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REVIEW ARTICLE

Solubility properties of human tooth mineral and pathogenesis of dental caries

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Dental research over the last century has advanced our understanding of the etiology and pathogenesis of caries lesions. Increasing knowledge of the dynamic demineralization/remineralization processes has led to the current consensus that bacteria-mediated tooth destruction can be arrested or even to some degree reversed by adopting fluoride and other preventive measures without using restorative materials. Our experimental approach provided new insight into the stoichiometries and solubility properties of human enamel and dentin mineral. The determination of the solubility product constant on the basis of the stoichiometric model $(Ca)_{5-x}(Mg)_q(Na)_u(HPO_4)_v(CO_3)_w(PO_4)_{3-y}(OH,F)_{1-z}$, verifies the difference in their solubility properties, supporting the phase transformation between tooth mineral and calcium phosphates in a wide range of fluid compositions as found in the oral environment. Further refinement of the stoichiometry and solubility parameters is essential to assess quantitatively the driving force for de- and remineralization of enamel and dentin in the oral fluid environment. Prediction of the effects of a combination of inhibitors and accelerator(s) on remineralization kinetics is also required. In order to develop devices efficient for optimizing remineralization in the lesion body, it is a critical question how, and to what extent, fluoride can compensate for the activity of any inhibitors in the mineralizing media.
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Keywords: caries; enamel; dentin; solubility; remineralization

Introduction

Dental caries is an infectious disease that is ubiquitous in all countries and populations around the world (Fejerskov and Nyvad, 1996). Caries does not develop in the absence of bacterial plaque, but the presence of microbial deposits is not sufficient for enamel and dentin lesions to develop. According to the Keyes' host-agent-environment model, the necessary causes of caries include susceptible teeth, cariogenic microflora such as the mutans streptococci and lactobacilli, and dietary fermentable carbohydrates (Nikiforuk, 1985; Newbrun, 1989). For a long time, the caries process has been thought to be irreversible, resulting in the permanent loss of tooth substance and eventually the development of a cavity. However, the current concept regarding cariogenesis is that a caries lesion, either clinically invisible or detectable, is the accumulation of numerous episodes of de- and remineralization, rather than a unidirectional demineralization process (Koulourides, 1966; Silverstone, 1977; Larsen and Fejerskov, 1989). Remineralization is the process by which partly-dissolved crystals are induced to grow by accretion of the common ions (i.e. calcium and phosphate ions forming part of the mineral) from solution, so that remineralization is an important natural repair process, countering cariogenic challenges, to maintain the balance between processes of mineral loss and gain (Fejerskov, 1997; Ten Cate, 1999). Indeed, the reversibility or healing of incipient caries lesions has been reported by many laboratories and is becoming common knowledge in the community of dental practitioners. The detailed chemistry and biology regarding the dynamic aspects of demineralization and remineralization taking place at the tooth-pellicle-plaque-saliva interface have been reviewed recently in several excellent publications (Fejerskov *et al*, 1996; Robinson *et al*, 2000). In this communication, I will first describe briefly the current state and histopathologic features of caries lesions in a Japanese population and then focus attention to the stoichiometric structure and solubility properties of tooth mineral and their relevance to the pathogenesis and prognosis of enamel and dentin caries lesions.

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Clinical diagnosis and histopathologic validation of incipient caries lesions

Many epidemiological surveys indicate that the severity of carious manifestations has been dramatically reduced

