Published in final edited form as:

J Dent Res. 2008 December ; 87(12): 1133–1137.

Enamel Proteases Reduce Amelogenin-Apatite Binding

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Abstract

Organic matrix degradation and crystal maturation are extracellular events that occur simultaneously during enamel biomineralization. We hypothesized that enamel proteases control amelogenin-mineral interaction, which, in turn can affect crystal nucleation, organization, and growth. We used a recombinant amelogenin (rP172), a homolog of its major cleavage product (rP148), and a native amelogenin lacking both N- and C-termini (13k). We compared apatite binding affinity between amelogenins and their digest products during proteolysis. We further compared binding affinity among the 3 amelogenins using a Langmuir model for protein adsorption. Amelogenin-apatite binding affinity was progressively reduced with the proteolysis at the C- and N- termini by recombinant pig MMP-20 (rpMMP20) and recombinant human kallikrein-4 (rhKLK4), respectively. The binding affinity of amelogenin to apatite was found to be in the descending order of rP172, rP148, and 13k. Analysis of our data suggests that, before its complete degradation during enamel maturation, stepwise processing of amelogenin by MMP-20 and then KLK4 reduces amelogenin-apatite interaction.

Keywords

amelogenin; MMP20; KLK4; enamel; apatite

INTRODUCTION

Enamel formation is a dynamic process involving crystal maturation, which is concomitant with matrix proteolysis (Smith *et al.*, 1989). To date, two types of enamel proteases have been identified: the metalloproteinase enamelysin (MMP20) (Bartlett *et al.*, 1996) and the serine proteinase kallikrein 4 (KLK4) (Simmer *et al.*, 1998). Both MMP20 (Kim *et al.*, 2005) and KLK4 (Hart *et al.*, 2004) gene mutations cause autosomal hypomaturation amelogenesis imperfecta (AI) in humans. Previous studies have identified the cleavage sites within the full-length amelogenin for both enzymes (Ryu *et al.*, 1999, 2002); however, the functional significance of such proteolytic activities and the detailed molecular mechanisms of interactions of proteolytic products with the mineral during enamel biomineralization are yet to be elucidated.

The interaction of amelogenin with enamel apatite is a key issue in enamel biomineralization. Many *in vitro* studies have been conducted to investigate the molecular mechanisms of amelogenin function, including the adsorption and binding properties of amelogenin onto apatite crystals, and its effect on crystal morphology, growth, and organization (Aoba *et al.*, 1987; Wen *et al.*, 1999a; Wallwork *et al.*, 2001; Iijima and Moradian-Oldak, 2004; Beniash

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et al., 2005). It is now well-accepted that amelogenin plays critical roles in controlling the organization and morphology of enamel apatite crystals.

The aim of the present study was further clarification of the mechanisms of proteolytic activities of enamel proteases on amelogenin-mineral interaction, and their function in amelogenesis. We hypothesized that amelogenin-mineral interaction is controlled by processing of amelogenins, with consequent reduction in the binding affinity to apatite. To model the stepwise and dynamic proteolytic events during enamel biomineralization, we used 3 different isoforms of amelogenins to analyze their proteolytic processing systematically, with and without synthetic hydroxyapatite (HAP) crystals. We used recombinant pig MMP-20 (rpMMP20) and recombinant human kallikrein-4 (KLK4) as sources of enzymes.

MATERIALS & METHODS

Protein Expression and Purification

The full-length recombinant pig amelogenin (rP172), an engineered mutant lacking the hydrophilic C-terminal 24 amino acids (rP148), and the recombinant pig MMP20 (rpMMP20) were prepared as previously described (Simmer *et al.*, 1994; Ryu *et al.*, 1999; Sun *et al.*, 2006). Recombinant rP172 has amino acids 2-173 of the native porcine amelogenin P173, while rP148 has amino acids 2-149. In both, the phosphate group on serine 16 is lacking. Recombinant human KLK4 (rhKLK4), expressed in a murine myeloma cell line (NSO), was purchased (R&D Systems Inc., Minneapolis, MN, USA). Human KLK4 shares 80% homology with pig KLK4, and they function similarly (Yousef and Diamandis, 2001; Ryu *et al.*, 2002, unpublished observations).

The 13k amelogenin was extracted by a method described previously (Yamakoshi *et al.*, 2004). Examination of the isolated protein by gel electrophoresis showed a major peak of the 13k amelogenin, with a minor peak of an 11-kDa protein (data not shown). Edman degradation confirmed the N-terminal sequence of “13k” (LHHQIIPVV) and “11k” (LQPHHH).

