Chemical Diversity of Apatites

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Abstract. Apatites can accommodate a large number of vacancies and afford multiple ionic substitutions determining their reactivity and biological properties. Unlike other biominerals they offer a unique adaptability to various biological functions. The diversity of apatites is essentially related to their structure and to their mode of formation. Special charge compensation mechanisms allow molecular insertions and ion substitutions and determine to some extent their solubility behaviour. Apatite formation at physiological pH involves a structured surface hydrated layer nourishing the development of apatite domains. This surface layer contains relatively mobile and exchangeable ions, and is mainly responsible for the surface properties of apatite crystals from a chemical (dissolution properties, ion exchange ability, ion insertions, molecule adsorption and insertions) and a physical (surface charge, interfacial energy) point of view. These characteristics are used by living organisms and can also be exploited in material science.

Introduction.

Apatite can be defined as a crystallographic structure [1]. It corresponds to the chemical formula:

$$[A(1)]_2[A(2)]_3(BO_4)_3X,$$

where A are generally bivalent cations distributed on two crystallographic sites, and BO_4 and X are respectively trivalent and monovalent anions. The high-symmetry members of the apatite family crystallise in the hexagonal system (space group $P6_3/m$). Many mineral compounds crystallise with the apatite structure and more than 75 chemically different apatites have been recently discussed and reviewed [1]. In addition to biological uses, apatites are involved in catalysis, optical devices, solid electrolytes and in environmental remediation. This paper focuses on the chemical and structural characteristics of apatite biominerals and biomaterials.

Calcium phosphates are synthesised by many organisms, ranging from isolated cells to invertebrates and vertebrates. The formation of calcium phosphates in primitive organisms is believed to enable the storage and regulation of essential elements such as calcium, phosphate and possibly magnesium. The morphology of precipitates in these organisms (small intracellular nodules of amorphous calcium phosphates often located in mitochondria) complies with the necessities for rapid mobilisation and intracellular control of the concentration of these elements. The excretion of these nodules out of the cell allowed new biological functions to be added to the primitive ion reservoir function. For example calcium phosphates are still used by a few organisms to eliminate toxic elements as in gastropods *Littorina littorea*, [2] and allow them to survive in polluted environments. This function uses subtle chemical properties of apatites, and especially their ability to incorporate foreign ions. The formation of extracellular calcium phosphates and the control of the calcification processes (and of the architecture of such calcifications) culminate in vertebrates and result in new functions for mineralised tissues (structuration of soft tissues, muscle attachment and mobility, protection of vital organs, defence and attack organs) in addition to the

primitive ion reservoir function. During the slow process of evolution, these biological uses involved progressively elaborated characteristics and specificities of calcium phosphates chemistry.

Biological calcium phosphates

Unlike calcium carbonate and other biominerals, biological apatites show a broad range of chemical compositions. This diversity has generated several theories and hypotheses nourished by the lack of information available on mineralised tissues. In primitive organisms, calcium phosphates are generally amorphous. In elaborated organisms, especially in vertebrates, calcium phosphates are crystallised and their crystallisation mode (generally as apatite) as well as the location, size and morphologies of crystals are well controlled. On the contrary, a wide diversity exists among pathological calcifications, where apatites but also whitlockite, octacalcium phosphate, brushite and calcium pyrophosphates have been identified [3]. Since the first structural determinations by X-ray diffraction [4], the constituent of hard tissues of vertebrates (bone, dentine, enamel) is frequently identified to hydroxyapatite, generally written as [5]:

$Ca_{10} (PO_4)_6 (OH)_2$ (I).