in most industrial populations in the past few decades (Manji and Fejerskov, 1994; Bowen, 2002). The early clinical sign of enamel caries is the appearance of a "white-spot lesion", which is histopathologically featured by subsurface demineralization. The body of the subsurface lesion beneath an apparently intact surface layer may have lost as much as 50% of its original mineral (Silverstone, 1973). The current problem emerging in clinical dentistry is that, whereas new caries lesions continuously develop throughout life, most of the lesions at the incipient stage are prone to overlooking by visual inspection (Wenzel *et al*, 1991; Weerheijm *et al*, 1992; Lussi, 1993). Figure 1 shows examples of small dentin caries that were found in our recent histopathologic examination of coronal and root caries in a pool of extracted permanent teeth in a Japanese population (Nakahara *et al*, 1998; Nakahara *et al*, 2000). Our histological validation of 87 premolars and molars confirmed their caries experience without exception and the occurrence frequency of caries was found to range from four to 25 lesions per tooth (745 lesions in total and 8.6 lesions per tooth on average). It is also of note that the so-called hidden caries, defined as caries beneath an intact tooth surface without visual evidence of a carious attack (Weerheijm *et al*, 1992), amounted to one-third of the histologically validated lesions. A significant portion of these hidden lesions occurred in pits and fissures of the occlusal groove-fossa system and, more importantly, the majority of the hidden caries lesions in fissures and pits had already extended into dentin; in some cases, dentin demineralization and translucent changes had penetrated through entire dentinal tubules to the pulp end. Electron microprobe analysis of these hidden caries lesions showed an increase in fluoride content at the enamel surface covering the underlying demineralized lesions. These findings are in agreement with the statement of Weerheijm *et al* (1997) that fluoride may mask the spread of caries in enamel and dentin by encouraging remineralization only at the surface layer, thereby burying the underlying lesions. Many studies on remineralization *in vitro* also demonstrated that surface-softened enamel remineralizes readily in the presence of fluoride in media, giving rise to the mineralized, less porous surface layer, whereas remineralization of subsurface demineralized lesions is becoming more difficult because of the restricted permeability of calcium and phosphate ions through the relatively mineralized surface layer (Pearce *et al*, 1995; Reynolds, 1997; Larsen and Richards, 2001). Thus innovation of technologies and measures for early diagnosis and management of the incipient caries lesions is still an urgent task to improve public health.

Demineralization/remineralization and reversibility of incipient enamel caries

Teeth are exposed to a continuous de- and remineralization process in the oral environment (Figure 2). In particular, intermittent changes in plaque composition give rise to de- and remineralization cycles at the tooth-plaque interface (Gao *et al*, 2001). In the presence of fermentable carbohydrate, organic acids (e.g. lactate, acetate and others) are produced by plaque microorganisms. These acids in an undissociated form diffuse through the pellicle into the tooth, resulting in the partial demineralization of tooth structure (Featherstone *et al*, 1979; van Dijk *et al*, 1983). The ions dissolved from the lesion body diffuse outward and inward through narrow pores and then become in part reprecipitated at the surface layer or the advancing front surrounding the demineralized lesion (Brown, 1974; Moreno and Zahradnik, 1974). If the demineralization progresses predominantly over periods, or the rate of demineralization outstrips the rate of remineralization, the net result is a gradual loss of tooth mineral, leading to irreversible cavity formation. However, when the rate of remineralization (or crystal growth on the existing crystal surfaces as a template) exceeds the rate of transport of ions out of the tooth surface, the surface layer can be retained with the continuous renewal. The

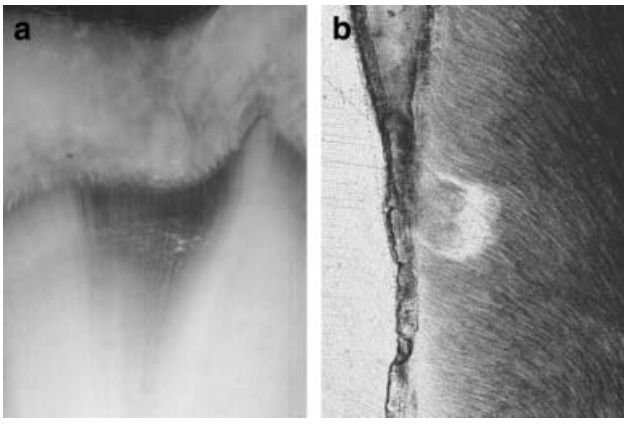

Figure 1 Microscopic appearance of dentin caries lesions that have occurred in the occlusal fissure and at the cementum-enamel junction. Both caries lesions were invisible clinically

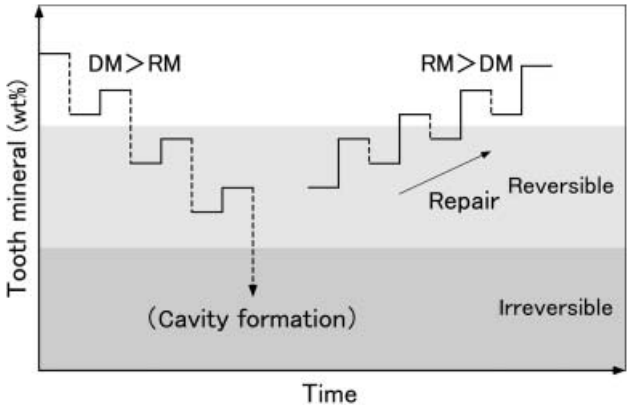

Figure 2 Schematic illustration of the dynamic processes of demineralization (DM) and remineralization (RM) and the possible consequences for the loss and gain of tooth mineral based on the DM/RM balance. The demineralized lesion is now considered to be reversible, to certain extent, by remineralization, while the predominant demineralization results in the irreversible cavity formation

thermodynamic principle implies that this dynamic equilibrium between the loss and gain cycles requires the establishment of the necessary physicochemical conditions, i.e. the medium at the tooth-plaque interface must become undersaturated with respect to the tooth mineral for demineralization, while remineralization or the precipitation of any new solid phase will not occur until a certain degree of supersaturation has been achieved (Nancollas, 1982).