Proteolysis of Amelogenins in the Presence of HAP

Amelogenins (rP172 or rP148) were dissolved in the working buffers (rP172 in Tris-HCl, pH 8. and rP148 in Bis-Tris, pH 6.8) at concentrations of 0.5-0.8 mg/mL, kept at 4°C overnight, then equilibrated at room temperature for 5 hrs, and centrifuged at 14k rpm. The rP172/rpMMP20 ratio was 800 (w/w), and the rP148 / rhKLK4 ratio was 400. For each experiment, 3 reactions were carried out simultaneously at room temperature, in 3 Eppendorf tubes, for a total of 8 hours' incubation: In tube A (“before”), HAP (National Institute of Standards and Technology, Gaithersburg, MD, USA) crystals and enzyme were added at times 0 hr and 4 hrs, respectively; in tube B (“during”), the additions were both at 0 hrs; however, in tube C (“after”), additions of HAP crystals and enzyme were at 4 hrs and 0 hrs, respectively. Pellets were washed by corresponding buffers 3 times, for 5 min each, and dissolved in acid for analysis. The control proteolysis without HAP was carried out under the same conditions as the corresponding experiments. Proteolysis products were analyzed by SDS-PAGE, HPLC, and Liquid Chromatography Tandem Mass Spectrometry (LCMS/MS).

Reverse-phase High-performance Liquid Chromatography (RP-HPLC)

Proteins and peptides were eluted with buffer A (0.1% TFA) and buffer B (buffer A + 60% acetonitrile) with a gradient of B, for 75 min or longer, in a C4 analytical column (Vydac, Hesperia, CA, USA). The liquid chromatograph workstation (ProStar/ Dynamics 6, version 6.41, Varian, Palo Alto, CA, USA) was used for quantitative analysis of the peak areas.

HAP-binding Experiment

Working buffers for amelogenin proteins were: Tris-HCl, pH 8, for rP172; Bis-Tris, pH 6.8, for rP148; and Tris-HCl, pH 7.2, for “13k”. The apatite-binding experiments ($N = 3$) were carried out with HAP crystals with a specific surface area of $18.3 \pm 0.3 \text{ m}^2/\text{g}$, according to a previously described protocol (Bouropoulos and Moradian-Oldak, 2003). Aliquots of supernatant were analyzed by means of the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA) on a nanodrop machine (NanoDrop Technologies, Wilmington, DE, USA). Calibration curves were constructed with standard solutions of the target protein. The amounts of protein (μmol) adsorbed *per* m^2 of HAP crystals were calculated by the difference between the initial (C_I) and the equilibrium (C_{eq}) protein concentrations ($\mu\text{mol}/\text{mL}$), by the following equation: $Q = [(C_I - C_{eq}) \times V]/(W \times S)$, where V is the volume of the solution (1 mL), W is the mass of the adsorbent (HAP), and S is the specific surface area of the adsorbent.

RESULTS

Proteolysis of Amelogenins in the Presence of HAP

We conducted these experiments to analyze amelogenin-HAP interactions during proteolysis, which presumably models the dynamic proteolytic events in the enamel extracellular matrix. For clarity, we term these experiments as “before” (Figs. 1A, 2A), “during” (Figs. 1B, 2B), and “after” (Figs. 1C, 2C).

rP172 Proteolysis by rpMMP-20—The major proteolytic products of rP172 cleaved by rpMMP20 in the absence of HAP have been previously reported as 2-148 (Ryu *et al.*, 1999; Moradian-Oldak *et al.*, 2001). This major product, with a mass of 16835 Da, could also be detected in the control experiment (no HAP, elution marked as “N” in Fig. 1), in the supernatant (elution marked as “S”), and on the HAP surface (elution marked as “P”) in all “before”, “during”, and “after” experiments. However, the ratios between peak areas corresponding to rP172 and 2-148 appeared to be different in each case. Without HAP, the ratio of peak areas between rP172 and 2-148 was 0.86, indicating that the quantity of rP172 was 86% of 2-148 (Fig. 1D). When HAP was added before rpMMP20 (the “before” experiment), the ratio was detected as 0.80 in supernatant and 1.26 on HAP surface, respectively (Figs. 1A, 1D). Compared with the ratio of 0.86 in control, the higher ratio of 1.26 on HAP surface suggested that more intact rP172 bound to HAP than its degradation product 2-148, indicating that rP172 has higher HAP-binding affinity. Similarly, the lower ratio of 0.80 meant that less rP172 remained in the supernatant compared with its major product, 2-148. The ratio of rP172 and 2-148 was higher than 0.86 on the HAP surface and lower than 0.86 in supernatant when HAP was added during or after the proteolysis (“during” and “after” experiments, Figs. 1B-1D). Thus, rpMMP20 cleavage of amelogenin resulted in the reduction of its binding to HAP crystals, regardless of the sequence of HAP addition to the proteolysis solution.