However, the chemical composition of biominerals does not correspond to this ideal formula: they all contain carbonate and the Ca/P ratio can vary substantially from the stoichiometric value. The composition of bone and of most apatite biominerals from normal or pathological mineralisations, cannot be represented by a unique chemical formula. Nevertheless, regarding the most abundant ionic species the following one, introducing a non-stoichiometry parameter (x), seems to describe correctly most biological apatites [6]:

$Ca_{10-x} (PO_4)_{6-x}(HPO_4^{2-} \text{ or } CO_3^{2-})_x (OH^- \text{ or } ½CO_3^{2-})_{2-x}$ with $0<x<2$ (II)

The loss of negative charges induced by the replacement of trivalent PO_4^{3-} ions by bivalent ions (mainly carbonate and hydrogenphosphate) is compensated by the creation of a cationic vacancy and an anionic vacancy in monovalent anionic sites. The carbonate ions occupying OH^- sites (historically referred to as type A carbonate) represent about 10% in mature enamel and probably less in bone. Most carbonate ions substitute for phosphate ions (type B carbonate). These two locations of carbonate species can be distinguished using spectroscopic methods, especially FTIR and Raman spectroscopies [7,8]. Most apatite biominerals contain HPO_4^{2-} ions observable, also, by spectroscopic techniques (FTIR, Raman and Solid State NMR) [9,10]. The quantitative determination of the amount of HPO_4^{2-} is, however, difficult and can only be approached by FTIR [11]. The occupancy of trivalent ionic sites either by phosphate ions or by bivalent ions (HPO_4^{2-} and CO_3^{2-}) is always close to 6 and the non-stoichiometry index of biominerals (x) can thus be determined from the Ca/(P+C) ratio (x = 10-6*[Ca/(P+C)]). Carbonate and HPO_4^{2-} ions play complementary roles: in young bones, regardless of the species, the amount of HPO_4^{2-} is very large and close to the maximum (x=2) whereas the carbonate content is very low. In old bones the amount of carbonate increases and that of HPO_4^{2-} decreases, however the substitution ratio of PO_4^{3-} ions by bivalent ions remains close to the maximum [12]. Bone composition at all ages in many vertebrates can be represented by the following formula:

$Ca_{8.3} (PO_4)_{4.3}(HPO_4^{2-} \text{ or } CO_3^{2-})_{1.7} (OH^- \text{ or } ½CO_3^{2-})_{0.3}$.

The composition of dentine mineral is probably very close. Tooth enamel composition on the contrary can vary, but the x value is always very low. A typical composition of human enamel can be represented by:

$Ca_{9.4} (PO_4)_{5.4} (HPO_4 \text{ or } CO_3)_{0.6} (OH \text{ or } ½ CO_3)_{1.4}$

The amount of OH^- ions in biominerals is variable and related to the amount of bivalent ions replacing PO_4^{3-} groups. Thus, it is very low in bone and dentine and OH^- groups can effectively be barely detected using spectroscopic techniques [13]. In mature enamel on the contrary OH^- ions are clearly observed.
The composition variations of apatite biominerals are related to the biological function of the tissue. Bone mineral has conserved the primitive function of ion reservoir and participates in homeostasis. The large amount of ionic vacancies reduces the cohesion of apatite crystals and increases their solubility thus facilitating the release of essential elements. On the contrary enamel crystals have to resist to acidic pH and therefore exhibit a composition close to stoichiometric hydroxyapatite with a minimum amount of vacancies. This adaptability is unique among biominerals and the same structure can exhibit very different properties depending on its composition and amount of vacancies. The reasons for the variation of the carbonate content with age in bone could be related to the rate of bone remodelling, but the role of this variation on the biological properties of the crystals has not yet been clarified.
An additional complexity of apatite biominerals has been revealed by spectroscopic techniques. Several studies have indeed shown the existence, especially in bone, of ionic environments different from those occurring in well crystallised synthetic apatites, called "non-apatitic" environments [8,9], considered to belong to a hydrated layer on the surface of the crystals. The ionic species of this layer exhibit a high mobility and they can be rapidly and reversibly exchanged in solution by other mineral species. Such a mobility could explain the rapid release, for example, of carbonate species in case of acidosis. The study of bone mineral at different ages indicates that the non-apatitic environments are more abundant in young bones than in mature or old bones [14]. The study of the formation and maturation of synthetic analogues of bone mineral suggests that the existence of non-apatitic environments is related to the formation mode of apatite at physiologic pH. They are believed to decrease the interfacial energy and to favour the nucleation of the crystals. The slow-forming apatite domains then develop at the expense of the hydrated domains and possibly incorporate some of the ions of the hydrated layer [15]. Once formed, the composition of the apatite domains is frozen and the incorporated species cannot be further exchanged. Thus, the reactivity of apatite nanocrystals analogous to bone mineral is mainly determined by thepresence and characteristics of the surface hydrated layer containing labile species in a non-apatitic environments. The irreversible evolution of the bone crystals towards a relatively inert and stable apatite unable to maintain active exchanges with body fluids could constitute a physico-chemical justification for bone remodelling in superior vertebrates. The preservation of the hydrated layer is also insured by the presence of ions such as carbonate, magnesium and pyrophosphates which inhibit the growth of apatite domains. The difference of carbonate and/or magnesium content in enamel and bone could therefore be related to different crystal growth controls: the existence of a large exchange surface area for bone is a necessity whereas it would be a drawback for the stability of enamel crystals.