The solubility diagram is useful to illustrate the thermodynamic aspects of the oral environment in relation to caries development and remineralization potential (Figure 3). In this diagram, saturation conditions with respect to the calcium phosphate salts are represented by solid lines corresponding to their solubility isotherms. With respect to a given calcium phosphate phase, the region above the line represents conditions of supersaturation, and the region below the line represents conditions of undersaturation. The rectangular zone shown in the diagram exhibits the range of solution composition understood to be encountered in saliva and plaque fluid under resting and stimulated conditions. Human salivary fluids are well known to be metastable or supersaturated with respect to hydroxyapatite, providing a driving force for remineralization (Driessens, 1982; Hay *et al*, 1982). With decreasing pH in the plaque fluid, the environment in contact with tooth mineral becomes undersaturated, triggering the loss of tooth mineral (Zhang *et al*, 2000). The diagram also manifests the unique aspect of calcium phosphate chemistry, that is, the presence of a singular point at about pH 4.1 where the corresponding solution composition is equilibrated simultaneously with respect to both hydroxyapatite and dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, called hereafter DCPD). This will be described later in relation to the implication of solubility properties of carbonated enamel and dentin apatites in the subsurface lesion formation.

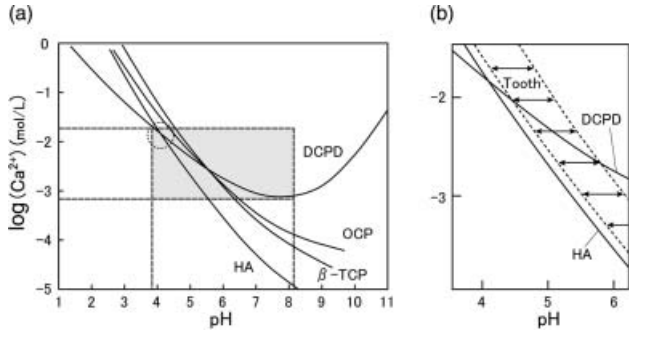

Figure 3 (**a**) Solubility isotherms for hydroxyapatite (HA), tricalcium phosphate (β-TCP), octacalcium phosphate (OCP), and dicalcium phosphate dihydrate (DCPD). Each line represents the solution concentration where crystals of that mineral phase are in equilibrium with the surrounding fluid (thermodynamic equilibrium). The shaded rectangle represents the range of fluid composition expected at the saliva-plaque-tooth interface. Note the closing point between the two isotherms for HA and DCPD (singular point; see Text) at the upper-left corner of the rectangle. (**b**) The solubility product constants of enamel and dentin mineral are much different from that of the pure hydroxyapatite, so that a singular point between tooth mineral and DCPD tends to shift toward more neutral pHs

According to the above-mentioned dynamic model of cariogenesis, it is reasonable to consider that the feature and prognosis of a caries lesion is determined by the balance between demineralization and remineralization, which in turn may be expressed by a function of the three variables, i.e. frequency, duration and reaction rates (Figure 4). The frequency and duration of demineralization and remineralization depend on nutritional status and diet habit such as timing and numbers of sucrose consumption, salivary flow and composition, fluoride exposure, oral hygiene, and underlying systemic disease so on (Nikiforuk, 1985; Burt and Ismail, 1986; Newbrun, 1989; Fejerskov *et al*, 1996). Quantitative information relevant to the frequency and duration of caries challenges has recently been accumulated using pH-recycling models *in vitro* (Jacobson *et al*, 1991; Ten Cate *et al*, 1995). Regarding the kinetic aspects, studies on crystal growth and dissolution of calcium apatites *in vitro* provided compelling evidence that the driving force for de- and remineralization is the degree of saturation (DS) of the fluid environment with respect to the concerned biomineral (Brown and Chow, 1981; Varughese and Moreno, 1981; Nancollas, 1982). The DS value with respect to a given calcium phosphate in an aqueous medium can be expressed as the ratio of the ionic activity product (IP) of the ionic lattice constituents (reflecting a given stoichiometry) in solution to its solubility product constant (Ksp) of the solid, i.e. DS = IP/Ksp. According to this expression, DS values of unity, higher than unity, and smaller than unity, indicate saturation, supersaturation, and undersaturation, respectively. As described later, it was also documented that dissolution and crystal growth behaviors of calcium phosphates are affected markedly by additives that can modulate reactions at relatively low concentrations (Hay *et al*, 1979; Moreno *et al*, 1987; Budz *et al*, 1988). In the last few decades, our objectives have been directed to define the driving force for de- and

