The acidic proline-rich proteins (PRPs), encoded by the PRH1 and PRH2 loci on chromosome 12p13.2 (4), are major saliva proteins (15). As polymorphic and multifunctional proteins (4, 15, 20), they are potential determinants of host susceptibility to dental caries (23, 24).

Acidic PRPs adsorb to hydroxyapatite surfaces, regulate calcium phosphate and hydroxyapatite crystal equilibrium (15), attach commensal Actinomyces and Streptococcus species to teeth (13, 21), and inactivate ingested plant polyphenols (tannins) (5). While the proline-poor N-terminal 30-residue domain confers hydroxyapatite and calcium binding (15), the proline-rich middle/C-terminal domain binds tannins via proline-rich repeats (5) and bacteria via the ProGln terminus (13, 21).

Acidic PRPs consists of large allelic (e.g., PRP-1 and PIF-s) and small posttranslational (e.g., PRP-3 and PIF-f) variants (4). The small acidic PRPs resulting from proteolytic cleavage at Arg106-Gly107 display poor bacterial adhesion activities but (4). The small acidic PRPs resulting from proteolytic cleavage and small posttranslational (e.g., PRP-3 and PIF-f) variants proline-rich middle/C-terminal domain binds tannins via proline-rich repeats (5) and bacteria via the ProGln terminus (13, 21). Acidic PRPs were used in this study to suggest turnover of acidic PRPs into innate-immunity-like peptides by oral Streptococcus and Actinomyces species.
The resolved PRP-1 and PRP-3 fractions were dialyzed against Tris-HCl buffer and subjected to a Macroprep High Q column (15 by 1.6 cm; Bio-Rad, Hercules, Calif.) using a linear gradient of 25 to 1,000 mmol of NaCl/liter in 50 mmol of Tris-HCl/liter, pH 8.0. The purified proteins were dialyzed against water, lyophilized, and stored at 220°C.

Streptococcus and Actinomyces strains (14, 17) were grown at 37°C for 18 h in 5 ml of Trypticase soy bean glucose limiting broth (1.7% peptone, 0.3% soy peptone, 0.15% yeast extract, 12.5 mmol of glucose/liter, and 12.5 mmol of NH4HCO3/liter in NaH2PO4-K2HPO4 buffer [1 mol/liter], pH 7.3) in an atmosphere with 5% CO2. Pelleted (17,000 x g for 5 min) cells were washed twice in M-DIL buffer (0.2 mol of Tris-HCl/pH 7.5)/liter, followed by addition of 20 μl (5 mM in dimethyl sulfoxide) of each substrate: H-Arg-Pro-pNA, H-Lys(Abz)-Pro-Pro-pNA, H-Pro-Glu(pNA), and H-Val-Pro(pNA). Bacterial cells and cell-free supernatants (obtained after pelleting of bacteria) were kept on ice prior to degradation experiments.

PRP degradation was assayed by mixing equal volumes (300 μl) of protein (0.6 mg/ml) and bacteria (2 x 10^9 cells/ml), both dissolved in mM-DIL buffer, followed by incubation at 37°C for various times (15 min, 4 h, 20 h, and 1 week). After pelleting (17,000 x g for 10 min) of bacterial cells, the supernatants were aliquoted, lyophilized, and stored at −80°C prior to native polyacrylamide gel electrophoresis (PAGE) and densitometric analyses as previously described except for the use of Tris-glycine 7.5% Ready gels (Bio-Rad) (7).

The substrate specificity of PRP degradation was measured essentially as described elsewhere (11). Briefly, 75 μl of bacterial suspension (2 x 10^9 cells/ml in M-DIL buffer) was diluted with 75 μl of 0.2 mol of Tris-HCl (pH 7.5)/liter, followed by addition of 20 μl (5 mM in dimethyl sulfoxide) of each substrate: H-Arg-Pro-pNA, H-Lys(Abz)-Pro-Pro-pNA, H-Pro-
PRP-derived peptide structures were established using a hybrid quadrupole time-of-flight mass spectrometer (Micromass, Manchester, United Kingdom) with a Z-configured nanospray source and gold-coated spraying needles (Protan, Odense, Denmark). Detection was all times in the positive ion mode.

