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Enamel proteins within two preferentially used animal models

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SUMMARY

Two different models have been used to study enamel proteins: rodent incisors and bovine or porcine tooth germs. In the present experiment proteins were sequentially extracted from forming enamel of rat incisors and bovine tooth germs and examined using SDS-PAGE.

The Coomassie-blue staining of amelogenins from both species revealed very similar patterns, which indicates a rather common processing, although developed at different rates. Non-amelogenin proteins behave differently when Concanavalin-A probing was used. Bovine non-amelogenins contain amido-black stainable proteins which are not recognized by lectin, contrary to rat enamel.

If those proteins are albumin or albumin derived, as recently suggested, the observed discrepancy might be explained by the non enzymatic glycation known to occur on circulating albumin. In that case it would be a consequence of the use of adult rats in which circulating albumin is partly glycated versus bovine foetuses in which albumin would not be significantly glycated. Finally both species contain glycoproteins within non-amelogenins, which remain to be more precisely defined.

KEY WORDS: Enamel, amelogenins, enamelins, non-amelogenin proteins.

RÉSUMÉ

Deux modèles ont été utilisés pour l'étude des protéines de l'émail: les incisives des rongeurs et les germes dentaires de bovins ou de porcs. Dans cette expérimentation, nous avons extrait de façon séquentielle les protéines de l'émail en formation provenant d'incisives de rat et de germes dentaires bovins et nous les avons étudiées par électrophorèse sur gel de polyacrylamide en présence de SDS.

La coloration des amélogénines par le bleu de Coomassie révèle dans les deux espèces des images électrophorétiques semblables qui sont révélatrices d'un processus de transformation analogue bien que se développant à des vitesses différentes.

Les protéines non-amélogénines se comportent de façon différente lorsque leur révélation est effectuée par l'intermédiaire d'un système Concanavaline-A et péroxydase. Les non amélogénines d'origine bovine contiennent des protéines colorées par l'amido-black qui ne sont pas reconnues par la lectine contrairement à ce qui se produit dans l'émail de rat. Si ces protéines sont de l'albumine ou dérivées de l'albumine comme cela a été récemment suggéré, la différence observée pourrait s'expliquer par un processus de glycation non enzymatique de l'albumine circulante. Dans ce cas, les résultats obtenus seraient la conséquence de l'utilisation d'un modèle adulte en ce qui concerne le rat dans lequel l'albumine circulante est partiellement glycatée en opposition avec des foetus bovins ou la glycation de l'albumine ne serait pas significative.

Enfin, les deux espèces considérées contiennent des glycoprotéines dans la famille des non-amélogénines qui restent à définir de façon plus précise.

MOTS CLÉ: Email, amélogénines, énamélines, protéines non-amélogénine.

INTRODUCTION

During the present decade, the organic matrix of developing enamel has been fully investigated leading to considerable progress in our knowledge of enamel formation. Two different models have been used: rodent incisors from rat, mouse, hamster or rabbit on the one hand, developing bovine, porcine, and human molars on the other (Robinson et al., 1977; Termine et al., 1980; Fincham et al., 1982a; Zeichner-David et al., 1983; Slavkin et al., 1988; Deutsch et al., 1984; Strawich and Glimcher, 1985; Rosenbloom et al., 1986; Fukae and Tanabe, 1987; Limeback, 1987; Bronckers et al., 1988; Menanteau et al., 1988; Ogata et al., 1988). The rodent model is suitable because the various stages of enamel development are easily recognized on a same tooth. The other model provides large amounts of material, necessary for primary biochemical investigations.

It has been demonstrated that the duration of enamel formation and maturation processes are subject to wide variations from weeks to months (Robinson et al., 1986). The entire mechanism requires intense processing of the newly deposited matrices leading to a nearly complete removal of the organic material. It is likely that some enamel proteins are submitted to enzymatic degradation. Recent evidence of the presence of amelogeninases within the matrix has been provided also (Moe and Birkedal-Hansen, 1979; Shimizu et al., 1979; Carter et al., 1984; Crenshaw and Bawden, 1984; Menanteau et al., 1986; Overall and Limeback, 1988; Carter et al., 1989). The purpose of the present paper was to take stock of the data obtained using the most popular methodology (SDS PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis) concerning rat incisors and bovine developing molars.

MATERIAL AND METHODS

Forming bovine enamel was scrapped off from freshly-extracted molars of 2-8 months old foetuses and prepared free of cellular contaminations according to a previously described method (Menanteau et al., 1986).

Forming enamel from rat incisors was scrapped off using the boundary which demarcates the white opaque area.