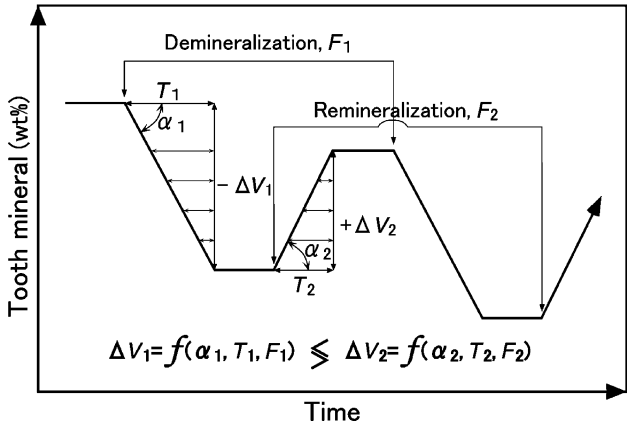

Figure 4 Schematic diagram of the coupled demineralization and remineralization processes which determine the development and prognosis of a caries lesion. The balance between the loss and recovery of tooth mineral volume ($\triangle V_1$ and $\triangle V_2$) is expressed by a function of duration (T_1 and T_2), frequency (F_1 and F_2) and rates (α_1 and α_2), where subscripts 1 and 2 stand for the processes of demineralization and remineralization, respectively

remineralization of enamel and dentin mineral based on their realistic composition and solubility properties, as well as to elucidate the regulatory mechanism of crystal growth in dilute supersaturated solutions in the absence and presence of various additives.

Stoichiometries and solubility properties of human enamel and dentin mineral

Knowledge of the stoichiometries and thermodynamic solubility products of enamel and dentin mineral is essential not only to compare their chemical stability but also to reasonably assess the driving forces for de- and remineralization taking place in various fluid environments. Hydroxyapatite, which has been considered a prototype of tooth mineral (Driessens, 1982), is represented by the stoichiometry $Ca_5(PO_4)_3OH$. In past periods, dental apatites were assimilated to a hydroxyapatite with a higher solubility than the synthetic salt (Margolis and Moreno, 1990; Shellis *et al*, 1993). However, as its name implies (Greek *apate*; deceit), the apatitic crystalline lattice has the propensity of accommodating multiple isomorphic substitutions without losing its apatitic nature (LeGeros, 1991; Elliott, 1994). Indeed, biogenic apatites in general display a wide range of varieties in their stoichiometries, as these apatite crystals are usually formed in media containing significant concentrations of cations (e.g. Na^+, K^+, Mg^{2+}) and anions (e.g. Cl^-, HCO_3^-, and F^-) as determined in the enamel fluid (Aoba and Moreno, l987). Acid phosphate HPO_4^{2-} is also a ubiquitous constituent of tooth and bone biominerals in mammals because acidic precursors are most likely involved in the initial stage of mineralization (Brown *et al*, 1987). Taking into account the major substitutions, namely, Na^+ and Mg^{2+} in place of Ca ion and CO_3^{2-}, HPO_4^{2-} and F^- ions at the PO_4 and OH sites, our interest was directed to adopt a general stoichiometry model for biogenic apatites consisting of tooth enamel and dentin, i.e. $(Ca)_{5\cdot x}(Mg)_q(Na)_u(HPO_4)_v(CO_3)_w(PO_4)_{3\cdot y}(OH,F)_{1\cdot z}$ (Aoba, 1997). The stoichiometric coefficients for each mineral were determined in combination with analytical procedures to distinguish the fraction of the ions in the bulk crystals from the pools of the corresponding ions on the crystal surfaces (Aoba *et al*, 1992a,b; Komatsu *et al*, 2001). The information obtained so far is given in Table 1, although the estimates for coefficients still involve gross approximations that should be refined. The important aspect related to the integrity of the tooth in acidic environments is that tooth apatites have incorporated substantial amounts of carbonate into the lattice positions during their formation.

