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Dissolution of Calcium Hydroxylapatite and its Application to Biological Demineralization*

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Several aspects of the study of the rate of dissolution of calcium hydroxylapatite in aqueous solution are discussed. From a chemical point of view, this system provides the possibility to study the chemical processes taking place at the crystal surface during the dissolution process, both in the pure system and in systems containing foreign substances. From a biological point of view, the study has led to a model for the formation of subsurface lesions in tooth enamel. An approach to a model for demineralization of bone tissue comes from the study of the effect of biologically relevant molecules on the dissolution process in vitro. Citrate ions are found to inhibit the dissolution process at low concentration, but accelerate this process at higher concentrations, where considerable complexing with calcium ions occurs.

NUCLEATION-CONTROLLED DISSOLUTION OF CALCIUM HYDROXYLAPATITE (HAP)

When crystals dissolve in an aqueous suspension two consecutive processes take place, a surface process and a bulk transport process. The surface process is the transformation of crystalline material to dissolved substance situated in the interface region, the solution adjacent to the crystals. The bulk transport (diffusion or convective diffusion) process is the transport of substance from the interface region to the bulk solution. The rate of dissolution of HAP microcrystals is controlled by the surface process, the rate of which is of the order 10^{-4}—10^{-5} times the expected rate for diffusion controlled dissolution. The overall rate of dissolution can be expressed[1,2] as

$$J = km_o F\,(m/m_o)\,g\,(C) \qquad (1)$$

We refer to the list of symbols for the definitions of these.

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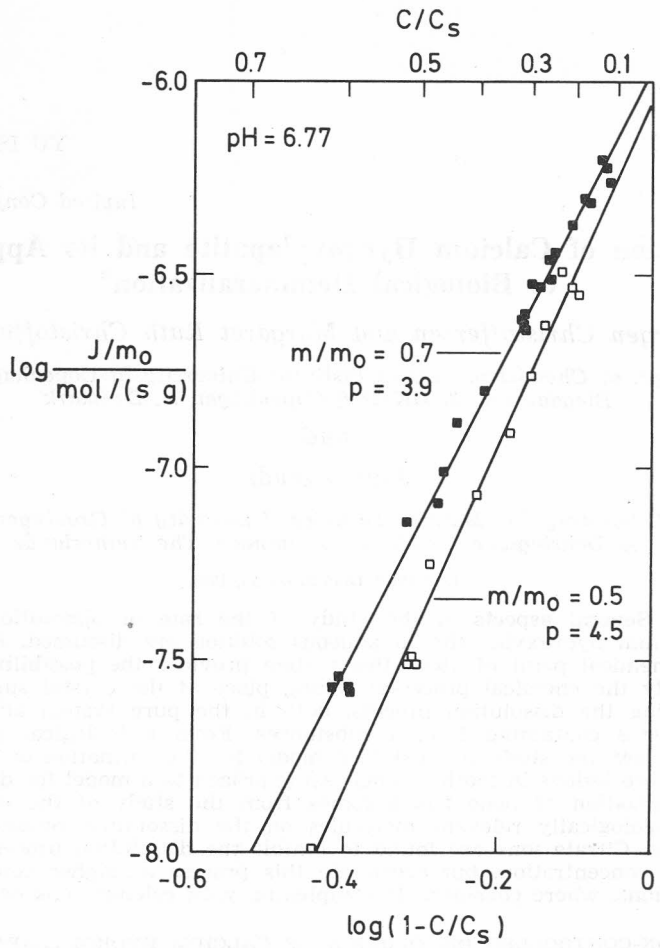

Figure 1. Plots of $log\ (J/m_o)$ against $log\ (1 - C/C_s)$ for pH 6.77. Closed squares: $m/m_o = 0.7$; open squares: $m/m_o = 0.5$. The slopes, p, of the straight lines through the points are shown in the figure.

Figure 1 shows typical plots of $log\ J/m_o$ against $log\ (1—C/C_s)$ for constant values of m/m_o and pH. The slope of such lines is 3—4, which indicates that the rate cannot be described by the unwinding of a spiral. A polynuclear dissolution mechanism can explain the rate of the surface process in the pH range 5—7.2 and in 30—90% undersaturation. Microscopic holes, dissolution nuclei, are formed in the crystal surface. These holes grow laterally and intergrow. For this mechanism the linear rate of growth perpendicular to the crystal surface can be expressed[2-7] as

$$dr/dt = k' v_+^{1/3} v^{2/3} \beta^{1/6} \exp(-\alpha/\beta) \quad (2)$$

The overall rate can similarly be expressed as

$$J = k'' m_o F (m/m_o) v_+^{1/3} v^{2/3} \beta^{1/6} \exp(-\alpha/\beta) \quad (3)$$