Enamel proteins were then sequentially extracted using the Termine et al. procedure (Termine et al., 1980). Guanidin-HCl extracts and Guanidin-EDTA extracts were desalted on a Bio-Gel P2 column and lyophilized extracts were then analysed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

SDS gels were 10% acrylamide and run under reducing conditions.

Some of them were directly stained with Coomassie blue. The others were electrotransferred onto nitrocellulose sheets (Towbin et al., 1979). The transferred proteins were revealed with Amido-black or Concanavalin-A peroxidase.

RESULTS

Enamel proteins were divided in two sets on the basis of their solubility according to Termine (Termine et al., 1980). In both forming enamel from rat incisors and developing enamel from bovine fetuses molars, the Gdn-HCL soluble material, amelogenins appeared as the groups of proteins ranging form 30 to 10 kD (Fig. 1 lanes 1 and 4).

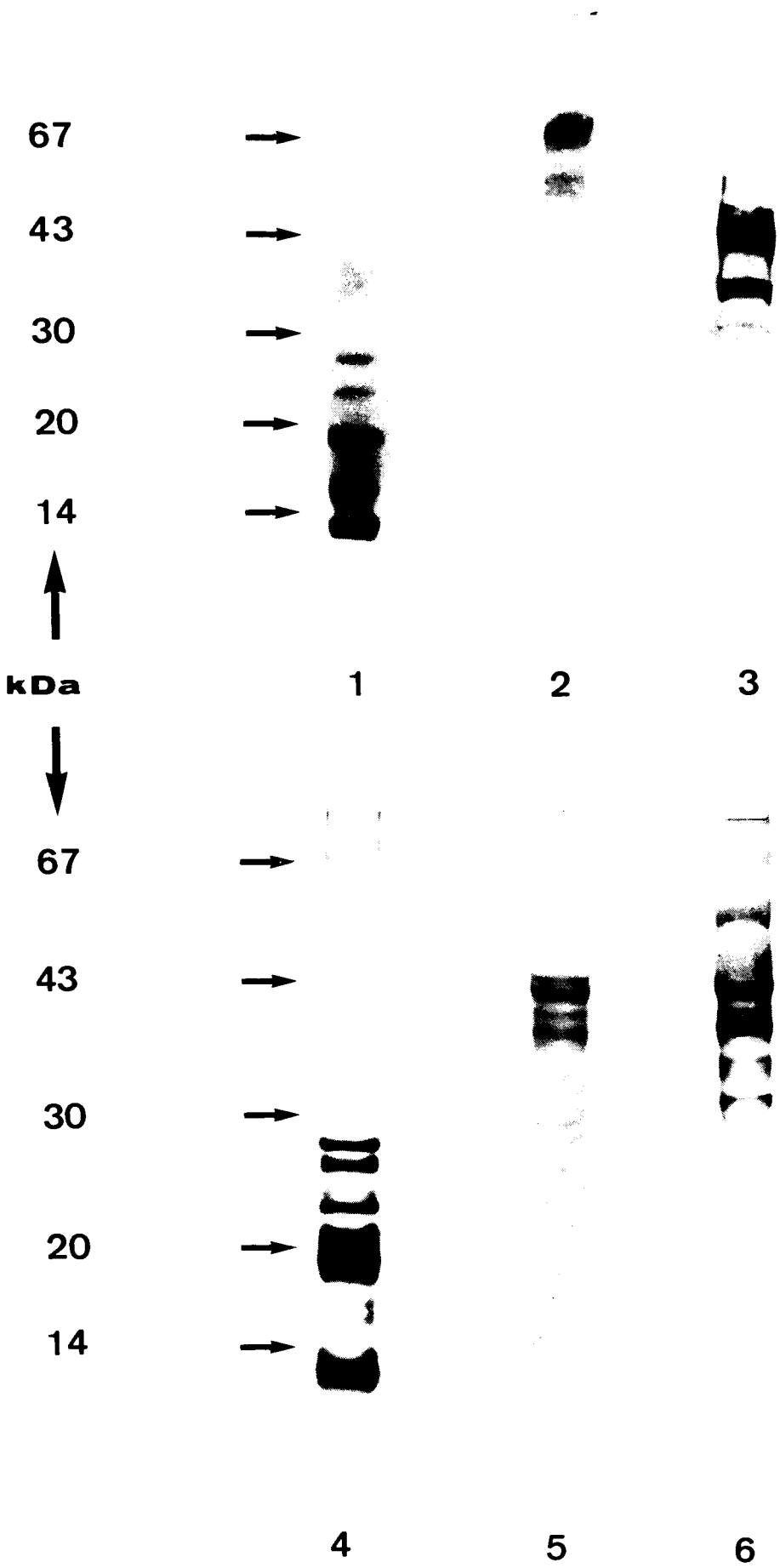
The non-amelogenin protein group was revealed either by Amido-black or by Concanavalin-A after transfer. The Amido-black staining showed similar patterns with proteins of relatively high molecular weight ranging from 40 to 70 kD (Fig. 1 lanes 2 and 5). Some Amido-black stained proteins were not revealed by Concanavalin-A in bovine molars (Fig. 1 lane 3) but revealed in rat incisors (Fig. 1 lane 6). In addition, Concanavalin-A revealed additional bands in both cases (Fig. 1 lanes 3 and 6).