Following analysis by mass spectroscopy, 5 fragments were detected on the HAP surface (Table). Detection of large quantities of 2 C-terminal fragments indicated their high binding affinity to HAP, which further confirms the reduction of amelogenin-apatite by C-terminal cleavage of rpMMP20.

rP148 Proteolysis by rhKLK-4—We first analyzed the proteolytic products of rP148 cleaved by rhKLK4 in the absence of HAP. At least 24 fragments were detected by mass spectrometry (unpublished observation). HPLC elution (Figs. 2A-2C, elution marked as “N”) showed one major product, and amino acid sequencing analysis of that product resulted in the N-terminal sequence of HPXTSXG. The mass spectrometric analysis detected a molecular mass of 13290 Da, corresponding to the amelogenin segment 32-149. The elution peak of this major product could also be seen in supernatant (elution marked as “S”) and the HAP surface

(elution marked as “P”) in all “before”, “during”, and “after” experiments, but with relatively different peak area counts (Fig. 2). Quantitative analysis of HPLC peaks revealed that the rP148 substrate bound HAP crystals with higher affinity than its degradation product (32-149), and less substrate was sustained in the supernatant (Fig. 2D). Mass spectrometric analysis of the polypeptide segments bound to apatite revealed 4 amelogenin fragments. Three of them were from the N-terminus and 1 from the C-terminus of rP148 (Table). The relatively small quantity showed the low binding affinity of these fragments to HAP.

Adsorption Isotherms for rP172, rP148, and 13k

The shape of the isotherm of rP172 (Fig. 3A) showed a flattening at low equilibrium concentrations (C_{eq}) and, subsequently, exhibited a transition to a plateau. The correlation coefficient (r^2) of this exponential increase was > 0.99 . We therefore described the isotherm of rP172 using the Langmuir model by application of the equation (Kresak *et al.*, 1977):

$$C_{eq}/Q = 1/NK + C_{eq}/N,$$

where N is the maximum number of adsorption sites *per* unit of surface area (mol/m^2) of adsorbent, and K is the affinity of the adsorbent molecules (L/mol). The correlation coefficient ($r^2 > 0.99$) of the linear form of the isotherm, which is a plot of C_{eq}/Q vs. C_{eq} , was a good fit (Fig. 3B). The affinity and adsorption sites were $K = 6.38 \times 10^5 \text{ L}/\text{mol}$ and $N = 8.24 \times 10^{-7} \text{ mol}/\text{m}^2$ respectively.

In contrast, neither the isotherm of rP148 nor “13k” can be described by the Langmuir model ($r^2 = 0.82$ and 0.85 , respectively, for the linear form) (Figs. 3C, 3D). The shape of the isotherm for rP148 was a reverse “S” shape, with an ascending direction without any transition or plateau (Fig. 3A). The isotherm of 13k was irregular (Fig. 3A), and “13k” had much lower affinity than rP172 and rP148 (note the difference in scale among B, C, and D). At a constant C_{eq} , in a comparison of the Q values of these 3 isotherms, it is apparent that $Q_{rP172} > Q_{rP148} > Q_{13k}$ (Fig. 3A).

DISCUSSION

It has been well-documented that effective proteolysis of the enamel extracellular matrix by MMP20 and KLK4 is critical for normal enamel biomineralization (Caterina *et al.*, 2002; Hart *et al.*, 2004; Kim *et al.*, 2005). Among other processes, a disturbance in proteolytic activity during enamel development may affect amelogenin-mineral interactions, leading to defective mineral formation.

At the early stage of enamel formation, the full-length amelogenin is processed immediately at the C-terminus by MMP-20 following secretion (Smith *et al.*, 1989; Moradian-Oldak *et al.*, 1994). Expression of KLK4 is apparent at the post-secretory and transition stages, where the “20k” fragment is more abundant (Yamakoshi *et al.*, 1994; Wen *et al.*, 1999b; Hu *et al.*, 2000, 2002). Therefore, for the proteolytic experiments, we used the full-length rP172 as a substrate for MMP-20 and the truncated rP148 as a substrate for KLK4. It is noteworthy that KLK4 had a much higher efficiency than MMP-20 in cleaving rP148, resulting in faster proteolysis of the recombinant substrate (unpublished observation).