Hydrogen ions may react with PO_4^{3-} groups in the crystal surface, causing the interaction between Ca^{2+} and the PO_4^{3-} groups to be weakened. This results in an increased exchange rate of phosphate groups between the two phases. The lateral growth rate can thus be described as H^+-ion catalysed. The overall rate can then be expressed[8,9] as

$$J = kX_{HP} F (m/m_o) (1 - C/C_s)^{2/3} \beta^{1/6} \exp(-\alpha/\beta) \quad (4)$$

An example of a plot of $ln\ J/m_o\ (1-C/C_s)^{2/3}\ \beta^{1/6}$ against $-1/\beta$ is, for constant values of m/m_o and pH, given in Figure 2. From the slopes of such lines the values of the Gibbs surface energy (surface tension) is found to be 45 ± 5 mJ/m^2. From the intercepts of lines as in Figure 2 with the line $-1/\beta = 0$, the acidity constant $\tilde{K}_{cr'}$ for the HPO_4^{2-} surface complex, can be determined

$$\tilde{K}_{cr} \equiv (H^+)_{aq} X_p / X_{HP} \quad (5)$$

Around pH 7, where the crystals are not electrically charged, $\tilde{K}_{cr}$ is found to be 10^{-7} mol/l. At lower pH ≈ 5, $\tilde{K}_{cr}$ is found to be 10^{-6} mol/l.

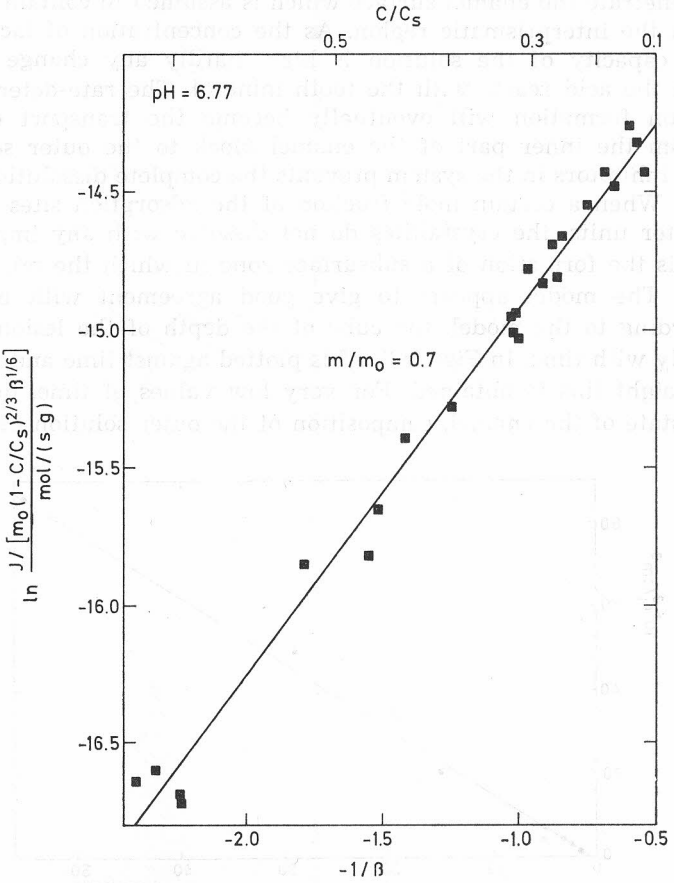

Figure 2. Plot of $ln\ J/[m_o\ (1 - C/C_s)^{2/3}\ \beta^{1/6}]$ against $-1/\beta$ for pH 6.77. This plot shows that the rate may be controlled by a polynuclear mechanism.

FORMATION AND REPAIR OF SUBSURFACE LESIONS

In biological systems inhibitors of crystal formation, growth and dissolution play an important role in controlling these processes. Many biological solutions, for example, remain supersaturated or undersaturated without the occurrence of pathological mineralization of demineralization. Of the biological mineralization and demineralization processes the easiest processes to study in vivo are formation and repair of subsurface lesions in tooth enamel. Artificial subsurface lesions can easily be formed. In recent years much work has been reported on the formation of such lesions;[10–12] less information is available for description of the corresponding repair processes.[13,14]

