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Characterization of the Binding Activities of Proteinase-Adhesin Complexes from *Porphyromonas gingivalis*

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Adhesins from oral bacteria perform an important function in colonizing target tissues within the dentogingival cavity. In *Porphyromonas gingivalis* certain of these adhesion proteins exist as a complex with either of two major proteinases referred to as gingipain R (arginine-specific gingipain) and gingipain K (lysine-specific gingipain) (R. N. Pike, W. T. McGraw, J. Potempa, and J. Travis, J. Biol. Chem. 269:406–411, 1994). With specific proteinase inhibitors, it was shown that hemagglutination by either proteinase-adhesin complex could occur independently of proteinase activity. Significantly, low concentrations of fibrinogen, fibronectin, and laminin inhibited hemagglutination, indicating that adherence to these proteins and not the hemagglutination activity was a primary property of the adhesin activity component of complexes. Binding studies with gingipain K and gingipain R suggest that interaction with fibrinogen is a major function of the adhesin domain, with dissociation constants for binding to fibrinogen being 4 and 8.5 nM, respectively. Specific association with fibronectin and laminin was also found. All bound proteins were degraded by the functional proteinase domain, with gingipain R being more active on laminin and fibronectin and gingipain K being more effective in the digestion of fibrinogen. Cumulatively, these data suggest that gingipain R and gingipain K, acting as proteinase-adhesin complexes, progressively attach to, degrade, and detach from target proteins. Since such complexes appear to be present on the surfaces of both vesicles and membranes of *P. gingivalis*, they may play an important role in the attachment of this bacterium to host cell surfaces.

Porphyromonas gingivalis has been implicated as one of the major organisms associated with destructive adult periodontal disease (35, 38). This bacterium, an asaccharolytic anaerobe requiring both amino acids and heme from its environment (23), has been shown to secrete numerous proteolytic activities, including both trypsin-like (11, 22, 36) and collagenolytic (2) cysteine proteinases, as well as those from other classes (12), all of which are used to obtain nutrients and/or to evade host defense mechanisms. We have recently isolated individual arginine- and lysine-specific proteinases from *P. gingivalis* (3, 29), which strongly suggests that its supposed trypsin-like activity is due to the synthesis of two independent proteinases. Both enzymes are apparently processed from high-molecular-weight precursors (27a, 28), and divergent proteolytic processing of their preproproteins was shown to comprise all the forms of trypsin-like enzymes found in *P. gingivalis* (30), thus greatly simplifying a very complicated picture (31). It has now been proposed by the International Union of Biochemistry that the naming of these enzymes be changed to account for their individual specificities. Thus, the arginine-specific cysteine proteinase from *P. gingivalis* will be described here as gingipain R and, similarly, the lysine-specific proteinase will be described as gingipain K.

The high-molecular-mass forms of gingipain R (94 kDa) and gingipain K (105 kDa) are thus complexes derived by proteolytic processing of polyprotein precursors (28), composed of a single protein with catalytic activity towards either arginine-X- or lysine-X-substrates joined noncovalently to a series of protein fragments which are responsible for adhesin and hemagglutinating activity (29). The hemagglutinin activity of *P. gingivalis* has been studied by various groups, and several proteins with this activity were isolated from both culture fluid (13, 17, 26) and membrane fractions (24, 25). Two of the purified hemagglutinins had strong amidolytic activity against benzoyl-L-arginine *p*-nitroanilide (BAPNA) (13, 24, 25), which has also been reported for crude fractions of *P. gingivalis* or whole cells (10, 14). Thus, there is significant precedence to support our initial observations relating both activities to a single protein complex. In the present study we have attempted to clarify the participation of the individual components of the complexes in hemagglutination (HA) and compared this with the characteristics of a crude vesicular fraction from *P. gingivalis*. In addition, we have investigated the binding and degradative properties of each purified enzyme.

MATERIALS AND METHODS

Materials. *N*α-*p*-Tosyl-L-lysine-chloromethyl ketone (TLCK), arginine-HCl, plasminogen-free fibrinogen (Fb), laminin, type I and III collagen, human immunoglobulin G (IgG), human IgA, and bovine serum albumin (BSA) were obtained from Sigma. Type IV collagen was prepared as described previously (9). Fibronectin (Fn) was purified from plasma with gelatin-Sepharose (40) and human serum albumin by cibacron blue-Sepharose affinity chromatography (37). Fragments D and E of Fb were generated by digestion with plasmin and purified as described elsewhere (6). 4-(2-Aminoethyl)-benzenesulfonyl fluoride (AEBSF) and leupeptin were acquired from Boehringer Mannheim. Human erythrocytes were obtained from human immunodeficiency virus- and hepatitis B-negative donor. *P. gingivalis* ATCC 53978 (W50) was purchased from the American Type Culture Collection, and the H66 strain was a gift from Roland Arnold, University of North Carolina. The inhibitor benzoyloxycarbonyl-Phe-Lys-CH_2OCO-(2,4,6-Me_3)phenyl · HCl (ZFKck) was a gift from A. Krantz, Syntex Corp., Palo Alto, Calif.