To date, much work has been conducted to collect meaningful information about the solubility and thermodynamic solubility product for tooth mineral (Patel and Brown, 1975; Larsen, 1986; Shellis *et al*, 1993). However, there still remain controversies or uncertainties about the solubility behavior of tooth mineral. For instance, Shellis (1996) indicated the heterogeneity of the solubility product constants (10^{-53} to 10^{-58}) for human enamel mineral, which may stem from the inhomogeneous structure and chemical composition of enamel. According to his solubility determinations in combination with scanning microscopic observations, it was shown that the solubility product of the bulk of human enamel was estimated to be around 10^{-58}, which corresponded to the mineral from the middle and outer intraprismatic areas of the enamel tissue, whereas more soluble material was found in the inner enamel intraprismatic layer ($>10^{-56}$) and in the prismatic junction ($>10^{-55}$). Another obstacle in the solubility determination of biogenic apatites is the incorporation of carbonate in the lattice sites. Most of the solubility measurements of tooth mineral have been conducted in the ternary system $Ca(OH)_2$-H_3PO_4-H_2O. Without controlling the gas environment during the mineral-solution equilibration, carbonate ions derived from the dissolved original crystals are lost as CO_2 from the equilibration system. This means that the solid-solution equilibration would not lead to any meaningful equilibrium but might bring about continuous dissolution of the original carbonated mineral and subsequent precipitation of a less soluble phase(s).

In order to avoid such incongruent equilibration, we initiated solubility measurements of enamel and dentin apatites in the system $Ca(OH)_2$-CO_2-H_3PO_4-H_2O (Moreno and Aoba, 1991; Aoba and Moreno, 1992; Komatsu *et al*, 2001). In solubility experiments, the equilibration of each of the pulverized samples was conducted with dilute phosphoric acid solutions and CO_2/N_2 atmospheres at 25°C. Equilibrium conditions

Table 1. Putative stoichiometry and solubility of human enamel and dentin mineral

Sample	*Stoichiometry*	*Solubility product*
Enamel	$(Ca)_{4.56}(Mg)_{0.03}(Na)_{0.11}(HPO_4)_{0.10}(CO_3)_{0.23}(PO_4)_{2.66}(OH,F)_{0.65}$	$8.5\ (\pm 0.9) \times 10^{-49}$
Dentin	$(Ca)_{4.22}(Mg)_{0.14}(Na)_{0.12}(HPO_4)_{0.13}(CO_3)_{0.36}(PO_4)_{2.49}(OH,F)_{0.39}$	$4.1\ (\pm 1.3) \times 10^{-45}$

Enamel and dentin samples were collected separately from human permanent molars. Both samples were pulverized and deproteinated by low-temperature ashing prior to use in solubility measurements.
Measurements of the solubility properties for both samples were conducted under the comparable conditions, i.e. 25°C, P_{CO_2} 1.86% but with the various initial concentrations of phosphoric acid (0.06–1.2 mM). The values of the solubility product constant for enamel and dentin mineral are expressed by the ionic activity products at equilibrium that were calculated according to their stoichiometries.

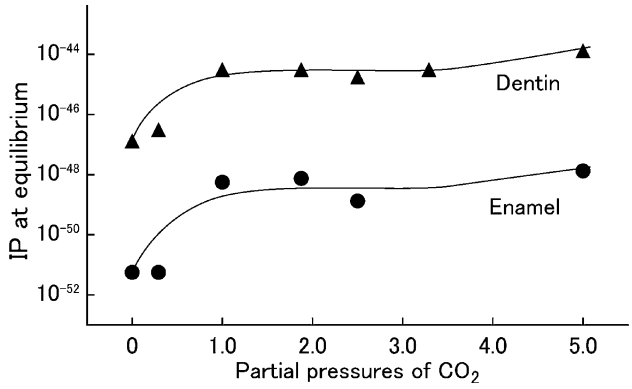

Figure 5 The discrete solubility properties of human enamel and dentin mineral collected from the permanent teeth as a function of the partial pressure of CO_2. Determination of the solubility product constant was repeated six times and the data are presented as the average of six independent determinations. The values of the solubility product constant for enamel and dentin apatites are expressed by the ionic activities product (IP) in solution at equilibrium based on their stoichiometries shown in Table 1

