RESEARCH REPORTS
Biological

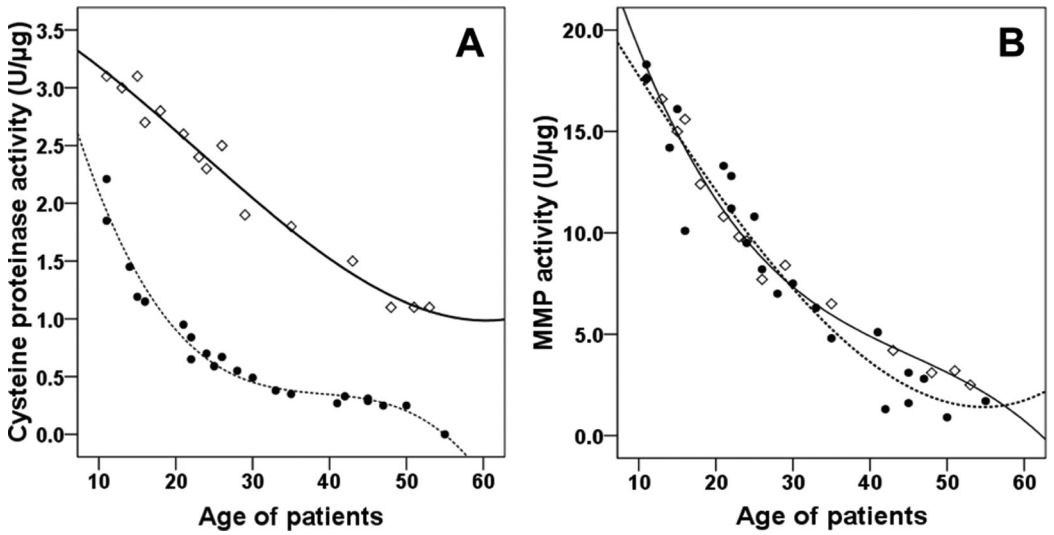
Cysteine Cathepsins in Human Carious Dentin

F.D. Nascimento[1], C.L. Minciotti[2], S. Geraldeli[3], M.R. Carrilho[1], D.H. Pashley[4], F.R. Tay[4], H.B. Nader[5], T. Salo[6,7], L. Tjäderhane[6,7]*, and I.L.S. Tersariol[2,5]*

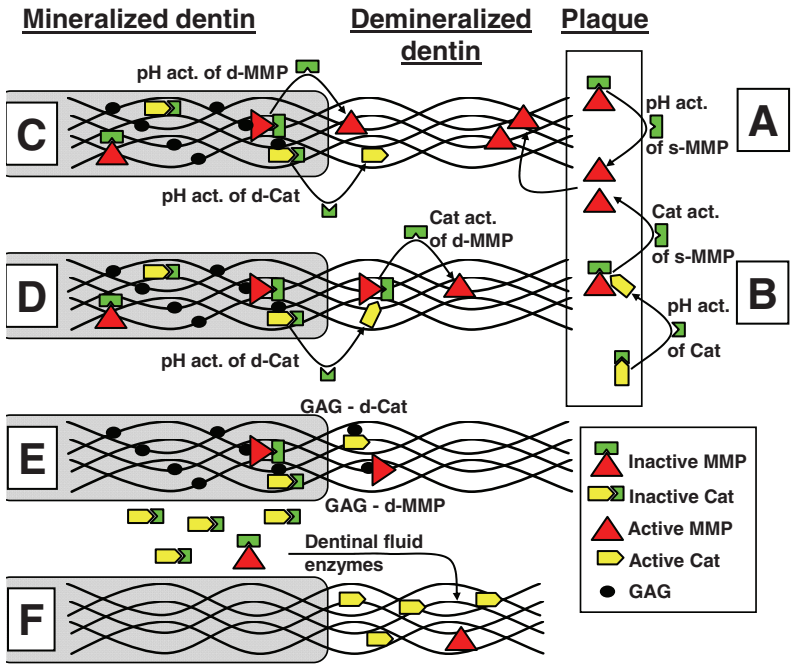
[1]Grupo de Estudos em Odontologia, Universidade Bandeirante de São Paulo (UNIBAN), Brazil; [2]Centro Interdisciplinar de Investigação Bioquímica, Universidade de Mogi das Cruzes, São Paulo, Brazil; [3]The University of Iowa College of Dentistry, Iowa City, IA, USA; [4]School of Dentistry, Medical College of Georgia, Augusta, GA, USA; [5]Universidade Federal de São Paulo (UNIFESP), Disciplina de Biologia Molecular, São Paulo, Brazil; [6]Institute of Dentistry, PO Box 5281, 90014 University of Oulu, Finland; and [7]Oulu University Hospital, Oulu, Finland; *corresponding authors, leo.tjaderhane@oulu.fi, ivarne@umc.br

J Dent Res DOI: 10.1177/0022034510391906

APPENDICES



Appendix 1, Figure. The relation between intact dentin (data extracted from the article by Tersariol *et al.*, 2010) and carious dentin enzyme activities. **(A)** Cysteine cathepsin activity in intact (white diamonds) and carious (black circles) dentin. The intact-dentin values have been multiplied by 10 to fit them into the same scale. **(B)** MMP activity in intact (white diamonds) and carious (black circles) dentin. The intact-dentin values have been multiplied by 10 to fit them into the same scale.