The existence of a hydrated layer with a composition different from that of bulk crystals can modify the description of biological apatites. The composition of the hydrated layer is not known with accuracy. It seems to contain essentially bivalent ions Ca^{2+}, HPO_4^{2-} and CO_3^{2-}. Thus, it can be inferred that the apatite domains are probably less rich in vacancies than suggested initially.

In addition to major elements, all hard tissues contain a multitude of minor elements. But they do not reach concentrations high enough to alter significantly the major elements composition. Among different trace elements trapped in bone, biologists distinguish essential elements with a positive biological activity, neutral and toxic elements. From a chemical point of view, it is often difficult to determine whether these trace elements belong to the organic matrix or to the mineral, and this point can lead to misinterpretations especially for elements present at very low concentrations. Considering the mineral fraction, trace elements may be incorporated in the apatite domains or in the hydrated layer where they remain exchangeable. Some elements like magnesium for example belong mainly to the hydrated layer [16] while others, like fluoride, are essentially

contained in the apatite domains. The first type of ions may contribute to bone-fluid equilibrium whereas the second type can only be released during a remodelling period. There is, however, partial recycling of ions during remodelling although this phenomenon is not well understood. These recycling rates are complex and related to the local precipitation conditions (local ionic concentrations, volumes, diffusion rates). They can however be roughly linked to the solubility products of the substituted apatitic phases. Thus fluoride and lead which give more insoluble apatites than regular bone apatite show a high recycling ratio, whereas Sr-containing apatites whose solubility seems higher than that of regular bone mineral would implicate a low recycling ratio. This phenomenon explains for instance the difficult elimination of fluoride and lead from bone and the easy elimination of strontium. These recycling ratios could be different in trabecular and cortical bone.

The formation of bone and enamel crystals is strictly circumvented to given domains. In addition to ionic crystal growth inhibitors, several macromolecules are involved or interfere with the crystal formation. Biological fluids are supersaturated with respect to stoichiometric hydroxyapatite. The chemical analysis of ultracentrifuged blood plasma gives an ionic product close to that of OCP [17]. This value is significantly lower than that of Simulated Body Fluids (SBF) [18] due to the existence of mineral ions bound to plasma proteins. The composition of biological fluids in the vicinity of forming enamel or forming or remodelling bone is not known, but it seems probable that there is a local control. Carbonic-anhydrase enzyme, for example, has been shown to play a role during the formation of enamel crystals. However, this does not seem sufficient to promote a spontaneous precipitation of calcium phosphates. Additional control is brought along by the existence of nucleation agents and an organic matrix able to orient crystal growth [19]. The interactions of organic molecules with apatite crystals are not known at a structural level. It can, however, be inferred that the hydrated surface layer on the crystals plays an important role.