were assumed when the solution composition did not change significantly for a period of 3–4 days; it usually took from 14 to 21 days. At equilibrium, the values of the individual ionic activities and the ionic activity product, $IP = (Ca^{2+})^{5\cdot x}\ (Mg^{2+})^{q}\ (Na^{+})^{u}\ (HPO_4^{2-})^{v}\ (CO_3^{2-})^{w}\ (PO_4^{3-})^{3\cdot y}\ (OH^{-})^{1\cdot z}$, were calculated based on the determined stoichiometric coefficients. The values of the solubility product constant for enamel and dentin apatites were obtained by averaging the IP values calculated from independent equilibrium solutions. In a series of equilibrations conducted under a wide range of partial pressures of CO_2 (P_{CO_2}), it was ascertained that the solubility behavior of human tooth mineral depends on the atmospheric condition. Most interestingly, the use of 1.0 through 3.3% P_{CO_2} during equilibration yielded relatively constant IP values at equilibrium for each solid, whereas there were obvious discrepancies in the IP values attained above and below the specified P_{CO_2} atmospheres (Figure 5). Currently no concrete information is available about CO_2 environment at dentin mineralization sites but it is notable that porcine enamel mineralization occurs in the enamel fluid where the P_{CO_2} is around 1.86% (Aoba and Moreno, 1987). These data lend support to the postulation that equilibration of any biomineral in the medium of composition similar to that in which the mineral has formed may involve only a congruent dissolution (Aoba and Moreno, 1992). In the last column of Table 1 are given the values of the solubility product constant calculated for human enamel and dentin apatites, determined using the constant partial pressure of CO_2 of 1.86%. The results show that there is a four orders of magnitude difference in the solubility product constant between the carbonated enamel and dentin apatites. Relative small s.d. values in these determinations also support the appropriateness of the equilibration system and the importance of controlling gas atmosphere in the solubility studies of carbonated biogenic apatites.

Effects of the solubility properties of tooth mineral on cariogenesis

From the solubility diagram shown in Figure 3, it is pertinent that hydroxyapatite is the most stable form of calcium phosphate (except for fluoridated apatites) at near-neutral pH, whereas DCPD becomes more stable under acidic conditions beyond the singular point at which the two solids are both in equilibrium with the solution. This supports the prediction that DCPD will form after dissolution of hydroxyapatite in the corresponding acidic media (Brown, 1974; Moreno and Zahradnik, 1974; Johnson and Nancollas, 1992). From a kinetic point of view, it is pertinent that the precipitation rate constant of DCPD is several orders of magnitude higher than that of hydroxyapatite. For instance, Zhang *et al* (1992) reported a three orders of magnitude difference in crystal growth rates at 37°C, such as 2.7×10^{-7} and 3.3×10^{-4} in terms of mol of salt/min/m^2 for hydroxyapatite and DCPD, respectively. These thermodynamic and kinetic considerations provide the basis for the current understanding of subsurface lesion formation, supposing that the kinetically favorable precipitation of DCPD would help to save in part a loss of outward-diffusing common ions from the body lesion and thereby maintain the integrity of the surface layer. Moreover, the fact that the realistic solubility product constants of tooth mineral (see Table 1) is substantially higher than that of the stoichiometric hydroxyapatite leads to the prediction that the quasi-equilibrium situation between tooth mineral and DCPD can be accomplished at higher pH values than the extreme acidic pH expected for pure hydroxyapatite-DCPD equilibrium. In addition to the precipitation of DCPD, another putative phase transition is the precipitation of fluoridated apatites when free fluoride ions are available for remineralization after dissolution of tooth mineral and/or from exogenous sources. Collectively, it is now accepted that a quasi-equilibrium situation is established between tooth mineral, DCPD and fluoridated apatites within the surface zone of a subsurface caries lesion when the external pH falls (Moreno and Zahradnik, 1974; Margolis and Moreno, 1985).

On the basis of the above-mentioned quasi-equilibrium situation, Figure 6 formulates the putative chain reactions taking place during demineralization/remineralization cycling. With the onset of demineralization, part of the original tooth mineral dissolves, generating DCPD under the acidic condition. With increasing pH in local environments, because of the release of hydroxide ions after demineralization and buffering capacity of saliva as well as extinction of organic acid production, DCPD redissolves and the next phase in the stability sequence starts being precipitated (Johnson and Nancollas, 1992; Shellis *et al*, 1997). Along with the presumed chemical reactions, electron microscopic studies of incipient enamel caries showed that the core of enamel crystallites, which corresponds to the vestige of the initially formed carbonate-rich domain, are especially susceptible to attack by hydrogen ions during

