Morphology and Size-Distribution of Sound and Acid-Treated Enamel Crystallites

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Citric acid dissolves crystallites of enamel by initially etching out approximately hexagonal holes in the core of the crystallites, parallel to their long axis. Such acid-treatment influences the crystallite diameter only slightly since the distribution of the diameters of crystallites with a hollow core is not essentially different from those found in sound enamel. In both cases, the average diameter is 37 nm. Crystallites having a central defect and an outer diameter of about 40 nm are split into two parts of approximately 15 nm in diameter following acid treatment. The central defect is caused exclusively by the acid and not by damage from the electron beam, nor by a combination of acid treatment and electron beam damage.

Key words: Dissolution — Enamel — Crystallite diameter.

Introduction

The transmission electron microscope (TEM) has shown convincingly that a considerable difference exists between the dissolution rates of the central core and the external shell of the hydroxyapatite crystallites in human enamel (Little, 1959; Frank, 1960; Nylen, 1964; Frazier, 1968; Jongebloed et al., 1973; Scott, 1974). Similarly, the scanning electron microscope (SEM) revealed preferential dissolution of the crystal core in synthetic hydroxyapatite crystals, indicating the role of dislocations (lattice defects) in the dissolution process of these crystals (Arends et al., 1971; Arends et al., 1973; Jongebloed et al., 1974).

For a qualitative and quantitative analysis of the dissolution process, knowledge of the normal size and shape of enamel crystallites is of the utmost importance. However, little agreement exists among investigators about the dimensions and shape of normal crystallites (Little, 1959; Rönnholm, 1962; Nylen, 1964; Frazier, 1968; Grove et al., 1972). Moreover, the enamel crystallites are certainly damaged by the electron beam, with which they are visualized in the TEM (Hirai and Fearnhead, 1972).

In this investigation, crystallites of sound and acid-treated enamel have been compared, with special attention being paid to the shape and size distribution of the crystallites and the influence of the electron beam on their morphology.

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Materials and Methods

The materials used in this study were sound molars, which were cleaned after extraction and stored in a thymol-containing solution at 4°C (Jongebloed et al., 1974). After washing and drying, the molars were partly embedded in methacrylate resin except for the buccal area. This was done for easier handling of the specimens during the preparative procedure. The buccal area was carefully ground flat and polished using 0.25 μm diamond paste. The polished areas were exposed to citric acid (30%) at 37°C for 120 sec. The samples were then washed for 1 h with running tapwater, and dried. The buccal area was sawn into small blocks of approximately 2 × 2 × 3 mm by means of a water-cooled stringsaw.

For the TEM these small blocks were embedded in araldite. With a diamond fraise the top of the block was given a pyramid shape, the cutting face being 0.5 × 0.5 mm. Ultrathin sections (approximately 50–80 nm thick) were made with a LKB-ultramicrotome equipped with a diamond knife (of 50° angle), taking care to use only the outer 5 μm from the material. The sections were then mounted on colloidon-carbon coated grids and examined in a Philips transmission electron microscope EM-300 at 80 kV. To evaluate the beam damage effect, some experiments were carried out in which electron micrographs were made successively after exposing the same spot to the electron beam for 1, 15 and 30 min, respectively. The irradiation conditions were: condensor (I) aperture 300 μm; condensor (II) aperture 200 μm; objective aperture 40 μm; emission 130 μA; H.T. 60 kV; magnification ×26000; condensor (I) in neutral position (4) and condensor (II) as normally chosen for making an electron micrograph (exposure time 0.5 sec).

The preparation for the scanning electron microscope (SEM) was similar until the drying procedure following the etching. The dried samples, after being mounted on stubs, were coated with carbon (20 nm) and gold (10 nm) respectively in a vacuum evaporator, with constant rotating and tilting of the samples. The material was finally examined in a Jeol JSM-U3 microscope, operated at 25 kV.

Results

1. Transmission Electron Microscopy

a) Sound Enamel. Fig. 1a, b and c represent TEM pictures of ultrathin sections of sound human enamel. As seen in Fig. 1a, at low magnification (50000 ×) the crystallites show a considerable variation in shape and diameter. The crystallites are needle-shaped, as can be seen in Fig. 1b in which they are cut approximately parallel to their longest axis. Cross-sections reveal a variety of shapes. Many have a roughly flattened hexagonal appearance (see also Fig. 8 and Table 1), although rectangular shapes are also observed. The approximate shape is shown schematically in Fig. 2. Because thickness (t) and width (w) can only be distinguished clearly if crystallite cross-sections are studied, then for practical reasons the crystallite diameter d was measured instead. For a large number of observations, the equation $d = \dfrac{w + t}{2}$ is valid. Crystallite length measurements were not carried out, because of the uncertainties involved in determining the length of individual crystallites in sectioned material.

Fig. 3 shows the distribution of the crystallite diameter (d) for sound enamel. Measurements, based on about 600 crystallites from 12 teeth, showed an almost symmetrical distribution around an average value of approximately 37 nm.

b) Acid-Treated Enamel. The results on acid-treated enamel are given in Figs. 4a, b and c. Fig. 4a shows a cross-section through a part of one prism, in which the crystallites with both orientations—parallel and perpendicular to the longest axis—are visualized at a relative low magnification. Figs. 4b and 4c show examples of the preferred dissolution of the crystal core at higher magnification for crystallites cut parallel and perpendicular to their c-axis. Most likely, I and II in