Growth of bacteria and preparation of vesicle fraction. Strains ATCC 53978

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TABLE 1. Effects of inhibitors and activators on HA by a vesicle fraction and purified proteinase-adhesin complexes from *P. gingivalis*

Activator/inhibitor (concn [mM])	Hemagglutinating titer (μg/ml)[a] of:		
	Gingipain K	Gingipain R	Vesicles
None	14.5	6	3
$CaCl_2$ (10)	14.5	6	3
Cysteine (1)	1.8	6	0.4
Cysteine (10)	3.6	24	3
Cysteine (1)/$CaCl_2$ (10)	1.8	6	0.4
Arginine (100)[b]	No HA[c]	No HA[c]	1.5
TLCK (1)[b]	7.2	6	250
Leupeptin (0.1)[b]	1.8	6	37.5
ZFKck (equimolar)[b]	7.2	6	37.5
AEBSF (1)[b]	1.8	6	0.4

[a] Titers from three experiments with negligible standard deviations.
[b] Inhibitory compounds tested in the presence of 10 mM $CaCl_2$ and 1 mM cysteine.
[c] No HA was found for concentrations of the purified proteinase-adhesin complexes of up to 500 μg/ml.

(W50) and H66 were grown in a total volume of 250 ml as described earlier (3) and then centrifuged (6,000 × *g*, 30 min, 4°C). The supernatant was clarified by ultracentrifugation (100,000 × *g*, 60 min, 4°C), and the precipitate, resuspended in 3 ml of buffer, was regarded as the vesicle fraction.

Purification of gingipain K and gingipain R. Low-molecular-mass gingipain R (50 kDa) was purified from strain H66 culture fluid as previously described (3), using a combination of gel filtration and ion-exchange chromatography, while gingipain K and gingipain R proteinase-adhesin complexes were isolated by the sequential use of gel filtration, arginine-Sepharose chromatography, and anion-exchange chromatography on Mono Q (29).

HA assays. HA measurements were carried out in Tris-buffered saline (8). Inhibitory compounds were tested in the presence of 10 mM $CaCl_2$ and 1 mM cysteine. The irreversible inhibitors TLCK, ZFKck, and AEBSF were incubated with hemagglutinins for 15 min at room temperature before being serially diluted in Tris-buffered saline. The reversible inhibitors arginine and leupeptin were serially diluted in Tris-buffered saline containing sufficient inhibitor to give the final concentrations shown in Table 1 and incubated for 15 min at room temperature before the addition of a 1% erythrocyte suspension.

Production of antibodies against proteinase-adhesin complexes. Antibodies against gingipain R and gingipain K were raised in chickens and rabbits by protocols described previously (5).

ELISA of proteinase-adhesin binding to matrix and plasma proteins. The enzyme-linked immunosorbent assay (ELISA) used was generally carried out as follows: proteins to be tested for binding to *P. gingivalis* proteinase-adhesin complexes were coated in phosphate-buffered saline (PBS) at 4°C for 16 h. Following this, wells were blocked with 0.5% (wt/vol) BSA in PBS (BSA-PBS) for 1 h at 37°C. Each incubation was followed by washing three times with 0.1% (vol/vol) Tween 20–PBS. After blocking, the wells were incubated with serial dilutions of either gingipain R or gingipain K, both previously inactivated by treatment with TLCK, in BSA-PBS at 37°C for 2 h; this was followed by addition of chicken anti-gingipain R or anti-gingipain K (1 μg/ml) in BSA-PBS for 1 h. Finally, the plates were incubated with anti-chicken IgY–horseradish peroxidase conjugate (1 μg/ml) in BSA-PBS for 1 h at 37°C, washed, and allowed to incubate with tetramethylbenzidine substrate solution. The reaction was terminated by the addition of 0.18% (vol/vol) H_2SO_4, and the product was read at 450 nm. The ELISA was modified to allow the calculation of K_d values as described by Friguet et al. (7), using biotinylated Fb to simplify the assay.

Analysis of the degradation of proteins by SDS-PAGE. Protein degradation was carried out in 0.2 M Tris-HCl–1 mM $CaCl_2$–10 mM cysteine, pH 8.0. After incubation at 37°C for a given time period, an aliquot was withdrawn and proteolytic activity was stopped by mixing with 1 mM TLCK. Proteins and degradation products were electrophoresed on 10% Tris-Tricine (34) and 7.5% Tris-glycine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels (19) and visualized by Coomassie blue R-250 staining.

