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TOOTH ENAMEL MICROSTRUCTURE

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CHAPTER 2

A short review of studies on chemical and physical properties of enamel crystallites

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ABSTRACT: Tooth enamel is composed of biological apatite which is characterized with low-crystalline, carbonate containing, 'non-stoichiometric' apatite. In this chapter, characteristics of tooth enamel crystallites was reviewed from the aspects of chemistry, crystallography, crystallite size and strains, formation and transformation, and assessment of diagenesis.

ZUSAMMENFASSUNG: Zahnschmelz wird aus biologischem Apatit gebildet, der durch einen geringen Kristalinitatsgrad, einen Karbonatgehalt und eine nicht stoichometrische Verteilnng gekennzeichnet ist. In dieser Arbeit werden die Apatitkristallite unter den folgenden Aspekten diskutiert: Chemie, Kristallographie, Kristallitgröße Bildung, Umbildung sowie Diagenese.

1 INTRODUCTION

It is well known that tooth enamel mainly consists of well oriented enamel crystallites. The enamel crystallites are made up by the so-called 'biological apatite', which is carbonated hydroxyapatite with a variety of ion-substitutions. To survey the differences in enamel crystallites among recent and/or fossil animals, it is necessary to understand the present knowledge of properties of enamel crystallites. This chapter aims to review the chemical and physical properties of enamel crystallites concisely with a brief historical review including the author's data.

2 CHEMICAL ANALYSIS OF ENAMEL (HISTORICAL REVIEW, DISTRIBUTION)

Tooth enamel is unique among vertebrate hard tissues in its high mineral content and its low content of organic matter and water. Chemical analysis has shown the inorganic content to be about 95% by weight. The remaining components are the water and organic matter (BRUDEVOLD & SOREMARK 1967).

Since water serves the important function of ion diffusion, analysis of water in tooth enamel has been carried out using a variety of techniques including thermo-

gravimetric analysis (TGA), infrared absorption spectroscopy (IR), electron spin resonance (ESR), magnetic nuclear resonance (MNR) as well as classic chemical analysis. It should be noted that the ability of enamel to retain foreign ions is further enhanced by the ability of hydroxyapatitc crystals to bind water and hydrated ions (NEUMAN & NEUMAN 1958).

One of the controversies on enamel mineral was the low Ca/P ratio, which is also found in dentine and bone mineral. Chemical analysis of tooth enamel mineral usually showed the Ca/P molar ratios lower than 1.60, whereas the ideal Ca/P molar ratio is 1.67 for hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. This was once called the 'non-stoichiometry' of biological apatite. Today, apatite is known as one of the typical 'solid-solution minerals'. The apatite crystal structure allows the substitution of F ions for OH ions. The following ionic substitutions are known in biological apatite: Mg^{2+} and Na^{+} for Ca^{2+}, CO_3^{2-} and HPO_4^{2-} for PO_4^{3-}, and CO_3^{2-} and Cl^{-} for OH^{-} (YOUNG & BROWN 1982). The minerals francolite and dahllite are carbonate-containing apatites, and by definition the latter mineral contains less than 1 wt% carbonate ions. Both minerals occur as the inorganic component of hone and teeth (MCCONNELL 1975).

Biological apatite may contain a considerable amount of carbonate ions, i.e. several percent in tooth enamel, both at the OH-site (A-site) and at the PO,-site (B-site) (MCCONNELL 1975, LEGEROS, 1991). The content of carbonate ions of tooth enamel apatite has been investigated intensively and now can be determined quantitatively using Fourier infrared absorption spectrometry (Fl-IR). An FT-IR study revealed that fossil proboscideans tooth enamel preserved a considerable amount of carbonate ions (Fig. 1). Human tooth enamel usually contains about 3 to 4% (LEGEROS 1991), and recent elephant tooth enamel also contains almost the same amount (Fig. I). Amount of carbonate ions in tooth enamel from the fossil proboscideans varied but did not correlate with geological time (Fig. I).

