The Distribution of Magnesium in Developing Rat Incisor Dentin

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Previous studies have shown that rat incisor dentin contains a considerable amount of magnesium that is distributed heterogeneously. The cementum-related dentin, especially its incisal portion, is richest in magnesium. It was the purpose of the present study to investigate the changes that occur in the magnesium content during dentin maturation.

Cross-sections were prepared from rat incisors at the apical, middle, and incisal levels. By means of an electron microprobe, tracings were made of the Ca-, Mg-, and P- signal frequencies. Comparison of corresponding dentin layers within and between the cross-sections showed that the Mg/P molar ratio was always higher in the cementum-related dentin (CRD) than in the enamel-related dentin (ERD) and increased from the apex toward the incisal edge. Especially in the incisal cross-section, an increase in Mg/P was found from the older (peripheral) toward the younger (central) dentin layers. As the Mg/P ratio varied from 0.07 to 0.33, the Ca/P ratio was found to fluctuate from 1.48 to 1.15. The two ratios appeared to be highly correlated ($r = -0.97$; $p < 0.001$), suggesting that Mg replaces Ca and is bound to phosphate.

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Introduction.

The contribution of magnesium to biomineralization is unclear. In a number of calcified tissues, such as rat incisor enamel (Hiller *et al.*, 1975), cutaneous calcinosis (Tochon-Danguy *et al.*, 1978), fracture callus, and turkey tendon (Quint *et al.*, 1980), the magnesium concentration strongly diminished as the mineralization process evolved. The presence of magnesium in the early stages was accompanied by an elevated carbonate content (Hiller *et al.*, 1975; Quint *et al.*, 1980).

In rat incisor dentin, the magnesium content increases as a function of time (Johnson, 1972; Steinfort *et al.*, 1990). In previous studies, we have demonstrated that in the rodent incisor two dentin portions can be distinguished, an enamel- and a cementum-related one (ERD and CRD, respectively), which differ with respect to their mineralization rates (Beertsen and Niehof, 1986) and the composition of their organic and inorganic matrices (Steinfort *et al.*, 1989, 1990). The ERD is harder and denser and contains more calcium and phosphate but less magnesium, as compared with the CRD. In addition, the ERD was found to be richer in phosphoproteins with a higher degree of phosphorylation than the CRD, which led us to suggest that different control mechanisms may exist with respect to mineralization in the two dentins. It was the purpose of the present study to investigate in more detail the incorporation of magnesium during dentin formation in the ERD and CRD.

Materials and methods.

Preparation of specimens.—Ten female Wistar rats (four months old) were anesthetized with ether and killed by an

overdose of Nembutal injected intracardially. Their lower right jaws were dissected out and stored frozen. Cross-sectional slices (1 mm in thickness) of the incisors, together with the surrounding tissues, were prepared at an apical, middle, and incisal level (Fig. 1) by use of a Microslice 2 MR (Metals Research Ltd., Cambridge, England) with a diamond saw blade under constant water cooling. After removal of the surrounding bone, the cross-sections of the incisors were dehydrated through a graded series of ethanol and propyleneoxide and embedded in epoxy resin (Epon LX112, Ladd), which was polymerized at 60°C. All specimens were embedded simultaneously in the same piece of epoxy resin, in such a way that the slices obtained from one tooth were next to each other. The incisal aspect of each slice was then serially polished with silicon carbide abrasive paper (nos. 240 to 600), followed by a 0.03- μm aluminum oxide micropolisher.

Electron microprobe analysis.—After the slices were coated with a carbon layer in a Balzer BEA 250 evaporator, the specimens were subjected to electron microprobe analysis in a JEOL Superprobe 733 (10 kV, 10 μA , beam area 1 μm^2 , ten s per

Fig. 1 — Rat lower incisor cross-sectioned at the apical, middle, and incisal levels. Electron microprobe tracings were made along the mid-sagittal line of each cross-section. ERD = enamel-related dentin. CRD = cementum-related dentin.

measurement, automatic background subtraction). Tracings of calcium, magnesium, and phosphorus were made at 3-, 7-, and 10- μm intervals (apical, middle, and incisal cross-sections, respectively) along the mid-sagittal line (Fig. 1). Of four slices obtained from the incisal level, tracings were also made along para-sagittal lines.

Signal count ratios of Mg, Ca, and P were calculated at intervals of about 25 μm . The signal counts along the outer and inner edges of the incisor showed considerable fluctuations. For this reason, a zone of 15 μm along the borderlines was excluded from the analysis. As a consequence, pre-dentin (width, *ca.* 15 μm) and cementum (width, *ca.* 2 μm) were not included in the present study.

Molar ratios were calculated from signal count ratios by comparison with tracings of standards of known composition (apatite and pyrope; chemical analysis by Micro-analysis Consultants, St. Ives, England).