The characteristics and the formation of hard tissues involve a sophisticated use of apatite crystals chemistry, especially concerning their surface and interface properties and their maturation ability. It is possible to obtain synthetic crystals similar to bone crystals and some of the progress made in the knowledge of bone mineral rely on the study of synthetic analogues.

Synthetic calcium phosphate apatites

The synthesis possibilities of apatites offer more flexibility than the biological conditions of formation and a wider range of chemical compositions and diversity is observed. Several types of ion substitutions and degrees of nonstoichiometry have been described and analysed in the literature and we will focus on those affecting the apatite lattice and the hydrated surface layer of nanocrystals.

Bulk apatite variability. Solid solutions have been observed for many apatite compositions. They consist mainly in the substitution of the original ions by ions with the same charge and they do not disturb the electrical charge distribution in the lattice. A second type of ionic substitutions involves ions with different electrical charges than the original ones.

Considering calcium phosphate hydroxyapatite, special attention has been given to substitutions among the monovalent OH^- ions of the structure, which can considerably modify its physical-chemical properties. These ions are located on the hexagonal screw axis of the structure, but their z coordinates vary for different apatite compositions [1]. As there are two cationic sites in the structure, their occupancy may also vary depending on the substituent involved. In general smaller ions with a high coordination number prefer to be located on type I sites although the ion distribution could possibly depend on the preparation conditions and on the nature of the monovalent anion. Ion segregation and heterogeneity of distribution may occur especially for apatites prepared at low temperature by precipitation methods. Ion concentrations may not be homogeneous throughout an apatite crystal, and of course among the crystals, depending on their formation and ageing conditions. Often ignored, this fact might affect the results obtained by

diffraction techniques, especially Rietveld refinement methods which generally do not consider a possible heterogeneity of composition.

All ions of the original apatite structure may be partly or totally substituted by ions with different charges. Thus, the bivalent cationic site can be occupied by monovalent or trivalent cations and may possibly be partly vacant. Generally, vacancies are preferably found in cationic sites II [20]. Similarly, the trivalent anionic site can be substituted by divalent and/or tetravalent ions. However, no significant amount of vacancies has ever been found in this type of site, probably because the XO_4 ions are the largest ones of the structure, which would collapse in the event of ample defects. The monovalent anionic sites can be occupied by divalent ions and/or be vacant. Once again, the ion distributions might not be homogeneous throughout a crystal and among crystals. From a biological point of view the most important substitutions involve the replacement of a trivalent phosphate ion by a bivalent HPO_4^{2-} or CO_3^{2-} ion as described in the previous section.
For apatites prepared in alkaline media, however, HPO_4^{2-} ions can no longer be present and carbonate-containing apatites contain an excess of Ca^{2+} ions with regard to formula (II) used for bioapatite. These apatites are best represented by the following formula [21]:

$$Ca_{10-x+u}\,(PO_4)_{6-x}\,(CO_3)_x\,(OH)_{2-x+2u} \text{ with } x \leq 2 \text{ and } u \leq x/2 \qquad \text{(III)}.$$

An alternative formula has been proposed for apatites involving on the contrary additional Ca deficiency with regard to formula (II) compensated for by monovalent ion vacancies, in the case of HPO_4^{2-}-containing apatites [22],

$$Ca_{10-x-y}\,(PO_4)_{6-x}\,(HPO_4)_x\,(OH)_{2-x-2y} \text{ with } x \leq 2 \text{ and } y \leq (1-x/2) \qquad \text{(IV)}.$$