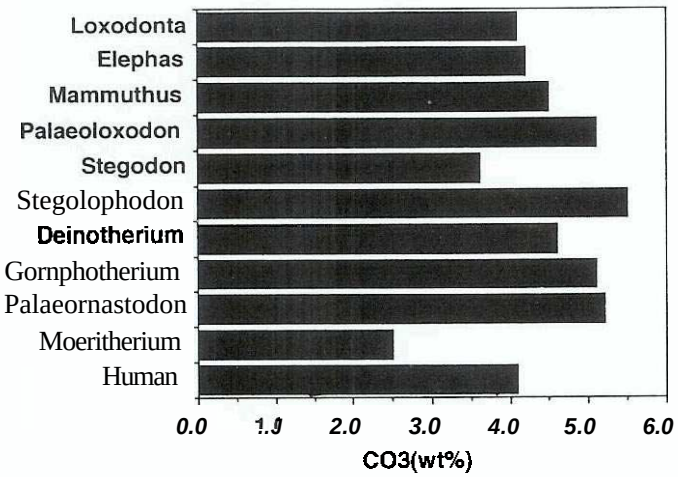

Figure 1. Carbonate content for the fossil proboscidean tooth enamel.

3 CRYSTALLOGRAPHY OF ENAMEL CRYSTALLITES (IDENTIFICATION AS APATITE)

Using a polarizing microscope, VALENTIN (1961) found that the birefringence of enamel was much stronger than that of dentine and cementum (SCHMIDT & KEIL 1971). In 1862, HOPPE observed that the optical sign changed from positive in immature enamel to negative in mature enamel (ref. in SCHMIDT & KEIL 1971). He also assumed the optical axis to be parallel to the enamel prism long axis. This would mean that the uniaxial apatitic crystallites are elongated parallel to their optical axes. It had not been believed that enamel crystallites consisted of a variety of apatite because of the considerable carbonate content which according to general concepts at the time could not enter the apatite lattice. However, MCCONNELL (1952) made a strong suggestion that biological apatite may contain carbonate ions in appreciable amount.
Identification of enamel crystallites was performed by GROSS (1926) using X-ray diffraction. He also showed the preferred orientation of enamel crystallites. It was four years later that the crystal structure of apatite was established by NARAY-SZABO (1930) and independently by MEHMEL (1930) using X-ray diffraction. However, it took another thirty years until the detailed crystal structure of apatite was resolved (KAY et al. 1964).

Crystallographic analysis of enamel crystallites revealed that the unit cell dimensions are about 0.9441 nm for the a-axis and 0.6884 nm for the c-axis, which are comparable to that of synthetic hydroxyapatite; 0.9421 nm and 0.6881 nm (ELLIOTT 1994). The unit cell dimensions of apatite vary due to ionic substitutions as in Table 1 (YOUNG & BROWN 1982).

Under the transmission electron microscope, enamel appears to consist of a mass of rod-like crystallites, oriented essentially with their long axes parallel to the direction of the enamel prisms and separated by exceedingly narrow spaces. The average crystallite size was measured as 160 nm in length and 20 nm in width for human enamel by transmission electron microscopy (RONNHOLM 1962). These values are in accordance with those of hippopotamus enamel determined by GLAS & OMNELL (1960) by X-ray diffraction.

The volume of individual enamel crystallites was estimated to average more than 200 times that of dentine and bone crystallites. The important study by NYLEN et al. (1963) showed that increased enamel calcification was due to an increase in crystal size. Shape and size of human enamel crystallites were carefully measured during maturation stage (DACULSI et al. 1984). Their's and other TEM studies showed that crystallites from the early formation stage were ribbon or lath-like in shape and very long in the c-axis dimension. Neighboring crystallites fused later during maturation.

Table 1. Enamel crystallite size and shape (variation in stages and among animals).

	One TE unit of H_2O	1 wt% CO_3 in B-site	1 wt% CO_3 in A-site	$(2OH^-)_x$ by $(O_2^-+[\ ])_x$	(OH), by Cl_x	(OH), by F_x
Aa (nm)	+0,0041	–0,00059	+0,0026	-0,00268	+0,0223×	–0,0052×
Δc (nm)	–	+0,00024	-0,00080	4,00129	–0,0166×	–0,00

