to minimize water vapor and possibly reduce pyrophosphate production from secondary sources. The vacuum-air-tube pyrolytic experiments, which incorporate increasing water vapor pressure as a major variable, were conducted to further assay this range of uncertainty.

The low-carbonate-containing DHAs ($\sim 0.2\%$) in Table II showed a barely significant increase in pyrophosphate with increasing water vapor pressure. This suggests that pyrolysis of low-carbonate-containing DHAs at 550 °C under either vacuum or air probably yielded near maximum pyrophosphate production because the higher water vapor pressure pyrolysis in tubes did not significantly increase pyrophosphate contents. Also, the lack of significant pyrophosphate increase between the vacuum and tube pyrolysis suggests that excess secondary pyrophosphate formation by, for example

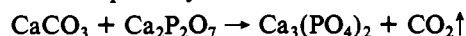


was minimal at 550 °C in about 2–3 atm of water vapor pressure.

The samples containing higher carbonate contents (~ 1 to 2%), overall, showed progressively higher pyrophosphate values and progressively lower carbonate values with increasing water vapor pressure. Pyrolytic mechanisms that involve CO_3^{2-} and HPO_4^{2-} loss by



or $\text{P}_2\text{O}_7^{4-}$ consumption by



would reduce pyrophosphate contents; however, the present results showed that increased carbonate loss during pyrolysis correlated with an increased pyrophosphate content rather than a decrease. Since the majority of the carbonate was retained under vacuum pyrolytic conditions and was still in phosphate positions from infrared analysis, vacuum pyrolysis of apatites containing both HPO_4^{2-} and CO_3^{2-} may yield HPO_4^{2-} values

more nearly correct than those of pyrolysis under air or atmospheres of higher water vapor pressure; however, further experimental data are needed to check this.

The HPO_4^{2-} contents of the fresh DHAs in this paper plot considerably lower, as a function of Ca/P ratio, than most HPO_4^{2-} values reported in the literature.^{8,9,31,39,40} The literature-cited acid phosphate values were obtained from DHAs pyrolyzed at 500–600 °C in air. The vacuum-air pyrolytic data indicate the lower acid phosphate contents observed here were not caused by vacuum pyrolytic conditions. Differences in conditions of the DHA preparation, washing, drying temperature, storage environment, surface area, carbonate content, and sample age at analysis time could account for the acid phosphate differences between samples here and those in the literature. The DHAs used in this study were separated from solution by filtering, briefly washed, dried by lyophilization after liquid-nitrogen freezing at temperatures no higher than 25 °C, stored over calcium sulfate, and analyzed within about 3 months. These same samples that had aged about 36 months had substantially increased in acid phosphate and apatite hydroxide contents. The increase in both HPO_4^{2-} and apatite OH^- in these aged DHAs that contained about one HOH per PO_4^{3-} suggests that HOH molecules split to furnish OH^- for further apatite hydroxylation and/or apatite formation and H^+ for additional HPO_4^{2-} formation. These solid-state changes occurred slowly at room temperatures (~ 20 – 25 °C). It is very probable that drying at usual temperatures (~ 110 °C) would have accelerated these changes; however, compositional changes with temperature were not explored.

In summary, it appears that the acid phosphate values obtained and used here in compositional computations were not experimentally low but instead reflected the DHA's acid phosphate contents at earlier stages of apatite maturation.

The principal objective of this study was to compare the results obtained from a complete chemical analysis on a number of solution-prepared DHAs of varying compositions with those predicted by the various defect apatite structure formulas. The data for the DHAs (Table IV) indicate that crystalline materials were obtained with Ca/P molar ratios varying from 1.40 to 1.62. Although the acid phosphate contents of the DHAs tend to decrease with increasing Ca/P ratio, there appears to be no strong correlation between Ca/P ratio, or any of the other measured parameters, and the hydroxide content. Instead, the average number of hydroxides per unit cell appears to be more dependent on the pH of the preparations and the age of the precipitates. As a consequence, previous experimental studies that predicted hydroxide content by indirect means, on the basis of measurement of the other lattice constituents, are generally in error, as are the compositional formulas that arose from those investigations. The compositional formula that seems to best represent the data (Table V) is that proposed by Kuhl and Nebergall,⁷ which takes into account two of the possible types of calcium vacancies that maintain electroneutrality. Their general formula III (Table I) assumes that a calcium vacancy can be electrically compensated by either loss of two hydroxides (Y type) or the loss of one hydroxide coupled to the addition of a hydrogen ion to the lattice (X type). The correlation between the measured hydroxide content and that predicted by formula III, in which measured HPO_4^{2-} and Ca^{2+} values were used for the X and Y terms, is good considering the magnitude of the errors involved. The correlation becomes even better, at least for the pH 9.75 and 10.00 samples, if the alternate formula IV is used, which allows for substitution of HPO_4^{2-} by CO_3^{2-} .