In fact formulae (III) and (IV) are similar and express the variability of calcium sites occupancy depending on the preparation pH.
Finally, another phenomenon has to be considered especially for H_2O-containing apatites: the internal hydrolysis of PO_4^{3-} ions:

$$PO_4^{3-} + H_2O \rightarrow HPO_4^{2-} + OH^- \qquad \text{(V)}$$

This reaction links the HPO_4^{2-} content and the OH^- content and can explain the formation of HPO_4^{2-} groups even in apatites obtained in alkaline conditions. The most general chemical formula for non-stoichiometric apatites can be written as:

$$Ca_{10-x+u}\,(PO_4)_{6-x-y}\,(HPO_4^{2-} \text{ or } CO_3^{2-})_{x+y}\,(OH)_{2-x+2u+y} \text{ with } 0 \leq x \leq 2 \text{ and } 0 \leq 2u+y \leq x$$

In the presence of sodium ions, the substitution of Ca^{2+} by Na^+ could also possibly compensate for, but only partly, the substitution of phosphate by carbonate ions [23].

Surface ion substitutions. Precipitated apatite crystals generally exhibit a structured hydrated surface layer containing mainly bivalent cations and anions. This feature should not be confused with the Stern double layer that forms on most mineral surfaces in solution. The hydrated layer of apatite is part of the crystal and its thickness can vary according to the age of the crystals and their conditions of formation. The non-apatitic environments present in synthetic apatites and biological apatites exhibit similar spectroscopic characteristics. However the study of freshly precipitated apatite has revealed that the structure of the hydrated layer is deeply affected by drying [24]. The non-apatitic environments observed in bone and synthetic dry analogues correspond in fact to a disordered hydrated layer resulting from the collapse of a very fragile structured hydrated layer existing only in wet media. The substitution possibilities in the hydrated layer are not well known but seem to be greater than in the core of the apatite structure. Magnesium ions for example, which hardly penetrate the apatite lattice, are easily and reversibly incorporated into the hydrated layer in

large amounts [16]. Ion substitutions in the hydrated layer considerably modify its structure although these alterations seem reversible in most cases [25].

Apatites and molecules. One characteristic of apatite compounds is the ability to incorporate individual molecules. It is generally believed that the vacancies in the apatite structure are occupied by water molecules and recent studies performed by solid state NMR have confirmed this ability. These molecules can be easily and irreversibly released on heating.

Other molecules can, however, be trapped in apatites. The example of molecular oxygen is one of the most interesting [26]. The oxygen molecules have been shown to form inside the structure through the decomposition of unstable precursor: peroxide ions and hydrogen peroxide molecules trapped in the structure during synthesis. An interesting feature is that the molecular oxygen remains trapped in the apatite until decomposition well above the temperature at which water molecules are released, probably because of diffusion hindrance due to its size. The trapping of carbon dioxide has also been observed during the thermal decomposition of biological apatites, but its location within crystals or in the crystals assembly could not be determined. Small organic molecules have also been incorporated in apatites during synthesis: formiate ions, and also glycine molecules [27]. The zwitterion, corresponding to glycine, was believed to have the carboxylate group on a monovalent anionic site and the amine group on a contiguous cationic (II) site.

The incorporation of organic molecules in the hydrated layer of apatite nanocrystals has not yet been studied, but adsorption studies have shown that the anionic groups of molecules substituted in a reversible way anions of the hydrated layer (carbonate or hydrogenphosphate ions). Concerning the parent structure of triclinic OCP, several hybrid organic-inorganic crystals have been prepared incorporating dicarborxylic acids [28].

Synthesis of ion-substituted apatites

Ion-substituted and non-stoichiometric apatites can be prepared in many different ways which cannot all be described in this short presentation. Substitutions in the bulk of the apatite crystals should be distinguished from surface substitutions. Two main difficulties can be identified: the heterogeneity of the substitution and the possible formation of amorphous phases.