RESULTS

Inhibition of HA. In an earlier study (29), a single-chain 50-kDa form of gingipain R, devoid of protein fragments believed to confer HA activity, was examined and found to have no such activity. This indicated that, at least for this form of the enzyme, the proteinase domain alone was not sufficient to carry out this function. In order to further test the role of the proteinase active site, specific activators and inhibitors of gingipain R (95 kDa) and gingipain K (105 kDa) were tested for their effect on HA. Unless otherwise mentioned, all studies of purified enzymes refer to these forms of each proteinase. These results were compared with those obtained with vesicles from strain ATCC 53978, in order to ascertain the role of proteinase-adhesin complexes in a crude bacterial fraction, as well as a possible relationship to overall function of the *P. gingivalis* membrane.

As shown in Table 1, cysteine at 1 mM was stimulatory for HA by vesicles and gingipain K but had no effect on gingipain R, while at 10 mM it was less effective in stimulating gingipain K and vesicles and was inhibitory for gingipain R. Calcium had no effect on HA by any of the samples tested but was previously shown to stabilize the low-molecular-mass form of gingipain R (3); therefore, in further inhibition studies the HA buffer always contained 10 mM $CaCl_2$ and 1 mM cysteine. Arginine (100 mM), which was previously found to be an inhibitor of the hemagglutinins from *P. gingivalis* (17, 26, 29), completely abolished HA by gingipain K and gingipain R. This compound was much less inhibitory of HA by vesicles, even at higher concentrations, in agreement with previous results which had shown that vesicles were less susceptible to inhibition by arginine than were purified hemagglutinins (18). Lysine was a much weaker inhibitor of HA (29), and other amino acids were not effective.

Leupeptin, which specifically inhibits gingipain R relative to gingipain K, did not inhibit HA by either proteinase-adhesin but was a very effective inhibitor for vesicles. ZFKck, which is specific for gingipain K when used at limiting concentrations for proteinase inhibition, had a weak effect on HA by gingipain K and none on that by gingipain R but was highly active on vesicles. The more nonspecific proteinase inhibitor TLCK, which inhibits both gingipain R and gingipain K, did not inhibit HA by gingipain R, had a small effect on gingipain K, and had a strong inhibitory effect on vesicle HA activity. Other proteinase inhibitors, such as the serine proteinase inhibitor AEBSF, were not effective in inhibiting HA by any fraction. Higher concentrations of any of the above inhibitors did not affect the results.

Inhibition of HA by proteins. Initially, investigations were made to determine whether HA by gingipain K and gingipain R could be inhibited by a range of proteins. The results (Table 2) showed that several were able to reduce HA by both proteinase-adhesin complexes, as well as by vesicles. Indeed, the only noninhibitory proteins found were IgG and IgA. Significantly, low concentrations of several proteins such as Fb, Fn, and laminin inhibited HA by the proteinase-adhesin com-

TABLE 2. Inhibition of HA by human plasma and tissue proteins

Plasma protein	HA-inhibitory concn [μg/ml (nM)][a] of:		
	Gingipain K	Gingipain R	Vesicles
Serum albumin	62.5 (920)	125 (1,840)	NI
IgA or IgG	NI	NI	NI
Collagen I or III	62.5 (219)	62.5 (219)	125 (438)
Collagen IV	62.5 (113)	62.5 (113)	125 (226)
Fn	16 (35)	16 (35)	32 (70)
Fb	8 (23)	32 (92)	16 (46)
Laminin	16 (18)	16 (18)	2 (2.4)

[a] Values represent the lowest concentration of test protein which was able to completely inhibit HA. NI, noninhibitory for HA, by which it is implied that HA occurred in the presence of the highest concentration (500 μg/ml) of protein used.