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It is obvious that formula I, which assumes that the stoichiometric number of hydroxides is necessary for lattice stability, is in error. Also in apparent error is formula II that assumes only X-type vacancies over the entire range $0 \leq X \leq 2$. A similar conclusion can be made about the compositional formulas that are restricted to Ca/P molar ratios either greater than 1.50 (formula V) or less than 1.50 (formula VI). Formula VI makes the further assumption that at least one hydroxide ion must be present for lattice stability, yet it is apparent that many DHAs were prepared with less than one hydroxide per unit cell.

It is interesting that the number of X-type vacancies tended to decrease as the Ca/P ratio increased whereas the number of Y-type vacancies remained relatively constant at a value of approximately 0.5 per unit cell. This may explain why the DHAs prepared under mild aqueous conditions appeared able to only incorporate about half the maximum amount of hydroxide ion in the crystal lattice. It is impossible to determine, however, whether structural constraints initially excluded the calcium or the hydroxide ions from their positions in the lattice, resulting in the loss of an electrically equivalent amount of the counterion. X-Type vacancies were most prevalent in precipitates formed at lower pH. This was probably reflective of the chemical environment in which the crystalline phases were formed, which was relatively higher in acid phosphate and lower in hydroxide ion and consequently may not be related to the structural requirements of the DHAs.

Another interesting feature of the data in Table IV, often ignored in the consideration of the structure of DHAs, is the presence of a considerable amount of water in the lyophilized samples. On a molecular basis, the number of water molecules is comparable to that of the phosphate ions, which are generally thought to be primarily responsible for maintaining structural integrity of the apatite lattice.⁴¹ The high surface

areas of these DHAs (~ 130 – 170 m²/g) could easily accommodate a considerable portion of the water; nevertheless, even small amounts of the water in the lattice could interfere with filling of the hydroxide and/or calcium vacancies and result in the observed vacancy in at least half the hydroxide positions. Contractions in the *a*-axis dimensions of the DHAs heated in the 200–400 °C range have been correlated with dehydration and loss of structural water.⁴²

The complete chemical compositions of the DHAs reported here were compared to various defect apatite formulas, and possible structural defects in their apatite lattices were inferred on the basis of a lattice model composed of stoichiometric HA with a negligible surface contribution. The large surface contributions of about 130–170 m²/g for these small-size DHAs and the unknown extent of compositional and structural coherence at their surfaces cause uncertainty in the degree of structural imperfection and defects inferred. With neglect of surface contributions, the implicated degree of defects in the DHAs studied here with Ca/P ratios greater than 1.5 is conceptually more realistic than that with ratios considerably less than 1.5. It may be difficult for a HA lattice to maintain its structural integrity with the degree of inferred structural defects for the DHAs with Ca/P ratios much less than 1.5. A less defect and more structurally integral model in this low Ca/P ratio region is a partially hydrolyzed OCP lattice or interlayered OCP–HA lattice.⁴³

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Registry No. Hydroxylapatite, 1306-06-5.

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Contribution from the Department of Chemistry,
Florida Atlantic University, Boca Raton, Florida 33431

Eight-Coordinate Complexes of Molybdenum with 1,1-Dithio Ligands. Correlation of Redox Potentials with Ligand Substituent Parameters and Spectroscopic Properties

DORIS A. SMITH and FRANKLIN A. SCHULTZ*

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Electrochemical half-wave potentials and charge-transfer absorption, EPR, and X-ray photoelectron spectral properties are reported for a series of eight-coordinate molybdenum complexes with dithiocarbamate, thioxanthate, and 1,1-disubstituted ethylenedithiolate ligands. The complexes undergo reversible Mo(VI)/Mo(V) and Mo(V)/Mo(IV) electron transfers with $E_{1/2}$ for a given charge-transfer step spanning a range of 2 V for the ligands studied. Shifts in $E_{1/2}$ are correlated successfully with parameters expressing the electron-donating or -withdrawing capabilities of ligand substituent groups. Charge-transfer absorption energies and Mo(V) EPR spectral properties experience relatively little change with ligand structure. However, Mo 3d and S 2p XPE binding energies exhibit significant changes with ligand structure and correlate well with electrochemical half-wave potentials. Results are interpreted in terms of a qualitative molecular orbital model for eight-coordinate Mo(VI), -(V), and -(IV) complexes in which reversible electron transfers occur within a d_{xy} orbital of nearly pure metal character. The energy of this orbital increases or decreases in response to the amount of negative charge inductively donated from the ligands to the metal through orbitals not involved in the electrochemical charge-transfer process. However, the character of the Mo d_{xy} orbital and its position relative to other orbitals in the molecular manifold appear to change very little as ligand structure is varied.

Introduction

Interest in the chemistry of molybdenum complexes with sulfur-containing ligands arises from the knowledge that extensive Mo–S bonding occurs in molybdenum-containing enzymes.¹ Dithio acid and 1,1-dithiolate ligands^{2–4} provide

transition metals with a sulfur-rich coordination environment. Mononegative dithiocarbamate,^{4–8} dithiobenzoate,^{9,10} and

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