Christoffersen and Arends[15] have suggested a model for in vitro subsurface lesion formation in tooth enamel in contact to a solution with a high buffer capacity, pH $\approx$ 4—5, (lactic acid is often used), and containing an inhibitor for the dissolution process of HAP, for example methylene diphosphonate ions, MDP. With this outer solution having a large volume, the concentrations of calcium and phosphate ions can be assumed constant. The acid, in the form of hydrogen ions or as undissociated lactic acid and the inhibitor penetrate the enamel surface which is assumed to contain small holes, probably in the interprismatic region. As the concentration of lactic acid and the buffer capacity of the solution is high, hardly any change in pH will occur when the acid reacts with the tooth mineral. The rate-determining step in the lesion formation will eventually become the transport of dissolved mineral from the inner part of the enamel block to the outer solution. The presence of inhibitors in the system prevents the complete dissolution of enamel crystallites. When a certain mole fraction of the adsorption sites are covered with inhibitor units, the crystallites do not dissolve with any important rate. The result is the formation of a subsurface zone in which the mineral density is reduced. The model appears to give good agreement with experimental data. According to the model, the cube of the depth of the lesion, r_l^3, should vary linearly with time. In Figure 3, r_l^3 is plotted against time and a reasonable fit to a straight line is obtained. For very low values of time, depending on the initial state of the enamel, composition of the outer solution etc, the model

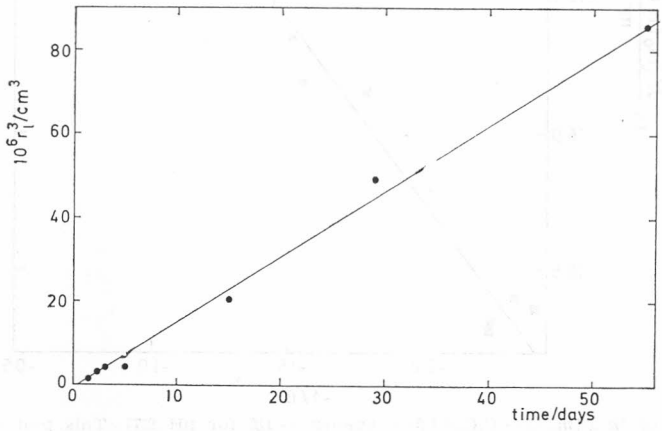

Figure 3. Third power of lesion depth versus time (ref. [11]).

cannot be used. Accurate experimental data are lacking for description of this transient period.

Many investigators are at present studying lesion repair. We have suggested[16] a model explaining why lesions can be repaired from the base of the lesion upwards, and not only repaired near the top. The model is based on the ratecontrolling process being the surface process by which the crystallites grow when a enamel block containing a subsurface lesion is in contact with a suitable supersaturated solution. For this model the small holes in the enamel surface through which substance can be transported between the outer solution and the inner part of the lesion, have to be larger than the linear dimension of the enamel crystallites. Smaller holes will be filled with mineral prior to repair of deeper lying parts of the original lesion. For the further understanding and description of enamel subsurface lesion formation and repair, and for a beginning to a physico-chemical understanding of bone formation and resorption, there is an urgent need for good experimental data describing, not only adsorption isotherms of inhibitors, but also the effects the inhibitors have on crystal formation, growth and dissolution processes, i. e. data illustrating the interplay between adsorption and crystal growth processes.

INHIBITION OF THE DISSOLUTION OF HAP

Despite the importance of crystal growth inhibitors in biological systems and in many industrial processes, hardly any theory exists for the inhibition of crystal processes, except for the work by Cabrera and Vermilyea.[17] We have discussed[8] a number of cases of Langmuir-like adsorption isotherms for which the kinetic effects of inhibitors can be described by an equation of the type

$$J_o/J_L = 1 + K_{kin}\, C_L \tag{6}$$

J_L and J_o are the crystal growth or dissolution rates determined with and without the inhibitor present, keeping all other parameters constant as far as possible. C_L is the concentration of inhibitor in the solution and K_{kin} is a constant, which can be related to the equilibrium adsorption constant. K_{kin} will in general increase as equilibrium with respect to the growth or dissolution process is approached. The effect of many inhibitors for the dissolution process of HAP is quite sensitive to small changes in pH around pH 7. This is the case, for example, for 3-Phosphoglyceric acid, 3-Phosphoserine and phosphoethanolamine, whereas the effects of 3-Amino-1,1,3-propanetricarboxylic acid (γ-carboxy-glutamic acid), pyrophosphoric acid and methylenediphosphoric acid (MDP) are not sensitive to changes in pH around pH 7.

It has recently been shown,[18] that the adsorption of MDP on HAP can be described as being bidentate, corresponding to each MDP molecule on the crystal surface occupying two phosphate sites. The adsorption isotherm is found to have the form

$$K_L = \frac{x}{(1-x)^2\, C_L} \tag{7}$$

in which x is the mole fraction of surface phosphate sites occupied by MDP. In the concentration range where the kinetic effect of MDP can be measured, the effect can be represented by eq. (6).