One of the most frequently used ways for the preparation of apatite minerals is double decomposition, in aqueous medium, between a solution of a calcium salt and a solution of a phosphate salt. Cationic substituents can be added to the calcium solution and anionic substituents to the phosphate solution. The co-precipitation then leads to substituted apatites. In addition to the possible formation of foreign phases, this method does not necessarily lead to a homogeneous distribution of cations and anions in the apatite lattice, which depends on the precipitation conditions. Rapid precipitation favours homogeneous distribution, however, even in this case, some heterogeneity may appear. Concerning carbonate for example, the rapid precipitation at physiological pH and room temperature produces a first precipitate with very few carbonate ions located mainly in the hydrated layer. It is only during ageing in solution that the carbonate content increases and that the ions can enter the apatitic lattice. Concerning magnesium, in the same conditions, an amorphous phase may form. The incorporation of most ions can be accomplished by this method and several substituted apatites can be obtained without major difficulties. In the case of slow precipitation processes at higher temperature, the composition of the precipitate depends on the order of precipitation. For example, carbonate apatites obtained at a given pH and temperature may differ depending on whether the calcium solution is added to the phosphate-carbonate solution ("direct" or "LeGeros" method) or the phosphate-carbonate solution is added to the calcium solution ("reverse" method) [29]. Generally the reverse method gives the best results for anion-substituted apatites, whereas the direct method is better suited for cation-substituted apatites. Although for mixed cations giving insoluble phosphate salts and for mixed anions giving insoluble calcium salts, the substitution ratio is generally that of the precipitation solutions, most phosphate-soluble cations and calcium-soluble anions enter in the apatite lattice with difficulty. The incorporation of a

significant amount of chloride ions in Ca-P apatites, for example, necessitates a large excess of this ion in both solutions.

The ion compensation mechanisms, and/or multiple substitution, facilitate the introduction of foreign species; for example the incorporation of silicate ions is easier in the presence of trivalent ions such as La^{3+}. Similarly sodium is preferably taken up in type B carbonate apatites where carbonate ions replace part of the phosphate ions [23].

Methods other than direct precipitation from aqueous solutions may be used to obtain substituted apatites (acid-base precipitation, alkalinisation of acidic solutions, hydrolysis of unstable intermediates, high-temperature reactions, non-aqueous reactions, sol-gel methods, mechano-synthesis). An interesting method is based on gas-solid ion exchange reactions [5, 16]. These reactions essentially concern the monovalent ions and type A carbonate. Due to their position, in narrow channels, these ions can be substituted by gas-solid exchanges at high temperatures (900-1000°C) without alteration of the crystals or ceramic characteristics. Partly or totally chlorinated, fluoridated or carbonated apatites can be obtained. The main advantage of high-temperature reactions, in the domain of stability of the substituted apatites (i.e. generally 900-1300°C) is to favour the homogeneity of ion distributions within crystals and to permit the formation of highly crystalline apatites and of ceramics; the main drawback is the loss of surface characteristics and the impossibility to treat the unstable non-stoichiometric apatites.

Specific methods can be used to substitute ions in the hydrated layer on the surface of precipitated apatites. These methods are based on their ion exchange ability. The sample is put in direct contact with the solution of the ions to be substituted for calcium or hydrogenphosphate. The exchange rate is generally very fast (a few minutes). The exchangeable amount depends strongly on the characteristics of the surface of the solid phase and on the nature of the ion. For example, for the same concentration in solution, the uptake of Sr^{2+} ions is much larger than that of Mg^{2+}. Freshly precipitated apatites with a rather thick hydrated layer can take up more foreign ions than aged precipitates for the same specific surface area. As mentioned earlier some of the ions of the hydrated layer may be incorporated in the subjacent apatitic lattice and then lose the ability to be reversibly exchanged. However, some ionic species which inhibit apatite lattice growth such as carbonate and magnesium may delay the process of incorporation and preserve reverse exchange properties. The conditions of preparation and drying of the nanocrystals also affect their ability to trap foreign ions in the surface layer. For example, dried and even lyophilised samples lose part of their exchange ability. This phenomenon is probably related to the fusion of adjacent crystals upon drying and the loss of surface contact with the solution. The exchange rate also depends on other characteristics such as the concentration of the ions in the exchange solution and the liquid-to-solid ratio.