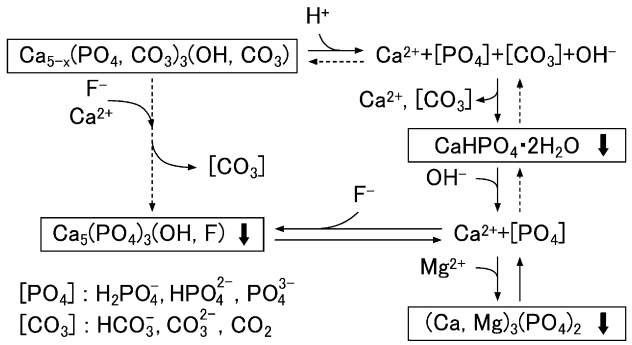

Figure 6 The postulated phase transition taking place during the demineralization/remineralization cycles. For the sake of simplicity, we accept here two assumptions: (i) the original tooth mineral is expressed as Ca-deficient apatites substituting carbonate at both PO_4 and OH lattice sites and (ii) the involvement of octacalcium phosphate as an intermediate phase is excluded. The outcome of the sequential reactions is the generation of more stable crystals that give resistance against further caries challenges

demineralization (Johnson, 1967). The partially dissolved crystals act as a template for remineralization or accretion of new apatite lattice components, and the newly acquired lattice portion preferentially concentrates fluoride and excludes carbonate (Larsen, 1986). This type of sequential reactions is likely a coherent process in any caries lesions, increasing the resistance of the surface enamel to subsequent acid attacks. Additionally, Mg-containing tricalcium phosphate (Whitlockite) tends to be precipitated in the dentin containing high Mg ion. It was documented that large rhombohedral crystals or caries crystals fill the caries-attacked dentinal tubules (LeGeros, 1991; Shellis *et al*, 1997).

Endogenous/exogenous modulators of caries lesion formation and its reversibility

As mentioned above, the primary factor determining the development and reversibility of caries lesions is the thermodynamic saturation level in the media surrounding the tooth mineral. It is of paramount importance, however, to give attention to the participation of multifactors in controlling the kinetic aspects of de- and remineralization, leading to drastic changes in the prognosis and outcome of preventive and repair treatment of subsurface lesions. Remineralization of the demineralized subsurface lesions requires calcium and phosphate, which are primarily supplied from saliva and plaque fluid. In an attempt to develop efficient devices for promoting remineralization of the subsurface lesions, much interest has also been in the potential role of calcium phosphate solids in the plaque as a source of Ca and phosphate ions upon acidification (Shellis and Duckworth, 1994). Partial dissolution/precipitation of these solids would buffer the activities of free calcium and phosphate ions in the plaque fluid helping to maintain a state of supersaturation with respect to enamel mineral. The aim of adding calcium phosphate salts to toothpaste is also to achieve the calcium and phosphate enrichment for remineralization of early caries lesions. However, it was realized that, when the driving force for remineralization is too high, the resulting rapid precipitation on the surface may block the surface layer pores, thereby leaving the repair or consolidation of the body lesion incomplete (Pearce *et al*, 1995). In order to overcome this situation, the use of inhibitors for nucleation and crystal growth of calcium phosphates has been recommended, because stabilizing the supersaturated solution would help to generate high-concentration gradients of calcium and phosphate ions and ion pairs into the subsurface lesion. The presence of salivary proteins and peptides are known to keep the saliva supersaturated with respect to the tooth mineral thus preventing mineral losses by dissolution, as well as preventing undesirable mineral accretion on the tooth surfaces (Hay *et al*, 1979). Pyrophosphate, phosphonates, and synthetic phosphorylated peptides (Reynolds, 1997) are examples of practical stabilizing agents. Most of the inhibitors have strong adsorption affinities to apatite surfaces, and their inhibitory activities can be explained by blocking the growth sites (Nancollas, 1982; Moreno *et al*, 1987). In connection with the adsorption-mediated effects of additives, even some of the organic acids (e.g. acetate) with low affinities might adsorb onto tooth mineral, giving rise to modulation effects on dissolution kinetics. The previous empirical and theoretical approaches showed that the rate of enamel demineralization is not solely a function of the thermodynamic driving force, but is also dependent on the organic acid concentration as well as the pH (Margolis *et al*, 1999).