The proteolytic products of rP172 and rP148 by the 2 enzymes corroborate previous results *in vitro* and *in vivo* (Moradian-Oldak *et al.*, 2001; unpublished observation). These include fragments 46-149 and 48-149, which are similar to native “13k” (46-148), and fragments 63-149 and 64-149, which are similar to the native “11k” (64-148). As the major component of the outer enamel layer, “13k” could be the cleavage product by both MMP20 and KLK4 (Moradian-Oldak *et al.*, 2001; Fukae *et al.*, 2007).

Our systematic proteolytic experiments allowed for a comparative binding analysis of amelogenin and its proteolytic products during proteolysis. We observed larger quantities of substrates bound to HAP in both proteolysis cases of rP172 by rpMMP20 and rP148 by rhKLK4. We detected both C- and N-termini of proteolytic products to be bound to HAP. The above findings confirmed that the amelogenin-binding affinity to mineral was reduced following its cleavage by enamel proteases at both C- and N- termini, regardless of the sequence of addition of HAP to the digest mixture.

We obtained further direct evidence by performing the systematic binding experiments of the 3 amelogenins using the Langmuir model for protein adsorption. The adsorption isotherms described the relationship between the concentrations of the protein at the HAP surface as a function of its solution concentrations. By comparing the Q values among the 3 amelogenins ($Q_{\text{rP172}} > Q_{\text{rP148}} > Q_{\text{“13k”}}$), we demonstrated that the “13k” had a significantly lower apatite-binding affinity than did rP148. The adsorption isotherm of rP172 on synthetic hydroxyapatite crystals fitted the Langmuir model. These results were consistent with those of a previous study on rM179, indicating that the full-length amelogenin adsorbed onto the surfaces of apatite crystals as binding units with defined adsorption sites (Bouropoulos and Moradian-Oldak, 2003). However, the adsorption isotherms of rP148 and “13k” did not follow such a model. Analysis of these collective data suggests that interactions between the full-length amelogenin and apatite crystals occur with relatively high affinity and are highly selective (Bouropoulos and Moradian-Oldak, 2003). Following the cleavage of the C- and N-termini, there is a progressive loss in affinity and selectivity in amelogenin-apatite interactions. The present *in vitro* experimental observations support our hypothesis that stepwise processing of amelogenin during enamel formation controls amelogenin-apatite interactions.

Although the present conditions for proteolysis are not exactly the same as the physiological conditions (*i.e.*, amelogenin concentration, enzyme/substrate ratio, the presence of nonamelogenins), analysis of our *in vitro* data provides relevant information on amelogenin-protease-HAP interactions during the dynamic process of enamel formation and maturation. We propose that such interactions control the morphology of enamel crystals following the initiation of organized nucleation. It has been demonstrated that the affinity of amelogenin is still higher to the (010) side faces of crystals, even after cleavage of the C-terminus. Such selective binding will promote the crystals to grow in length (along the *c* axis) and thickness (along the *a* axis) faster than in width. Finally, we propose that cleavage of the N-terminus, by either MMP20 or KLK4, leads to disassembly of the nanospheres, increasing amelogenin solubility and accessibility for further degradation, a critical step in amelogenesis required for the completion of maturation (Ryu *et al.*, 1999; Sun *et al.*, 2006). We envisage that the knowledge gained from our *in vitro* experiments will provide a solid base for interpretation of the *in vivo* animal model studies where MMP-20, amelogenin, and KLK-4- null and transgenic mouse strategies are applied, and will contribute to the basic knowledge required for the design and development of enamel-like material.

ACKNOWLEDGMENTS

The study was funded by NIH-NIDCR grants DE-015644 and DE-013414. We thank Mr. David Maltby of the Mass Spectrometry Laboratory of the School of Pharmacy at the University of California, San Francisco, for the mass spectrometry analysis (Project # 403). We thank Lakshminarayanan Rajamani for his valuable discussion and Weston Carpiaux for proofreading the manuscript.