ZAF correction.—The molar ratios mentioned above, as determined by electron microprobe, are subject to variations in density and composition of the dentin material. In order to determine the extent to which this occurred, the composition of epoxy-resin-embedded dentin was calculated for a series of mineral contents ranging from 10 to 75% (wt/wt) and Mg/Ca molar ratios varying between 0.03 and 0.30. ZAF corrections were then calculated by the Proza correction program (Bastin and Heijligers, 1990) for dentin—on the basis of C, N, O, Cl, P, Ca, and Mg—and for the standards.

Corrections between -0.1 and $+1.8\%$ for Ca/P and between $+5.4$ and $+6.4\%$ for Mg/P were found as the dentin varied from highly mineralized and low in magnesium toward poorly mineralized and high in magnesium.

Because these values influence the results to only a minor degree, it was considered unnecessary to calculate corrections for all individual measurements. The ratios presented in the results are the uncorrected data.

Statistics.—Because the ratios calculated showed skewed distributions, the results were tested by Wilcoxon's signed-rank test for paired observations and Spearman's correlation coefficient of rank correlation.

Results.

Distribution of Mg/P and Ca/P (mol/mol) in dentin within cross-sections.—In the middle and incisal, but not in the apical, cross-sections, the Mg/P ratio tended to increase from the outer dentin toward the pulp (Fig. 2). However, in the dentin most adjacent to the pulp, Mg/P tended to decrease again. This effect was most prominent in the CRD. The increase in Mg/P was statistically significant at the incisal level, especially in the ERD (ERD, $r = 0.94$; $p < 0.001$; CRD, $r = 0.55$; $p < 0.001$).

The Ca/P (Fig. 3) ratio showed inverse tendencies with respect to the Mg/P ratio. At the middle and incisal levels, a decrease from the outer dentin toward the pulp was observed, followed by an increase in the dentin most adjacent to the pulp. The decrease in Ca/P was statistically significant at the incisal level (ERD, $r = -0.95$; $p < 0.001$; CRD, $r = -0.54$; $p < 0.001$).

The ERD contained more Ca/P ($p < 0.01$) and less Mg/P ($p < 0.01$) than the CRD when corresponding measuring sites were compared. This was the case at all three levels.

Tracings along para-sagittal lines (not shown) revealed similar distribution patterns of Mg/P and Ca/P as found for the mid-sagittal lines. The inclinations, however, were less steep because of the oblique intersections of these lines with the concentric dentin zones.

Comparison of Mg/P and Ca/P (mol/mol) in dentin between

cross-sections. Effects of time.—Comparison of the outer dentin zones of the apical, middle, and incisal levels showed an increase in Mg/P with dentin aging. The same was observed when the middle zones of the middle and incisal levels were compared with each other (Fig. 2, open arrows; $p < 0.01$). This was not the case for enamel.

With respect to Ca/P (Fig. 3, open arrows), a comparison of the outer dentin zones at the apical and middle levels showed a decrease in Ca/P in the CRD ($p < 0.01$). The same was observed when the middle zones of the middle and incisal levels were compared ($p < 0.05$). A similar tendency, although not statistically significant, was found for the ERD.

Effects of site.—Comparison between the dentin zones most adjacent to the pulp (marked "1.") at the apical, middle, and incisal levels (all of which were formed at the same time) revealed that Mg/P increased in the incisal direction (Fig. 2; $p < 0.01$). The reverse was found for Ca/P (Fig. 3; $p < 0.02$).

Relation between calcium and magnesium.—The distribution patterns of Mg/P and Ca/P were found to be highly complementary. The value of (Mg + Ca)/P varied only slightly with the distance from the pulp and was nearly the same in all cross-sections (not shown).

When Ca/P was plotted against Mg/P (Fig. 4), it appeared that the two ratios showed an almost perfect negative correlation [$r = -0.80$, -0.91 , and -0.97 ($p < 0.001$) for the apical, middle, and incisal levels, respectively]. At the incisal level, but not at the other levels, a negative correlation was also found between (Mg + Ca)/P and Mg/P ($r = -0.63$), which was hardly affected by ZAF correction ($r = -0.56$). The latter correlation indicates that although Mg/P and Ca/P are inversely related, Mg is not merely replacing Ca on a 1:1 molar basis.

Discussion.

The present study has shown that the difference in Mg/P ratio between ERD and CRD is already present in the youngest dentin layers and is maintained throughout the maturation process. These findings further substantiate the notion that intrinsic differences exist in the rodent incisor between the ERD and CRD with respect to formation and mineralization of dentin (Beertsen *et al.*, 1985; Beertsen and Niehof, 1986; Steinfort *et al.*, 1989, 1990).