4 INTERACTION WITH CIRCUMSTANCES (NUCLEATION, THERMODYNAMIC AND FOSSILIZATION)

Formation of biological apatite: It is a well known fact that precipitates formed under conditions close to physiological circumstances are amorphous calcium phosphate rather than crystalline apatite (DRIESSENS 1982). Freshly precipitated 'amorphous' tricalcium phosphate, $Ca_3(PO_4)_2$, is believed to be apatitic in nature and its formula can be written as $Ca_9(PO_4)_6{\cdot}nH_2O$ (POSNER et al. 1984), or $Ca_9[\ ](PO_4)_6[\ ]_2$, where [ ] is a vacancy (CORBRIDGE 1985). In the presence of water some hydrolysis occurs and the material eventually becomes crystalline $Ca_9[\ ](PO_4)_5(HPO_4)(OH)[\ ]$ (CORBRIDGE 1985). Although the amorphous calcium phosphate (ACP) precursor theory was once generally adopted to explain the formation of bone crystallites, there is strong doubt about its validity because a series of investigations failed to show evidence for it in hone and other hard tissues (GRYNPUS et al. 1984).

While apatite bas a vast stable area in the solubility diagram (NANNCOLLAS 1982), the first formed crystalline material may be octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6{\cdot}5H_2O$) which easily is transformed to apatite (BROWN 1962, BROWN et al. 1962, LEGEROS et al. 1989). The platy or ribbon habit of precipitated and biological apatites has been taken as evidence that these crystallites were formed via an OCP intermediate and may therefore still contain intercrystalline layers of OCP (BROWN et al. 1987). The OCP precursor theory explains well the non-stoichiometric nature of biological apatite with it's many ionic substitutions.

Transformation of biological apatite: Hydroxyapatite itself is stable up to at least 1000°C. and above 1500°C it decomposes into a mixture of tricalcium phosphate and tetracalcium phosphate (CORBRIDGE 1985). Biological apatite, including enamel crystallites, decomposes more easily and along a different pathway. They first loose the adhesive water in the temperature range below 120°C. With increasing temperature, the organic materials is almost completely burned out between 200°C and 600°C. In this temperature range, the inorganic phase of human enamel transforms into the combination of apatite and whitlockite, $Ca_3(PO_4)_2$, (SAKAE & HIRAI 1984, LEGEROS 1984), and apatite and CaO in the case of bone (LEGEROS & LEGEROS 1984). During this process, carbonate and water evolve from the inorganic phase. This water is attributed to the HPO_4 in the crystal structure of biological apatite.

Unit cell dimensions of enamel apatite crystal change during the thermal treatment according to the above mentioned ionic movements. Figure 2 shows that the changes of the unit cell dimensions for the fossil proboscideans tooth enamel are similar to that of human tooth enamel. The similar patterns in decreasing a-axis lengths suggested that chemical composition of the crystals was almost the same among these specimens.

X-ray diffraction analysis showed that the thermal decomposition pattern of tooth enamel varies among recent and fossil elephant teeth (SAKAE 1991). As the ratio of apatite to whitlockite after thermal decomposition may reflect the original chemical composition of the enamel (SAKAE & HIRAI 1984), the different Wh/Ap ratio can be used to detect distinct chemical compositions. Recent *Loxodonta* and *Elephas* molar enamel was transformed completely into whitlockite, while fossil *Deinotherium* molar enamel was transformed into whitlockite and alpha-tricalcium phosphate (Fig. 3).

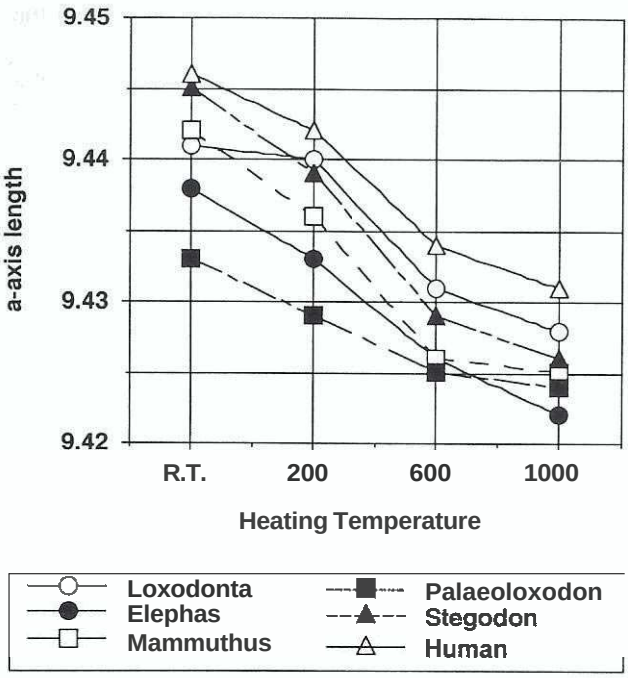

Figure 2. Changes of a-axis length (Å) of tooth enamel apatite with temperature.