Appendix 2, Figure. Schematic presentation of potential functional mechanisms of activation and function of cysteine cathepsins and MMPs in caries lesions. Green blocks with MMPs (red triangles) or cathepsins (yellow arrowheads) indicate inactivity of enzyme, either as a proform or in complex with specific (TIMPs, cystatins) or non-specific (*e.g.*, α2 macroglobulin) proteinase inhibitors. Activation of protein (indicated as removal of green blocks) may represent either elimination of inhibitor, transformation from latent to active form, or both. **(A)** pH changes in plaque (acidic pH neutralized by salivary buffers) convert salivary proMMPs (s-MMP) and/or MMP-TIMP complexes into active MMP (Tjäderhane *et al.*, 1998; Sulkala *et al.*, 2001); activated MMPs then degrade dentin matrix proteins (Tjäderhane *et al.*, 1998). **(B)** At least cathepsin B directly cleaves and inactivates MMP-specific tissue inhibitors TIMP-1 and TIMP-2 (Nagase, 1997), and may thus change the balance between MMPs and their inhibitors. Acidic pH in plaque activates salivary cysteine cathepsins (s-Cat), which in turn either proteolytically activate salivary proMMPs or degrade TIMP-inhibiting MMPs, or both, resulting in active MMPs. Therefore, cysteine cathepsins activated at the acidic phase of caries may further activate dentin-bound or salivary MMPs, which in turn would become functional after neutralization of pH by salivary buffers (Tjäderhane *et al.*, 1998). **(C)** Activation of dentin-bound MMP and/or cathepsin (d-MMP and d-Cat, respectively) by pH changes in caries lesions, resulting in dentin matrix enzymatic degradation. **(D)** Acid activation of dentin-bound cathepsin, with further activation of dentin-bound MMP by active cathepsin. **(E)** Glycosaminoglycan (GAG: black dot) activation (*e.g.*, Rozman *et al.*, 1999) and stabilization (Almeida *et al.*, 2001) of cathepsin (and possibly MMPs, as suggested by Ra and Parks, 2007), allowing for functional activity of cathepsins, even in neutral pH. Glycosaminoglycans (GAGs) are able to accelerate the conversion of zymogen forms of cysteine cathepsins into its mature forms at neutral pH, activating cathepsin B (Rozman *et al.*, 1999), cathepsin L (Ishidoh and Kominami, 1994), cathepsin S (Kopitar *et al.*, 1996), and congopain (Serveau *et al.*, 2003). The presence of glycosaminoglycans in the dentin extracellular matrix (Embery *et al.*, 2001) and their release during acid-demineralization and subsequent proteolytic degradation of acid-demineralized dentin matrix (Dung *et al.*, 1995) support the possibility that GAGs are involved in *in vivo* processing of cysteine cathepsins in carious dentin. Interestingly, procathepsin S is capable of autocatalytic activation not only at acidic pH but also at neutral pH in the presence of related glycosaminoglycans (Vasiljeva *et al.*, 2005). Also, we have showed that cathepsin B is stabilized at pH 7.4 by interaction with heparan sulfate and heparin glycosaminoglycans (Almeida *et al.*, 2001). **(F)** Dentinal-fluid-derived enzymes degrading demineralized matrix. Analysis of the data presented in this manuscript indicates that odontoblast- or pulp-tissue-derived cysteine cathepsins are more important in dentinal caries lesions. Analysis of previous data indicates that at least MMP-20 may also be functional in dentinal caries matrix degradation (Sulkala *et al.*, 2002).

APPENDIX REFERENCES

Almeida PC, Nantes IL, Chagas JR, Rizzi CC, Faljoni-Alario A, Carmona E, *et al.* (2001). Cathepsin B activity regulation. Heparin-like glycosaminoglycans protect human cathepsin B from alkaline pH-induced inactivation. *J Biol Chem* 276:944-951.

Dung SZ, Gregory RL, Li Y, Stookey GK (1995). Effect of lactic acid and proteolytic enzymes on the release of organic matrix components from human root dentin. *Caries Res* 29:483-489.

Embery G, Hall R, Waddington R, Septier D, Goldberg M (2001). Proteoglycans in dentinogenesis. *Crit Rev Oral Biol Med* 12:331-349.

Ishidoh K, Kominami E (1994). Multi-step processing of procathepsin L *in vitro*. *FEBS Lett* 352:281-284.

Kopitar G, Dolinar M, Štrukelj B, Pungerčar J, Turk V (1996). Folding and activation of human procathepsin S from inclusion bodies produced in *Escherichia coli*. *Eur J Biochem* 236:558-562.

Nagase H (1997). Activation mechanisms of matrix metalloproteinases. *Biol Chem* 378:151-160.

Ra HJ, Parks WC (2007). Control of matrix metalloproteinase catalytic activity. *Matrix Biol* 26:587-596.

Rozman J, Stojan J, Kuhelj R, Turk V, Turk B (1999). Autocatalytic processing of recombinant human procathepsin B is a bimolecular process. *FEBS Lett* 459:358-362.

Serveau C, Boulangé A, Lecaille F, Gauthier F, Authié E, Lalmanach G (2003). Procongopain from *Trypanosoma congolense* is processed at basic pH: an unusual feature among cathepsin L-like cysteine proteases. *Biol Chem* 384:921-927.

Sulkala M, Wahlgren J, Larmas M, Sorsa T, Teronen O, Salo T, *et al.* (2001). The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats. *J Dent Res* 80:1545-1549.

Sulkala M, Larmas M, Sorsa T, Salo T, Tjäderhane L (2002). The localization of matrix metalloproteinase-20 (MMP-20, enamelysin) in mature human teeth. *J Dent Res* 81:603-607.

Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T (1998). The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. *J Dent Res* 77:1622-1629.

Vasiljeva O, Dolinar M, Pungerčar JR, Turk V, Turk B (2005). Recombinant human procathepsin S is capable of autocatalytic processing at neutral pH in the presence of glycosaminoglycans. *FEBS Lett* 579:1285-1290.