EFFECT OF CITRATE IONS ON THE RATE OF DISSOLUTION OF HAP

Citrate ions have long been known to affect processes involved in the formation of calcium phosphates. Brecević and Füredi-Milhofer[19] have reported that citrate ions adsorb on colloidal calcium phosphate precipitates and slow down the transformation of these particles to octa-calcium phosphate. The effect of citrate ions on the rate of dissolution of HAP is shown in Figure 4,

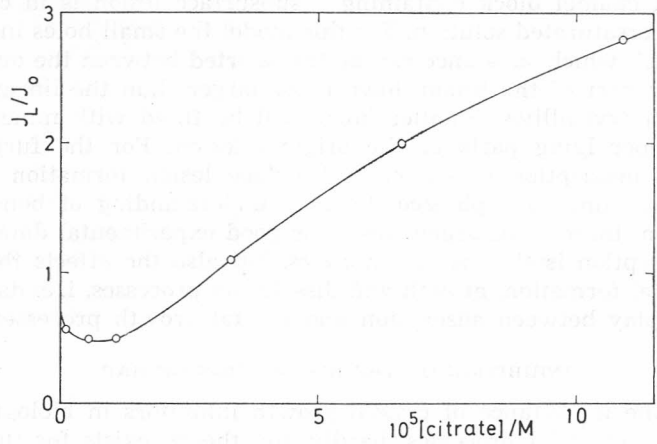

Figure 4. J_L/J_o, the rates of dissolution of HAP with and without citrate ions, plotted against the total concentration of citrate, pH 7.15, $m_{Cr} = 5$ mg, $V = 0.91$ l, total concentration of calcium 1.43×10^{-5} M, Ca/P = 1.67.

in which J_L/J_o is plotted against the total concentration of citrate ions in the system. J_L and J_o are the rates of dissolution with and without citrate, all other reaction parameters being constant. From the plot is seen that citrate ions inhibit the dissolution of HAP crystals if the citrate concentration is less than about 3×10^{-5} M. The largest inhibitory effect of these ions is obtained for a citrate concentration of about 10^{-5} M. At concentrations larger than 3×10^{-5} M citrate ions cause an increased rate of dissolution. The solution composition in these experiments is given in Table I. The accelerating effect at citrate concentrations larger than 3×10^{-5} M can be explained as being due

TABLE I

*The Solution Composition and the Relative Rate, J_L/J_o, for the Effect of Citrate on the Rate of Dissolution of HAP**

10^5 [citrate] (M)	10^5 [$CaCi^-$] (M)	10^5 [HCi^{2-}] (M)	10^5 [Ci^{3-}] (M)	10^5 [Ca^{2+}] (M)	J_1/J_o
0.11	0.03	0.01	0.03	1.40	0.56
0.55	0.22	0.04	0.23	1.21	0.48
1.09	0.42	0.09	0.53	1.01	0.49
3.30	0.88	0.36	2.01	0.55	1.1
6.60	1.12	0.82	4.60	0.31	2.0
10.90	1.24	1.46	8.15	0.19	2.8

* $[Ca]_{total} = 1.43 \times 10^{-5}$ M = 0.25 C_s, pH = 7.15, Ca/P = 1.67. The concentrations of the ions, $CaCi^-$, HCi^{2-}, Ci^{3-} and Ca^{2+}, are calculated assuming other equilibria than $HCi^{2-} \rightleftarrows Ci^{3-} + H^+$ and $Ca^{2+} + Ci^{3-} \rightleftarrows CaCi^-$ to be insignificant. The equilibrium constants for these reactions are taken to be $10^{-6.4}$ M and $10^{4.9}$ M^{-1}, respectively. 0.5 μmol/l of the total citrate is assumed to be on the surface of the crystals.

to ion-pair formation between calcium ions and citrate ions. The specific surface area of the crystals was 32 m^2/g, determined by N_2/He gas adsorption using a Quantasorb® surface area analyzer. From the specific surface area the amount of phosphate ions in the surface of the crystals was calculated to be about $1{,}6 \times 10^{-4}$ mol/g. This calculation is explained in ref. [8]. For 5 mg crystals as used in the experiments, the amount of citrate ions that can be adsorb on the crystal surface is expected to be of the order of 0.5 μmol. At low citrate concentration only a minor amount of the calcium ions will complex with the citrate in solution. At low concentrations of citrate ions, these ions may adsorb onto the crystal surface and cause a decreased rate of dissolution. As the citrate concentration is raised, the effect of adsorption is counter-acted by the complexing of calcium ions in solution with citrate ions. The latter process will cause an increase in the rate of dissolution of HAP, corresponding to an increase in the affinity of the dissolution process.