From the foregoing consideration, one can claim that, whereas the use of inhibitors is indispensable to stabilize remineralization solutions with higher degrees of supersaturation, such inhibitors also slow down the kinetics of remineralization or apatite growth. Consequently, it is becoming a consensus that fluoride as an accelerator of precipitation and crystal growth should also be a constituent of these remineralizing solutions. The biological action or chemical outcome of the multi-molecular interaction is usually a function of the associated factors, e.g. concentration, competitive binding strength or affinity and rate constants. From a practical point of view, it is a critical question as to how, and to what extent, fluoride can compensate for the inhibitory activity of any inhibitors in the mineralizing media. Some well-designed studies provided evidence that it is possible to predict the apatite growth reactions in a given experimental system using a certain inhibitor and fluoride based on coverage of the unique adsorption sites on the crystal surface by inhibitor molecules (Margolis *et al*, 1982; Moreno *et al*, 1987).

Caries-reducing effects by fluoride

It is becoming accepted that the decreasing trend in caries prevalence and incidence is due, at least in part, to the increased availability of fluoride in water supplies and toothpaste, and/or by clinical applications (Bratthall *et al*, 1996). The fact that fluoride can be incorporated readily into the crystalline lattice of tooth mineral,

resulting in a tissue less soluble in an acid environment, has been the scientific cornerstone for caries prevention. The major concept prevailing in the past was that the caries-inhibitory effect of fluoride was because of its incorporation in tooth mineral during the development stages or of the tooth prior to eruption. This led to the extensive systemic use of fluorides in caries prevention. However, it is now realized that the primary caries-preventive mechanisms of the action of fluoride are posteruptive through topical effects that can interfere with the dynamic equilibrium at the interface between mineral surface and oral fluid (Wong *et al*, 1987). Free fluoride ions in solution can react with apatite crystals or biominerals in several different ways depending on their concentrations and the solution composition (Ten Cate and Featherstone, 1991; Aoba, 1997). In a supersaturated solution where fluoride is available together with calcium and phosphate ions, fluoride ions are incorporated readily into the apatite lattice through precipitation and growth reactions. The substitution of F^- for OH^- in the lattice brings about a reduction in the volume of the unit cell, so that the chemical stability of the apatite lattice is greatly enhanced by virtue of the electrostatic bond between fluoride and the adjacent ions (Elliott, 1994). Fluoride can augment the rate of remineralization by reducing the solubility of precipitating fluoridated apatites and thereby increasing the driving force for apatite formation without any changes in the concentrations of common ions in the mineralizing media (Moreno *et al*, 1974). In this context, fluoride is referred to as a unique accelerator of apatite crystal growth. Fluoride also has a strong affinity for apatite surfaces because of its small ionic size and strongly electronegative character. Fluoride adsorption acts as a potent protection mechanism against acid dissolution of the crystal surface. Indeed, pH-cycling studies showed that fluoride ions in the media induce only short periods of remineralization enhancement but yield demineralization protection throughout acid challenges (Ten Cate *et al*, 1995). This explains the successful use of fluoridated toothpaste and mouth rinses, as well as fluoridated water, for the protection of tooth integrity at the solution-solid interface. At the same time, caution should be given in regards to the fact reported by an epidemiological survey showing that fluoride in drinking water reduces dental caries, but does not eradicate it, or that fluoride inhibits primarily caries progression but has a relatively small effect on caries incidence (Fejerskov *et al*, 1996).

Conclusive remarks

Caries progression *vs* reversal is a delicate balance between pathological factors (bacteria and carbohydrates) and protective factors (saliva, mineral ions, fluoride). The protective effect is ascribed to the depression of demineralization and/or enhancement of remineralization. The frequent delivery of fluoride to the tooth surface is currently the most efficient measure leading to caries arrestment and reversal. The paradigm shift that the incipient non-cavitated enamel caries can be healed without using restorative materials has resulted in a modification of the guiding principle in clinical dentistry, from the classic theory of 'extension for prevention' to the 'minimal intervention dentistry' that pursues to retain as much as possible of the natural tooth structure (Tyas *et al*, 2000). This paradigm shift, on one side, increases the demands for clinical dentists regarding their diagnostic accuracy to differentiate sound teeth from incipient or arrested caries, as well as their capability to predict the lesion progression and prognosis. In particular, the pits and fissures of the occlusal groove-fossa system are still undoubtedly responsible for the high caries susceptibility. Thus there is an emerging need for sensitive clinically applicable methods for early detection and quantification of caries lesions in pits and fissures, as well as longitudinal monitoring of these lesion responses to preventive measures. Seminal work on the solubility and plaque environment in fissures has been published (Zaura *et al*, 2002). Future perspectives of caries prevention and reversal could be found in elucidating the details of structural and compositional changes in fissures associated with caries challenges and remineralization regimens.

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