REFERENCES

- Aoba T, Tanabe T, Moreno EC. Function of amelogenins in porcine enamel mineralization during the secretory stage of amelogenesis. *Adv Dent Res* 1987;1:252–260. [PubMed: 3504174]

- Bartlett JD, Simmer JP, Xue J, Margolis HC, Moreno EC. Molecular cloning and mRNA tissue distribution of a novel matrix metalloproteinase isolated from porcine enamel organ. *Gene* 1996;183:123–128. [PubMed: 8996096]
- Beniash E, Simmer JP, Margolis HC. The effect of recombinant mouse amelogenins on the formation and organization of hydroxyapatite crystals in vitro. *J Struct Biol* 2005;149:182–190. [PubMed: 15681234]
- Bouropoulos N, Moradian-Oldak J. Analysis of hydroxyapatite surface coverage by amelogenin nanospheres following the Langmuir model for protein adsorption. *Calcif Tissue Int* 2003;72:599–603. [PubMed: 12704567]
- Caterina JJ, Skobe Z, Shi J, Ding Y, Simmer JP, Birkedal-Hansen H, et al. Enamelysin (matrix metalloproteinase 20)-deficient mice display an amelogenesis imperfecta phenotype. *J Biol Chem* 2002;277:49598–49604. [PubMed: 12393861]
- Fukae M, Yamamoto R, Karakida T, Shimoda S, Tanabe T. Micelle structure of amelogenin in porcine secretory enamel. *J Dent Res* 2007;86:758–763. [PubMed: 17652206]
- Hart PS, Hart TC, Michalec MD, Ryu OH, Simmons D, Hong S, et al. Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. *J Med Genet* 2004;41:545–549. [PubMed: 15235027]
- Hu JC, Zhang C, Sun X, Yang Y, Cao X, Ryu O, et al. Characterization of the mouse and human PRSS17 genes, their relationship to other serine proteases, and the expression of PRSS17 in developing mouse incisors. *Gene* 2000;251:1–8. [PubMed: 10863090]
- Hu JC, Sun X, Zhang C, Liu S, Bartlett JD, Simmer JP. Enamelysin and kallikrein-4 mRNA expression in developing mouse molars. *Eur J Oral Sci* 2002;110:307–315. [PubMed: 12206593]
- Iijima M, Moradian-Oldak J. Control of octacalcium phosphate and apatite crystal growth by amelogenin matrices. *J Mat Chem* 2004;14:2189–2199.
- Kim JW, Simmer JP, Hart TC, Hart PS, Ramaswami MD, Bartlett JD, et al. MMP-20 mutation in autosomal recessive pigmented hypomaturation amelogenesis imperfecta. *J Med Genet* 2005;42:271–275. [PubMed: 15744043]
- Kresak M, Moreno E, Zahradnik R, Hay D. Adsorption of aminoacids onto hydroxyapatite. *J Colloid Interface Sci* 1977;59:283–292.
- Moradian-Oldak J, Simmer PJ, Sarte PE, Zeichner-David M, Fincham AG. Specific cleavage of a recombinant murine amelogenin at the carboxy-terminal region by a proteinase fraction isolated from developing bovine tooth enamel. *Arch Oral Biol* 1994;39:647–656. [PubMed: 7980113]
- Moradian-Oldak J, Jimenez I, Maltby D, Fincham AG. Controlled proteolysis of amelogenins reveals exposure of both carboxy- and amino-terminal regions. *Biopolymers* 2001;58:606–616. [PubMed: 11285557]
- Ryu OH, Fincham AG, Hu CC, Zhang C, Qian Q, Bartlett JD, et al. Characterization of recombinant pig enamelysin activity and cleavage of recombinant pig and mouse amelogenins. *J Dent Res* 1999;78:743–750. [PubMed: 10096449]
- Ryu OH, Hu JC, Yamakoshi Y, Villemain JL, Cao X, Zhang C, et al. Porcine kallikrein-4 activation, glycosylation, activity, and expression in prokaryotic and eukaryotic hosts. *Eur J Oral Sci* 2002;110:358–365. [PubMed: 12664466]
- Simmer JP, Lau EC, Hu CC, Aoba T, Lacey M, Nelson D, et al. Isolation and characterization of a mouse amelogenin expressed in *Escherichia coli*. *Calcif Tissue Int* 1994;54:312–319. [PubMed: 8062146]
- Simmer JP, Fukae M, Tanabe T, Yamakoshi Y, Uchida T, Xue J, et al. Purification, characterization, and cloning of enamel matrix serine proteinase 1. *J Dent Res* 1998;77:377–386. [PubMed: 9465170]
- Smith CE, Pompura JR, Borenstein S, Fazel A, Nanci A. Degradation and loss of matrix proteins from developing enamel. *Anat Rec* 1989;224:292–316. [PubMed: 2774208]
- Sun Z, Ahsan MM, Wang H, Du C, Abbott C, Moradian-Oldak J. Assembly and processing of an engineered amelogenin proteolytic product (rP148). *Eur J Oral Sci* 2006;114(Suppl 1):59–63. [PubMed: 16674664]
- Wallwork M, Kirkham J, Zhang J, Brookes S, Shore R, Wood S, et al. Binding of matrix proteins to developing enamel crystals: an atomic force microscopy study. *Langmuir* 2001;17:2508–2513.
- Wen HB, Moradian-Oldak J, Fincham AG. Modulation of apatite crystal growth on bioglass by recombinant amelogenin. *Biomaterials* 1999a;20:1717–1725. [PubMed: 10503973]