The adhesion-blocking activity of synthetic ArgGlyArgProGln (Biomolecular Resource Facility, University of Lund, Sweden) was measured by mixing equal volumes (15 μl) of suspensions of bacteria (10^6 cells/ml) and PRP-1-coated latex beads (16) on a glass plate for 2 min in the presence and absence of pentapeptide. Aggregation was scored visually, and in some experiments the pentapeptide was added to already established bacterium–PRP-1-latex bead aggregates.

The ability of the pentapeptide to counteract the sucrose-induced decrease of dental plaque pH was measured using dental plaque from one healthy donor who had refrained from eating and oral hygiene for 12 h. Sampled plaque was washed twice, suspended (16 mg/ml) in sterile distilled water, and added (90 μl) to microtiter wells, followed by addition of (i) 10 μl of distilled water (control); (ii) 5 μl of sucrose (7.0 mM) plus 5 μl of distilled water; (iii) 5 μl of sucrose (7.0 mM) plus 5 μl of ArgGlyArgProGln (100 mM); and (iv) 5 μl of ArgGlyArg-ProGln (100 mM) and 5 μl of distilled water. The microtiter wells were incubated at 37°C for 1 h and continuously monitored using a pH electrode.

PRP degradation by commensal Streptococcus and Actinomyces species. Native alkaline PAGE of acidic PRPs (PRP-1 and PRP-3) after incubation with washed bacterial cells revealed PRP degradation by certain species and in the following order (Table 1; Fig. 1a): S. gordonii, S. sanguis, and A. odontolyticus > S. anginosus > S. mitis, and S. oralis. In contrast, S. mutans, S. sobrinus, A. naeslundii genospecies 1 and 2, and A. viscosus lacked PRP degradation activity.

Native alkaline PAGE distinguished four qualitatively distinct degradation patterns (patterns I to IV) with N-terminal peptides migrating close to PRP-3 but otherwise deviating bonding patterns (Fig. 1a; Table 1). A strain representative of each of the four degradation patterns cleaved Pro-containing substrates (S. gordonii SK12, S. mitis SK304, S. anginosus SK215, and A. odontolyticus T-5-G, respectively) with similar, narrow (types I to III) or deviating, broad (type IV) substrate specificities the S. gordonii strain SK33 devoid of PRP degradation did not cleave Pro-containing substrates). The broad substrate specificity of A. odontolyticus (type IV) paralleled its high cleavage rate of both PRP-1 and PRP-3 compared to streptococci (types I to III) with a low PRP-3 cleavage rate.

PRP degradation by cell-free supernatants occurred also. Preparative gel electrophoresis of the cell-free supernatant of S. gordonii strain SK12 revealed PRP-degrading activity by at least two different protein fractions. There was no direct relationship between PRP degradation and adhesion to acidic PRPs or soluble immunoglobulin A1 protease activity.

Time dependency and structural features of PRP degradation. The pentapeptide obtained by degradation of PRP-1 by S. gordonii SK12 for various times were separated by gel filtration and analyzed by mass spectrometry (Fig. 1b to d and 2). The peptide peaks obtained after 15 min of incubation contained an N-terminal 105-residue peptide, Pyr1-Pro104Pro105 (peak 2), and a C-terminal 40-residue peptide, Gly111-Pro135Gln136, Gly111-Pro140Gln141, Gly111-Pro149Gln150, and Pro104-Pro129Gln150. The peptide peak appearing after 1 week of incubation (peak 5) contained oligopeptides and amino acids, as identified by peptide gel filtration.

Innate-immunity-like properties of synthetic Arg106Gly107 Pro135- Gly111-Pro135Gln136, Gly111-Pro140Gln141, Gly111-Pro149Gln150, and Pro104-Pro129Gln150. The peptide peak appearing after 1 week of incubation (peak 5) contained oligopeptides and amino acids, as identified by peptide gel filtration.

This study suggests turnover of acidic PRPs into innate-immunity-like peptides by proteolytic activity in commensal Streptococcus and Actinomyces species. Like degradation of casein by lactococci (19), degradation of acidic PRPs is time dependent and releases oligopeptides instantly and after prolonged degradation. The instant release of an Arg106Gly107 Pro135-Gly111-Pro135Gln136, Gly111-Pro140Gln141, Gly111-Pro149Gln150, and Pro104-Pro129Gln150 pentapeptide presumed to be instantly released by PRP degradation counteracted sucrose-induced decrease of dental plaque pH in vitro (Fig. 3a). The pentapeptide alone increased dental plaque pH. In addition, the pentapeptide desorbed bound cells and blocked adhesion of Actinomyces strain T14V, while strain LY7 with another PRP binding specificity was unaffected (Fig. 3b).