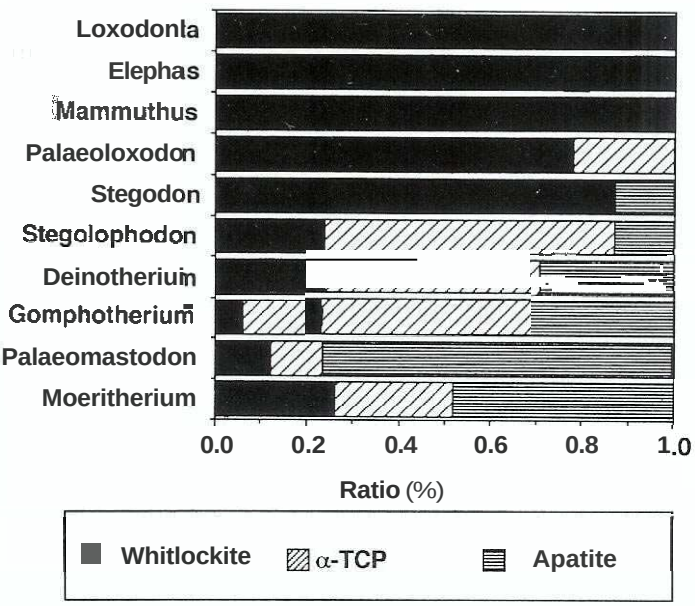

Figure 3. Crystal constitution for the proboscidean tooth enamel after heating at 1000°C.

These results indicate that the magnesium content of tooth enamel is greater in the recent proboscidea than it was in the ancient one.

Assessment of the effect of diagenesis on fossil tooth enamel: It is well known that diagenetic changes occur during the burial of vertebrate skeletons. PARKER & TOOTS (1980) divided the chemical elements involved in these changes into three groups:

1. Additions: Such as fluorine, silicon, manganese, iron and yttrium;
2. Losses: Sodium, magnesium, chlorine and potassium; and
3. Unaffected: Such as strontium.

These chemical changes during diagenesis may vary among different tissue types; enamel is the least changeable. Therefore, the observed decrease in magnesium content in fossil elephant tooth enamel may not be the effect of fossilization alone.

X-ray diffraction patterns for fossil mastodon enamel showed somewhat greater diffuse scattering than those ot human enamel, probably attributable to a combination of effects, i.e. greater departure from the ideal crystalline symmetry because of CO_3 groups within the structure as well as a greater range in size of the individual crystallites (MCCONNELL 1973). It may be noteworthy that the chemical composition of the mastodon enamel (dahllite) diverged from pure hydroxyapatite, i.e. CaO: 51.44%, MgO: 0.34%, Na_2O: 0.80%, K_2O: 0.05%, Fe_2O_3: 0.03%, CO_2: 2.72%, $H_2O^{(+)}$: 2.83%, $H_2O^{(-)}$: 0.80%, P_2O_5: 39.92%, Cl: 0.42%, F: 0.03% (MCCONNELL 1973).

Broadening of the X-ray diffraction peak is indicative of crystal size and strains effects, and biological apatites show appreciable X-ray diffraction peak broadening which is attributable to ionic substitutions in it's crystal structure. The broadening varied among the layers of human tooth enamel (SAKAE 1988); the peak broadening was larger for the enamel inner layer than for the outer layer indicating that crystallite size was larger in the outer layer of enamel.

Whitlockite formation after heat treatment was greater in the inner layer than in the outer layer (SAKAE 1988), suggesting that magnesium was more concentrated in the inner layer. ROBINSON et al. (1981) documented that the amount of magnesium and carbonate ions increased towards the inner layers of human tooth enamel. The increase in crystallinity, or crystal perfection, towards the outer layers of human tooth enamel was in accordance with the elemental distribution. Biological apatite, including tooth enamel apatite, generally contains a large amount of ionic substitutions such as Mg for Ca and CO_3 for PO_4 and OH. These ionic substitutions cause the lowering of crystallinity due to the ionic size difference.