Physical-chemical consequences of ion substitutions

Ion substitutions essentially affect the solubility of apatites and their surface properties.

Solubility of ion-substituted apatites. The data concerning the solubility of ion-substituted apatites are scarce and cover only part of the wide range of substitution capabilities. Besides, there is often confusion between the amount dissolved in certain conditions and the solubility. Obviously the amount dissolved depends on the dissolution rate and whether a solubility equilibrium is reached is not always certain. In addition the dissolution of ion-substituted apatites is generally non-congruent and the solid and solution which are in equilibrium do not show the same ionic ratio. The solubility products are thus rather difficult to determine and some discrepancy may appear. One of the most widely studied systems is fluoride-hydroxide calcium phosphate apatites. Minimum solubility has been found for F/OH ratios close to 1 [30], possibly corresponding to the occurrence of O-H—F hydrogen bonding. However these results were questioned as the composition of the

surface layer could differ from that of the bulk. In any case, it is accepted that the fluoride substitution for OH- in Ca-P apatites results in a considerable decrease in solubility.

The solubility of biological, and more generally non-stoichiometric apatites, seems rather complex. From a theoretical point of view, the presence of ionic vacancies reduces crystal cohesion and might result in increased solubility. However, for bone mineral and synthetic analogues experimental solubility studies do not give a constant solubility product. The calculated solubility product (Ksp) depends on the amount dissolved and this special behaviour has led to the concept of metastable solubility equilibrium (MSE) [31]. Each sample can be characterised by a collection of Ksp values, related to the relative amounts dissolved. The global behaviour, however, seems consistent with theoretical expectations. For example, when the carbonate content increases the solubility curves move towards higher Ksp. The origin of this phenomenon has not yet been clearly identified, the nanocrystalline nature of the samples, the heterogeneity of composition of the crystals, the presence of strains, and/or the existence of a surface hydrated layer could be involved.

The effect of other types of substitutions of biological interest is less well known. From a theoretical viewpoint, the replacement of trivalent PO_4^{3-} by tetravalent SiO_4^{4-} can be compensated for by the introduction of a trivalent ion replacing Ca^{2+}, the creation of a monovalent ionic vacancy, or by coupled substitution in the trivalent anionic sites (SiO_4^{4-} and SO_4^{2-} for example). In the first case, the cohesion of the crystal should increase and the solubility should decrease which is effectively observed for britholites (apatites containing both silicate and trivalent ions). In the other cases, the global charge of the lattice ions does not change and the effect on solubility cannot be forecast. Mg^{2+} ions have been claimed to increase the solubility of apatites. In fact Mg^{2+} ions can only substitute a few percent of Ca^{2+} ions in well-crystallised Ca-P apatites. Although higher Mg contents can be reached in nanocrystalline apatites, it seems probable that the Mg^{2+} ions are essentially located on the surface of the crystals in the hydrated layer and could thus involve MES.

One of the main difficulties concerning nanocrystalline apatites is to determine the HPO_4^{2-} and OH^- contents which are often totally ignored but probably play an essential role in solubility. Thus, it has been shown that apatites freshly precipitated in carbonate-containing solutions, at physiological pH, mainly contain HPO_4^{2-} as foreign ions and a very small amount of carbonate; however during aging (maturation) in the mother solution, which contains an excess of carbonate and phosphate species, they progressively incorporate increasing amounts of carbonate ions without changing the Ca/(P+C) ratio. These experiments indicate that HPO_4^{2-}-containing apatites are less stable (more soluble) in these conditions than CO_3^{2-}-containing apatites with similar vacancies content, although their formation rate is faster. This evolution is comparable to that of bone mineral during ageing.