FIG. 2. Mass spectrum of a peptide mixture from degradation of PRP-1 for 20 h by S. gordonii SK12 (cf. peak 4 in Fig. 1b). All ions (m/z) are labeled with a letter indicating the identity of the peptide structure (Fig. 1c). Superscripts indicate the charge state of the peptide. The insert shows an expansion of the m/z scale for the quadruply charged ion 4F at m/z 984.50, corresponding to a mass of 3,930 Da (Fig. 1c).
peptide structures released by strain SK12 indicated cleavage at peptide bonds formed at Pro or Gln residues: \( \text{Pro}^{96}_{\text{Pro}^{99}} \text{Arg}^{103}_{\text{Pro}^{104}} \text{Pro}^{105}_{\text{Gln}^{110}_{\text{Gly}^{111}}} \text{Gln}^{131}_{\text{Gly}^{132}} \text{Pro}^{135}_{\text{Gln}^{136}_{\text{Gly}^{137}}} \text{Gln}^{141}_{\text{Gly}^{142}}. 

The synthetic Arg\(^{103}\)Gly\(^{106}\)Arg\(^{109}\)ProGln\(^{112}\) pentapeptide possessed innate-immunity-like properties. (i) It blocked the sucrose-induced decrease in dental plaque pH in vitro. Like sialin, an Arg-containing tetrapeptide in saliva (10), the pentapeptide containing two Arg residues increased the dental plaque pH in the absence of sucrose, suggesting acid neutralization by Arg catabolism to ammonia (arginine deiminase (ARG)) to dental plaque suspended in microtiter wells. (b) Reversal of aggregates induced by strain LY7 with another PRP binding specificity by 5 mM (3.0 mg/ml) of ArgGlyArgProGln (black bars). The formation of aggregates in the absence of pentapeptide is also shown (white bars).

![Image](https://viaasm.asm.org/Downloaded_from?via=1&issue=NOTES&vol=INFECT.IMMUN.5428)

**FIG. 3.** Ability of synthetic Arg\(^{103}\)Gly\(^{106}\)Arg\(^{109}\)ProGln\(^{112}\) to counteract the sucrose-induced decrease in dental plaque pH (a) and to desorb attached *Actinomyces* cells (b) in vitro. (a) Plaque pH following addition of distilled water (Plaque), 0.35 mM (0.12 mg/ml) sucrose (Plaque + sucrose), and 0.35 mM sucrose plus 5 mM (3.0 mg/ml) ArgGlyArgProGln (plaque + sucrose + ArgGlyArgProGln) to dental plaque suspended in microtiter wells. (b) Reversal of aggregates of *A. naeslundii* strain T14V and PRP-1-coated latex beads (but not of aggregates induced by strain L7 with another PRP binding specificity) by 5 mM (3.0 mg/ml) of ArgGlyArgProGln (black bars). The formation of aggregates in the absence of pentapeptide is also shown (white bars).

protective properties. Notably, Arg seems to affect the ecological relationship between *S. sanguis* and *S. mutans* (25), and carries-susceptible and carries-resistant subjects seem to differ in ability to increase the dental plaque pH after acidification (1)

Innate-immunity-like properties of acidic PRPs are in line with the association between allelic variation in acidic PRPs, saliva-mediated adhesion, and carries susceptibility; the Db variant, with a 21-amino-acid internal tandem repeat, is associated with altered saliva adhesion of bacteria and carries susceptibility (23, 24). Interestingly, the pentapeptide contains the ArgGlyArgProGln motif (AT hook present in transcriptional factors (3), and innate immunity peptides have been found to affect transcription (6). Both intracellular effects by salivary histatins (18) and bactericidal activity of a poly-t-proline II conformation of the proline-rich tandem repeat sequences of salivary MG2 (2) have been demonstrated. Nevertheless, further studies supporting the concept of PRP-derived innate immunity peptides should demonstrate their presence in oral biofilms, biological functions, and nature of underlying bacteria and endopeptidases.

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