Regulation of pH during enamel development

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Summary

During enamel development dental enamel is mineralized by deposition of massive amounts of hydroxyapatite crystals formed from calcium and phosphate ions. Each unit of hydroxyapatite crystal [Ca10(PO4)6.2OH] formed during this process releases ~8 H+ protons ions into the enamel space. In theory, this process results into a decrease in pH in the enamel matrix space, an effect that will peak in maturation stage when crystal formation enhances sharply. In order to deal with the drop in pH, the ameloblasts have been proposed to buffer acid using bicarbonate. In this thesis we focused on the possibility that maturation stage ameloblasts buffer protons. We hypothesized that (1) ameloblasts express a set of different ion transporters/exchanger(s) to secrete bicarbonates similar as typical transport epithelia to neutralize the protons released during crystal formation in the enamel space; and (2) insufficient neutralization of the protons released during the formation of crystals in enamel of mice with null mutation of such pH regulators are responsible for formation of hypomineralized immature enamel.

In **Chapter 2** and **Chapter 3**, we tested the hypothesis that ameloblasts secrete bicarbonates into the enamel space in order to neutralize the protons. We investigated the possible involvement of the SLC4 and SCLC26A family of ion-transporters/exchangers in transporting bicarbonate into the enamel space matrix. We have found that the mineral density of enamel in *Nbce1*$^{-/-}$ teeth was lower compared to that in the wild-type littermates (chapter 2). In *CFTR*$^{-/-}$ and *Ae2*$^{-/-}$ mice the expression of Nbce1 protein in enamel organs was strongly elevated. The data favored a role of Nbce1 in pH regulation by responding to acids. We also showed that SLC26A3/Dra and SCL26A6 are localized in the apical membranes of the ameloblasts (chapter 3). However, both *Dra*$^{-/-}$ or *Slc26a6*$^{-/-}$ mice had a normal dental or skeletal phenotype and showed no changes in mineral density, as measured by micro-computed tomography. Western blot analysis indicated that enamel organs of *Slc26a6*$^{-/-}$ mice contained elevated levels of Dra and pendrin protein. From these results we concluded that there are strong compensatory reactions by other members of the SLC26A family in response to the deletion of one of them (i.e. SLC26A1, SLC26A3, SlC26A4, and SLC26A6).

Accumulation of Na^+ and K^+ in hypomineralizing enamel in *CFTR*$^{-/-}$ and *Ae2*$^{-/-}$ mice suggested that maturation ameloblasts transport both types of ions into enamel and also remove these from the enamel space by yet unknown mechanisms. Potential transporters capable of reabsorbing K^+ and Na^+ belong to the Na^+: K^+:2Cl^- (NKCC) cotransporter family. In **Chapter 4**, we therefore examined whether mouse maturation stage ameloblasts express Na^+: K^+:2Cl^- cotransporter1 (NKCC1) involved in ion transport. Our results showed that in mouse incisors ice Nkcc1 is expressed in the outer enamel epithelium and the papillary layer but not in the ameloblast layer. The height of dental epithelium in upper incisors of *Nkcc1*$^{-/-}$ mice at advanced maturation was shorter and enamel mineral density was slightly lower than in wild-type controls. The enamel organs of *Nkcc1*$^{-/-}$ mice contained more pendrin, Slc26a6

and Nbce1 protein suggesting compensation of *NKcc1*$^{-/-}$ mutation. To study whether the Nkcc1 is functional in ameloblasts, we examined effects of bumetanide (an inhibitor of NKCCs) on cell volume in ameloblast-like HAT-7 cell cultures. The regulation of cell volume was impaired by bumetanide, indicating that NKCC1 is capable of significantly contributing to cell volume regulation of the enamel organ and papillary layer.

In **Chapter 5**, we tested the hypothesis that development of enamel fluorosis in human teeth is associated with insufficient neutralization of the extra protons released during exposure of developing enamel to fluoride. This concept was suggested by animal experimental studies in which exposure to fluoride caused formation of hypermineralization lines. In this chapter, we tested whether this concept was also true for human enamel by examining whether human fluorotic teeth contain hypermineralized lines. Our micro-CT study showed that exposure of mice and humans to fluoride basically gives the same effect in developing enamel: formation of hypomineralized enamel with presence of hypermineralized lines.

These studies described in this thesis allowed us to determine several new ion (co) transporters necessary for enamel formation. Additionally, we have made progress in understanding the importance of pH regulation for proper enamel formation. Based on the studies discussed in this thesis a more complete model for ion and bicarbonate transport into/out of the enamel space has been proposed which was, discussed in **Chapter 6**., These studies by improved our understanding of the factors that lead to development of the mechanism underling enamel hypomineralization.