Fossil elephant tooth enamel exhibited the same inner-to-outer pattern as human tooth enamel, i.e. that crystallinity, measured by the peak broadenings, is higher in the outer layers than in the inner layers (Fig. 4). The possible cause for the crystallinity deviation was the differing amount of ionic substitutions mentioned above. The X-ray diffraction study revealed that there was a similar relationship in the a-axis length between the inner and outer layers of the fossil proboscidea tooth enamel and those of human tooth enamel (Fig. 5).

These two relationships between the inner and outer layers of tooth enamel for the fossil pmboscidea strongly suggest that these fossil specimens keep their original crystallographic properties.

The fact that the fossil tooth enamel kept their original properties supports the belief that tooth enamel is the least leached material during diagenesis as suggested by

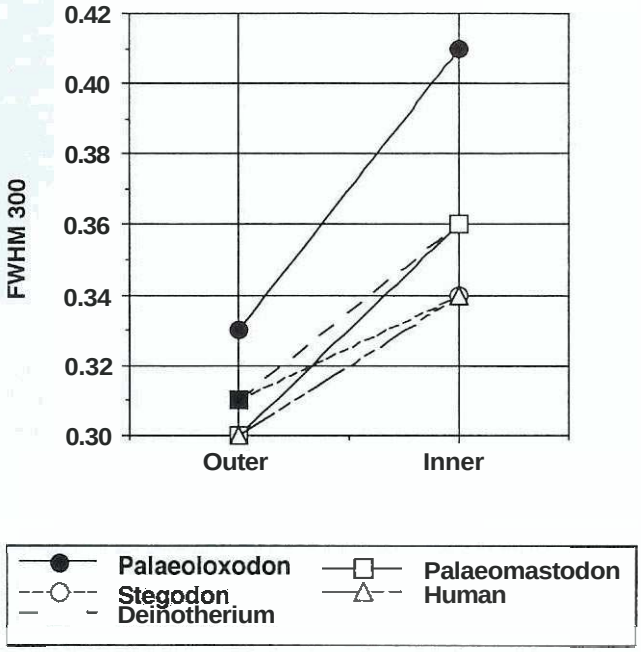

Figure 4. Comparison of X-ray diffraction peak broadenings between the inner and outer layers of tooth enamel for the fossil proboscidean together with human tooth enamel. FWHM: Full width at half maximum.

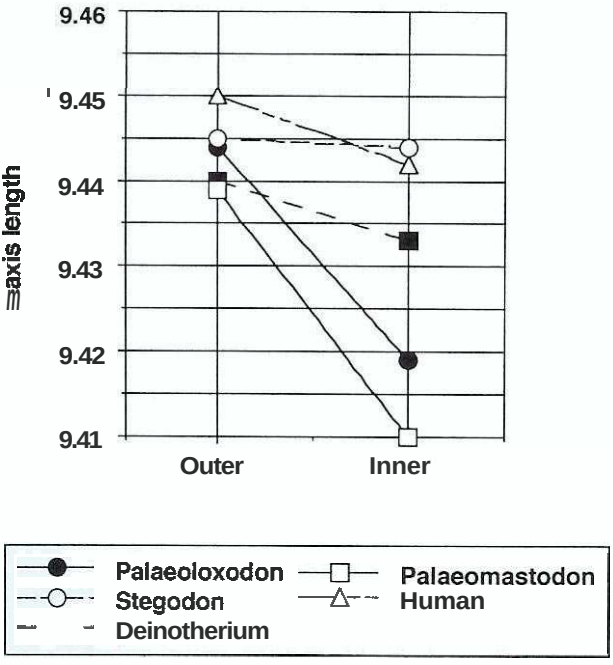

Figure 5. Comparison of a-axis length (Å) between the inner and outer layers of tooth enamel for the fossil proboscidean tooth enamel apatite.

SAKAE et al. (1991) that the older elephant tooth enamel was transformed into a low Wh/Ap ratio crystal combination after the heat treatment. They also documented that the a-axis length of the fossil elephant enamel crystallites was shorter in the older one; 0.9431 nm for ***Moeritherium*** as opposed to 0.9441 nm for ***Loxodonta.*** These results may, however, reflect that the fossil elephant tooth enamel had a somewhat different chemical composition from the recent one. Further study is needed to clarify the evolutionary changes in tooth enamel crystallites.

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