DISCUSSION

Although the developmental time frame of the two different considered species differed, the electrophoretic patterns of amelogenins showed similar molecular transformation of the genetic products. The progressive degradation of amelogenins in keeping with enamel mineralization is well documented with regard to a lot of different species (Fincham et al., 1982b; Menanteau et al., 1984). The mechanism remains uncertain although there is increasing evidence in favour of an enzymatic process (Moe and Birkedal-Hansen, 1979; Shimizu et al., 1979; Carter et al., 1984; Crenshaw and Bawden, 1984; Menanteau et al., 1986; Overall and Limeback, 1988; Carter et al., 1989). If some specific amelogeninases are responsible for the degradation of amelogenins some kind of mechanism should control the rate of hydrolysis. A range of possibilities could account for the different speeds observed in enamel maturation.

▶ Fig. 1: SDS-PAGE of enamel proteins within developing bovine enamel (lanes 1,2, 3) and forming enamel from rat incisors (lanes 4, 5, 6).
Lanes 1 and 4: Coomassie blue staining of amelogenins.
Lanes 2 and 5: Amido-black staining of electro-transferred non-amelogenins.
Lanes 3 and 6: Con-A peroxidase probing of electro-transferred non-amelogenins.

Electrophorèse sur gel de polyacrylamide en SDS des protéines de l'émail bovin en formation (lignes 1, 2, 3) et de l'émail en formation d'incisives de rats (ligne 4, 5, 6).
Pistes 1 et 4: Coloration au bleu de Coomassie des amélogénines.
Pistes 2 et 5: Coloration au Noir-Amido des protéines non-amélogénines transférées.
Pistes 3 et 6: Révélation à la Concanavaline A et péroxydase des protéines non amélogénines transférées.



Amelogenin processing should be under cellular control likely to act in different ways: genetic regulation of enzymes synthesis, protection of the proteins from the proteolytic activity, accessibility of the enzymes to the substrates, synthesis and removal of a protease inhibitor as suggested earlier (Termine et al., 1980) or differential activation of the enzymes through effectors. Identical schemes could be postulated for other enzymes possibly involved in the degradation process, such as phosphatases, glycohydrolases...

Some discrepancies have appeared with regard to the properties of the constituants of the mineral compartment related to non-amelogenins proteins. They should be examined to the light of recent data and their foreseeable developments in the moving field of the so-called «enamelins». Non amelogenins have been recently identified in porcine and bovine developing enamel almost partly as serum proteins mainly albumin and degradative products of albumin (Limeback and Chu, 1988; Menanteau et al., unpublished data 1988). This discovery introduces new incertainties as regards the origin of the organic constituants of the mineral compartment of enamel.

Such feature is consistent with the behavior of bovine non-amelogenins with the lectin concanavalin-A since albumin is not a glycoprotein. With respect to rat non-amelogenins the case is more intriguing because all amido-black stained proteins have been recognized by Concanavalin-A. This might be due to non enzymatic glycation, a feature demonstrated in rat albumin (Day et al., 1979) although this phenomenon normally concerns a part of the circulating protein only. In both bovine and rat enamel, additional glycoproteins have been revealed by Concanavalin-A, but remain to be identified.

The present data confirm that, from a biochemical point of view, enamel maturation involves processes common to different teeth and species. However such conditions call upon a close cellular control of the velocity of the mechanisms involved in enamel protein processing.

REFERENCES

Bronckers, A.L.J.J., Lyaruu, D.M., Bervoets, T.J.M., Woltgens, J.H.M. — Autoradiographic, ultrastructural and biosynthetic study of the effect of colchicine on enamel matrix secretion and enamel mineralization in hamster tooth germs in vitro. *Archs Oral Biol., 33:* 7-16, 1988.