Surface properties of substituted apatites. Few works have been devoted to the surface properties of substituted apatites. It seems rational to consider that the surface of wet apatite minerals equilibrates with the surrounding medium and could be different from dry surfaces. Partial dissolution of stoichiometric apatite mineral could lead to the formation of surface HPO_4^{2-}. Although the surface composition could change with equilibration reactions, the effect of surface substituted ions is not known. It has been shown that the increase of the atomic weight of the ions leads to an increase of surface energy [32]. This phenomenon could change the adsorption behaviour although specific binding properties to the foreign ions also have to be considered.
Similarly, the surface charge of substituted apatites has rarely been investigated [33]. It is considered to be related to the hydrolysis of surface PO_4^{3-} or HPO_4^{2-} ions and to the adsorption of cations. The surface OH^- have also been postulated to be involved.

The adsorption properties of nanocrystalline apatites vary considerably with the maturation time and the change of the hydrated surface layer. Generally the number of adsorption sites decreases with the maturation time whereas the affinity constant deduced from Langmuir isotherms increases.

Biological consequences of ion substitutions in apatitic biomaterials

Several biological effects can be distinguished related to apatitic biomaterials composition: indirect effects (related to the modifications of the surface properties, textural properties or the materials solubility) and direct cellular effects related to ions release.

Indirect effects can be related to a series of causes. It has been shown for example that the biological activity of biomaterials was related to their propensity at nucleating a neoformed apatite layer analogous to bone mineral on their surface. The determination of the nucleation ability of biomaterials in SBF has even become a general testing procedure for expected biological efficiency [18]. Considering apatite surfaces their nucleation ability can be related to the number of active surface sites and to the supersaturation ratio. The number of active sites depends essentially on the specific surface area and the material history. The supersaturation ratio is directly related to the solubility of the apatite considered and strongly depends on ionic substitution. Fluoridated apatites should thus be more efficient than hydroxyapatite which in turn should be more efficient than non-stoichiometric apatites. Other effects are related to the modifications of surface or texture properties, but they are difficult to prognosticate due to the few elements available. It seems probable that most substituted apatites close to stoichiometry (sintered ceramics) cannot release ions and that their possible biological effect is related to changes in surface or texture properties (nucleation ability, surface charge, surface energy, exposed crystal faces, morphology, crystal size, porosity). The most obvious biological use of ion substitutions is related to the modulation of the solubility properties of the mineral phase. The vacancies content related to non-stoichiometry may thus considerably modify the apatite behaviour. It has been shown that the resorption rate of calcium phosphate biomaterials was roughly related to their solubility. Most stoichiometric apatites are non-bioresorbable and the resorption rate of non-stoichiometric apatites is directly related to their vacancies content as shown by the study of biomimetic cements [34]. The solubility of apatite nanocrystals seems however complex and it might vary with the maturation time of the material in vivo.

The spontaneous release of ions cannot occur from apatite materials with a solubility higher than the ionic product of body fluids which is close to the solubility product of OCP and higher than that of most apatites. However, a release may occur due to cell activity. The becoming of the released ions is not clear and depends probably on the local conditions as discussed for trace elements in bone. The existence of a hydrated layer would allow ions to be spontaneously released by ion exchange although this point has not yet been demonstrated in vivo. In vitro studies show that 90% of the foreign ions incorporated in the hydrated layer can be reversibly exchanged. From another point of view apatites susceptible to release large proportions of ions such as magnesium and carbonate know as apatite crystal growth inhibitors should not favour the formation of a neoformed layer from body fluids. It shall be mentioned that nanocrystalline apatite may mature in vivo and that the spontaneous release properties would depend on the stability of the hydrated layer.

Summary

The chemical diversity of apatites allow fine tuning of their physical-chemical and biological properties. The description of substituted and deficient apatites can generally be adequately reached but their consequences on the physical-chemical properties of the crystals have yet to be determined. The specific features of precipitated apatite nanocrystals with a structured hydrated layer on their surface add to the complexity of apatite structures but also opens new possibilities in material science which could not be analysed in this short review. The surface ion mobility allows processing at very low temperatures and confers to the nanocrystals properties such as electrical conductivity, ion exchange and molecular binding which could be used in the biomaterials field as well as in other domains.

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