BIOLOGICAL MINERALIZATION

In vertebrates bone resorption is by far the most important form of demineralization and presents many unsolved problems.[20] Dominguez and Raisz[21] have reported that in vitro acidosis causes increased bone resorption. The effect could be explained as mainly due to an increase in the rate of dissolution of devitalized bone, caused by the increase in the hydrogen ion concentration. Cell-mediated bone resorption appears to be practically independent of pH in the pH-range 7.0—7.5. Neither the enhanced bone resorption during metabolic acidosis, nor the lack of increased bone resorption during respiratory acidosis has been understood in terms of a model.

For future improvement in the description of in vivo bone resorption it is important to obtain information about how in vivo occuring ions and molecules affect in vitro dissolution of calcium phosphates. Ions affecting the rate of dissolution of HAP have been shown to behave quite differently. Citrate ions appear to reduce the rate of dissolution of HAP at low concentrations, but, due to their complex formation with calcium ions in solution, citrate ions increase the rate of dissolution when present in higher concentrations. Fluoride ions have been shown[22] to decrease the rate of dissolution of HAP, particularly at low values of pH, and to cause an increase in the rate of growth of HAP containing fluoride ions. Diphosphonates and diphosphate ions have been shown to inhibit both the rate of dissolution and the rate of growth of calcium phosphates. The influence on calcium phosphate dissolution of potential candidates for affecting in vivo bone resorption should thus be investigated over a wide range of concentration and solution composition.

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LIST OF SYMBOLS

a	mean diameter of an ion.
C	concentration of solute. In this paper only dissolution of HAP into a solution with $Ca/PO_4 \simeq 1.67$ is discussed.
C_s	equilibrium value of C at the actual value of pH.

C_L concentration of an inhibitor.

dr/dt linear rate of dissolution.

$F\,(m/m_o)$ function representing the surface area on which the dissolution takes place. This term may, in the general case, include certain kinetic factors.

$g\,(C)$ function representing the influence of concentration on the rate.

J overall rate of dissolution of HAP, dn_{HAP}/dt.

J_o overall rate of dissolution for a particular set of values of the rate controlling parameters, no inhibitor being present.

J_L overall rate of dissolution for the same set of values of the rate controlling parameters as used for definition of J_o, except an inhibitor is present.

k, k', k'' rate constants.

$k\,T$ Boltzmann constant times the absolute temperature.

K_L Langmuir adsorption constant.

K_{kin} Langmuir adsorption constant, determined from kinetic experiments.

$\tilde{K}_{cr}$ acidity constant for HPO_4^{2-} ions in the crystal surface.

m mass of crystals at time t.

m_o mass of cristals at time zero.

n_{HAP} amount of calcium hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$.

r linear dimension of crystals.

r_1 depth of sub-surface lesion i enamel.

v lateral rate of growth of a dissolution nucleus.

v_+ lateral rate of growth of a dissolution nucleus if no back reaction takes place

x mole fraction of adsorption sites occupied by an inhibitor.

x_p, x_{HP} mole fraction of phosphate groups in the crystal surface in the form of PO_4^{3-} and HPO_4^{2-} respectively.

dr/dt linear rate of dissolution.

α $\pi\, a^4\, \sigma^2 / 3\,(kT)^2$

β dimensionless dissolution affinity for a mean ion.

σ surface tension of HAP (Gibbs surface energy).

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SAŽETAK

Studij otapanja kalcij-hidroksiapatita i njegova primjena na biološku demineralizaciju

J. Christoffersen, M. R. Christoffersen i J. Arends

U ovom radu razmotreni su razni aspekti ispitivanja brzine otapanja kalcij-hidroksilapatita u vodenim otopinama. S kemijskog stajališta sistem je pogodan za proučavanje kemijskog procesa koji se zbiva na površini kristala za vrijeme njihova otapanja u čistim sistemima, kao i u nazočnosti inhibitora. Sa stajališta biologije ta su istraživanja rezultirala modelom stvaranja subpovršinskih lezija u zubnoj caklini. Proučavanje *in vitro* efekta biološki relevantnih molekula na proces otapanja upućuje i na model demineralizacije koštanog tkiva. Nađeno je da niske koncentracije citrat-iona inhibiraju proces otapanja, a više koncentracije ubrzavaju taj proces zbog znatnijeg kompleksiranja kalcij-iona.