Carter, J., Smillie, A.C., Shepherd, M.G. — Proteolytic enzyme in developing porcine enamel. In: Fearnhead, R.W., Suga, S. (eds), Elsevier, North-Holland, Amsterdam, Tooth enamel, 4: 234-239, 1984.

Fincham, A.G., Belcourt, A., Lyaruu, D.M., Termine, J.D. — Comparative protein biochemistry of developing dental enamel matrix from five mammalian species. *Calcif. Tissue Int., 34:* 183-189, 1982a.

Fincham, A.G., Belcourt, A., Termine, J.D. — Changing patterns of enamel matrix proteins in the developing bovine tooth. *Caries Res., 16:* 64-71, 1982b.

Fukae, M., Tanabe, T. — Nonamelogenins components of porcine enamel in the protein fraction free from the enamel crystals. *Calcif. Tissue Int., 40:* 286-293, 1987.

Limeback, H. — Isolation and characterization of pig enamelins. *Biochem. J., 243:* 385-390, 1987.

Limeback, H., Chu, W. — Porcine «enamelins» cannot be distinguished biochemically from porcine serum albumin and its acid break down products. *J. Dent. Res., 67:* 205, Abstr. 738, 1988.

Menanteau, J., Meflah, K., Strecker, G. — The carbohydrate moiety of mineral-bound proteins from fetal enamel: a basis for enamelins heterogeneity. *Calcif. Tissue Int., 42:* 196-200, 1988.

Menanteau, J., Mitre, D., Daculsi, G. — Aqueous density fractionation of mineralizing tissues: an efficient method applied to the preparation of enamel fractions suitable for crystal and protein studies. *Calcif. Tissue Int., 36:* 677-681, 1984.

Menanteau, J., Mitre, D., Raher, S. — An in-vitro study of enamel protein degradation in developing bovine enamel. *Archs Oral Biol., 31:* 807-810, 1986.

Moe, D., Birkedal-Hansen, H. — Proteolytic activity in developing bovine enamel. *J. Dent. Res., 58B:* 1012-1013, 1979.

Ogata, Y., Shimokawa, H., Sasaki, S. — Purification, characterization and biosynthesis of bovine enamelins. *Calcif. Tissue Int., 43:* 389-399, 1988.

Overall, C.M., Limeback, H. — Identification and characterization of enamel proteinases isolated from developing enamel. *Biochem. J., 256:* 965-972, 1988.

Robinson, C., Kirkham, J., Weatherell, J.A. — Control of crystal growth during enamel development: a possible role for fluoride. In: Leach, S.A. (ed.) Factors relating to demineralization and remineralization of the teeth. IRL Press limited, Oxford, England, 13-21, 1986.

Robinson, C., Lowe, N.R., Weatherell, J.A. — Changes in animo-acid composition of developing rat incisor enamel. *Calcif. Tissue res., 23:* 19-31, 1977.

Rosenbloom, J., Lally, E., Dixon, M., Spencer, A., Herold, R. Production of a monoclonal antibody to enamelins which does not cross-react with amelogenins. *Calcif. Tissue Int., 39:* 412-415, 1986.

Shimizu, M., Tanabe, T., Fukae, M. — Proteolytic enzyme in porcine immature enamel. *J. Dent. Res., 58B:* 782-789, 1979.

Slavkin, H.C., Bessem, C., Bringas, P.Jr, Zeichner-David, M., Nanci, A., Snead, M.L. — Sequential expression and differential function of multiple enamel proteins during fetal, neonatal, and early postnatal stages of mouse molar organogenesis. *Differentiation, 37:* 26-39, 1988.

Strawich, E., Glimcher, M.J. — Synthesis and degradation in vivo of a phosphoprotein from rat dental enamel. *Biochem. J., 230:* 423-433, 1985.

Termine, J.D., Belcourt, A.B., Christner, P.J., Conn, K.M., Nylen, M.U. — Properties of dissociatively extracted fetal tooth matrix proteins. I. Principal molecular species in developing bovine enamel. *J. Biol. Chem., 255:* 9760-9768, 1980.

Termine, J.D., Miyamoto, M.S., Kuettner, K.E. — Lysozyme, protease, and protease inhibitor proteins in fetal bovine enamel matrix extracts. *J. Dent. Res., 59:* 1523-1524, 1980.

Towbin, H., Staehlin, T., Gordon, J. — Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some application. *Proc. Natl. Acad. Sci. USA, 76:* 4350-4354, 1979.

Zeichner-David, M., Slavkin, H.C., Lyaruu, D.M., Termine, J.D. — Biosynthesis and secretion of enamel proteins during hamster tooth development. *Calcif. Tissue Int., 35:* 366-371, 1983.

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