- Wen HB, Moradian-Oldak J, Leung W, Bringas P Jr, Fincham AG. Microstructures of an amelogenin gel matrix. *J Struct Biol* 1999b;126:42–51. [PubMed: 10329487]
- Yamakoshi Y, Tanabe T, Fukae M, Shimizu M. Porcine amelogenins. *Calcif Tissue Int* 1994;54:69–75. [PubMed: 8118757]
- Yamakoshi, Y.; Hu, CC.; Fukae, M.; Tanabe, T.; Oida, S. A comprehensive strategy for purifying pig enamel proteins. In: Kobayashi, I.; Ozawa, H., editors. The 8th international symposium on biomineralization (BIOM2001). Tokai University Press; Niigata, Japan: 2004.
- Yousef GM, Diamandis EP. The new human tissue kallikrein gene family: structure, function, and association to disease. *Endocr Rev* 2001;22:184–204. [PubMed: 11294823]

Figure 1. rpMMP20 actions on rP172 in the presence of HAP. HAP crystals were added before (A), during (B), and after (C) proteolysis of rP172 and rpMMP20. The peak marked with an arrowhead shows the elution of fragment 2-148; the peak marked with an arrow shows the elution of rP172. (A) “Before” experiment; HAP addition before 4 hrs of proteolysis. (B) “During” experiment; HAP addition at the beginning of proteolysis. (C) “After” experiment; HAP addition 4 hrs after proteolysis. (D) Summary of the ratios between substrates and products of A, B, and C. Values were obtained from the counts of HPLC elution peak areas calculated by affiliated software. P, S, and N represent pellet, supernatant, and no HAP, respectively.

Figure 2. rhKLK4 actions on rP148 in the presence of HAP. HAP crystals were added before (A), during (B), and after (C) proteolysis of rP148 and rhKLK4. The peak marked with an arrowhead shows the elution of fragment 32-149; the peak marked with an arrow shows the elution of rP148. (A) “Before” experiment; HAP addition after 4 hrs of proteolysis. (B) “During” experiment; HAP addition at the beginning of proteolysis. (C) “After” experiment; HAP addition 4 hrs before digestion. (D) Summary of the ratios between substrates and products of A, B, and C. Values were obtained from the counts of HPLC elution peak areas calculated by affiliated software. P, S, and N represent pellet, supernatant, and no HAP, respectively.

Figure 3.

Adsorption properties of amelogenins on HAP (N = 3). (A) Adsorption isotherms of rP172, rP148, and “13k” on HAP. The plot shows surface adsorption as a function of the equilibrium concentration of the protein. (B–D) Linear adsorption isotherms for rP172 (B), rP148 (C), and “13k” (D) amelogenins on HAP.

Table

Amelogenin Fragments Detected on HAP Surface following Proteolysis (sequences based on mass spectrometric analysis)

	Range	Exp. mass (Da)	Cal. mass (Da)	Amino Acid Sequence
rP172 by rpMMP20	152-173	2535.2	2535.3	LLPDLPLEAWPATDGTKREEVD
	149-173	2881.4	2881.4	MQSLLPDLPLEAWPATDGTKREEVD
	2-63	7195	7195.1	PLPPHPGHPGYINFSYEVLTPWKYQNMIRHPYTSYGYEPMGGWLHHQIIPVVSQQTPQSHA
	2-148	16707	16707.2	-
	2-173	19572	19572.5	-
rP148 by rhCLK4	2-14	1395.7	1395.6	PLPPHPGHPGYIN
	15-24	1196.6	1196.4	FSYEVLTPK
	16-24	1049.5	1049.2	SYEVLTPK
	132-140	1042.5	1042.2	HPIQPLLPQ