The results of this study have further shown that the maturation of the outer and middle dentin layers is accompanied by a rise in the Mg/P ratio. In the CRD, this increase in Mg was accompanied by a concomitant fall in the Ca/P ratio. In the ERD, the decrease in Ca was less evident than the increase in Mg. Comparison of the youngest dentin zones at the three levels studied revealed that the highest Mg/P ratios and the lowest Ca/P ratios were found at the incisal level. With respect to the ERD, the latter finding is in line with the work of Johnson (1972). In a previous study (Steinfort *et al.*, 1990), we found that at least 99% of the calcium and phosphate is inorganic, which implies that the differences mentioned above are related mainly to the mineral and not to the organic matrix. This is also likely to be the case with magnesium, since the concentration of magnesium in the incisal segment of the dentin cylinder was found to be as much as 7% (w/v). Furthermore, it was shown that whereas the overall mineral density of the dentin cylinder showed a 30% increase from the apical toward the incisal segment, the increase in magnesium was five times as large (Steinfort *et al.*, 1990).

A striking observation was the almost-perfect negative correlation between the Mg/P and Ca/P ratios (Fig. 4), the Mg/P ratio ranging from 0.07 to 0.33 and the Ca/P ratio from 1.48

Mg/P (mol/mol)

Fig. 2 — Mg/P molar ratio along the mid-sagittal line (see Fig. 1) of the apical, middle, and incisal cross-sections. Measurements were made at 25- μ m intervals (mean + S.D.; $n = 10$). E = enamel; closed arrows = dentin-enamel junction; I = outer dentin zone; II = middle dentin zone; 1. = dentin zone most adjacent to the pulp. The open arrows indicate sequential maturation stages of the dentin ($p < 0.01$). The measurements of pre-dentin and cementum are not presented in this Fig.

to 1.15. Furthermore, the ERD contained less magnesium than the CRD. Both findings are in line with our previous work (Steinfort *et al.*, 1990). Low Ca/P ratios in rat incisor dentin have also been reported by Höhling *et al.* (1968). An increas-

ing Mg/P ratio from the outer dentin zone toward the center has also been observed in the teeth of man and the monkey (Shaw and Yen, 1972).

The rise in Mg in the aging outer dentin layers can be ex-

Ca/P (mol/mol)

Fig. 3 — Ca/P molar ratio along the mid-sagittal line (see Fig. 1) of the apical, middle, and incisal cross-sections. Measurements were taken at 25- μ m intervals (mean + S.D.; $n = 10$). E = enamel; closed arrows = dentin-enamel junction. I = outer dentin zone; II = middle dentin zone; 1. = dentin zone most adjacent to the pulp. The open arrows indicate sequential maturation stages of the dentin [(a) N.S.; (b) $p < 0.05$; (c) $p < 0.01$]. The measurements of pre-dentin and cementum are not presented in this Fig.

plained in two ways. The high negative correlation between Mg and Ca could signify that Mg is actually replacing Ca in pre-existent mineral crystallites. Another explanation is offered by the results of our previous work on the rat, which indicated

that older dentin is considerably harder and denser and contains more mineral than younger dentin (Steinfort *et al.*, 1990). Thus, it would seem that the increase of Mg in aging dentin is the result of the influx of a newly formed Mg-containing mineral,

which may be related to changes in the odontoblasts. The finding that the Mg enrichment in the central dentin core is more prominent than that observed in the outer and middle zones may support this view.

In calcified tissues, magnesium may be bound to phosphate (Glick, 1981; Featherstone *et al.*, 1983; Terpstra and Driessens, 1986) or to carbonate. In the latter case, magnesium and carbonate may exist as impurities at the surfaces of hydroxyapatite crystals (Hiller *et al.*, 1975) or as a separate mineral phase (Driessens and Verbeeck, 1985). Although it has been shown that carbonate occurs in bovine dentin (Quint *et al.*, 1980), we have not been able to determine its presence and distribution in the rat incisor by the methods used. Consequently, it is not possible to draw conclusions with respect to the binding of magnesium to either phosphate or carbonate. On the other hand, on the assumption that all magnesium would be bound to carbonate, this would leave unexplained the low Ca/P ratio and the high negative correlation between the Mg/P and Ca/P ratios. Thus, we consider it likely that a considerable amount of magnesium is bound to phosphate, either as magnesium-substituted hydroxyapatite at the surfaces of the crystallites or as a separate mineral phase.

Finally, it is interesting that the increasing amount of magnesium in aging rat incisor dentin deviates from what is seen in some other mineralizing tissues. As time progresses, the Mg/Ca ratio in cutaneous calcinosis, fracture callus, and mineralizing turkey tendon decreases (see "Introduction"). A similar decrease is found during early maturation of rat incisor enamel (Hiller *et al.*, 1975), whereas in bovine enamel, an initial increase is followed by a decrease (Robinson *et al.*, 1984). From this we conclude that the relationship between aging and magnesium content is variable and depends upon the type of tissue studied.

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Fig. 4 — Ca/P and (Ca + Mg)/P plotted against Mg/P (values obtained at 25- μ m intervals, along the mid-sagittal line at the incisal level; ERD plus CRD).