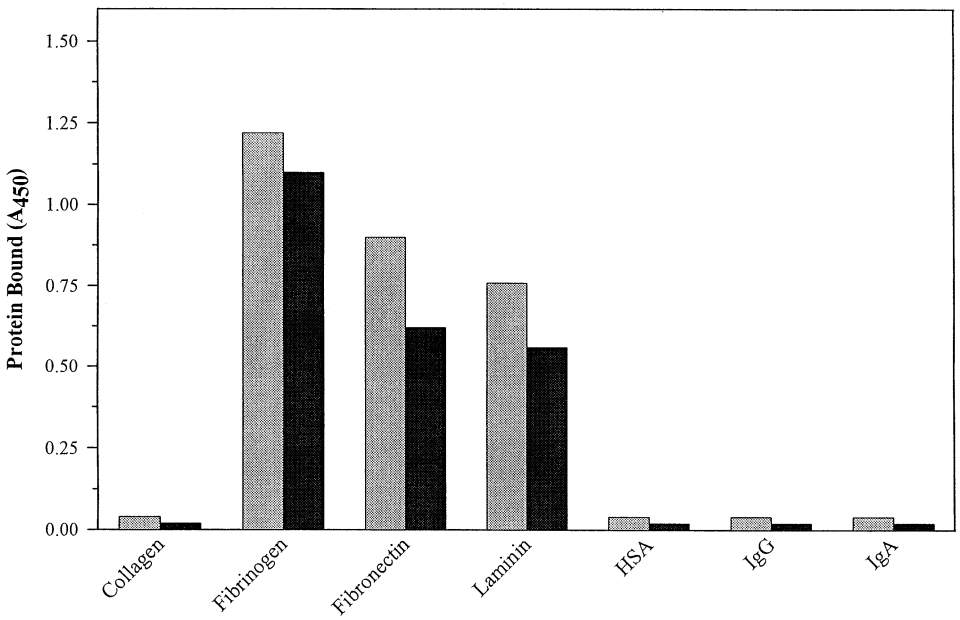

FIG. 1. Binding of gingipain R (light bars) and gingipain K (dark bars) to a range of proteins, as measured in an ELISA. Proteins were coated at 10 nM, and the ELISA was carried out as described in Materials and Methods. Values shown here are averages from three experiments, using a 4 nM concentration of each proteinase-adhesin. HSA, human serum albumin.

plexes, indicating that the complexes could not carry out this activity in infection sites, where higher concentrations of these proteins would be present. However, with the exception of Fb and laminin, higher protein concentrations were required to inhibit HA by vesicles. Indeed, both of these proteins were highly effective inhibitors, with the former being functional at concentrations far below physiologically relevant levels (<1/100 of physiological concentration) (1). Interestingly, laminin appeared to be a much stronger inhibitor of HA by vesicles relative to that of the gingipains, indicating once again that adhesins present on the surface of these particles functioned somewhat differently from the purified, soluble proteinase-adhesin complexes.

Adhesion of gingipain K and gingipain R to a range of proteins in an ELISA. The ELISA provided a much more sensitive assay than HA for measurement of the binding of the proteinase-adhesin complexes to the various proteins tested, allowing the elimination of the background found with higher enzyme levels. These results (Fig. 1) are therefore a more specific reflection of the interactions obtained at low concentrations of both enzyme and protein and thus of binding specificity. It may be seen that both proteinase-adhesin complexes adhere strongly to Fb and less substantially to Fn and laminin and displayed no adherence to other proteins tested. It should be emphasized that the results given in Fig. 1 were obtained with inactivated proteinases, supporting the concept that the active site appears to play no role in adhesion. This was further confirmed with either native or inactivated 50-kDa gingipain R, which in both cases was found to have no affinity for the proteins tested. These results, however, do not necessary mean that a specific protein recognition site is entirely absent on the catalytic domain, since it was recently determined that, in comparison to the catalytic domain of the proteinase-adhesin complex, this form of gingipain R is truncated on its C terminus (29a). Despite this uncertainty, it is clear that the interactions found were physiologically relevant, since these assays took place in the presence of BSA, which serves as a blocking agent for nonspecific protein-protein binding.

Characterization of the binding of gingipains to Fb. In ELISAs carried out to fully characterize the interaction of the gingipains with Fb, a competition format was used. This verified the finding that the gingipains preferentially bound to Fb relative to Fn and laminin, which were used as competitors in this system. It was found (data not shown) that while Fb could compete for binding of the gingipains at low concentrations in the presence of Fn and laminin, the other two proteins could not compete with Fb, even at high concentrations. Laminin and Fn appeared to bind the gingipains with similar affinities, however, since they competed equally in this assay.

Since it had been established that Fb predominated in the range of proteins tested here, further characterization of binding to this molecule was undertaken. In order to further test the effect of proteinase inhibition on adhesion, measurement of the interaction of the gingipains with Fb over time, while inhibited or uninhibited, was made. It was found (data not shown) that inhibited gingipains reached maximum binding to Fb at approximately 15 min and then remained constant. Active gingipains associated maximally within 15 min, but this was followed by a decrease over time. This most likely indicates degradation after adhesion, followed by detachment. Elevating the temperature to 37°C was found to accelerate binding and detachment by approximately 30% at each given time point. It was also noted (Fig. 2) that gingipain K had only a limited affinity for the plasmic fragment D of Fb and none for fragment E, while gingipain R had a relatively high affinity for plasmic fragment D but none for fragment E. This difference in affinity for the plasmic fragments may indicate that the two gingipains bind at different sites on Fb.

Several parameters were altered to assess the impact of solution conditions on the binding of the gingipains to Fb. The effect of pH in the range from 4 to 8.5 was found to be minimal, while ionic strength decreased binding by 20% at a concentration of either 4 M NaCl or 2 M $(NH_4)_2SO_4$ and by 90% at 4 M $(NH_4)_2SO_4$. Solvents such as ethylene glycol, which might be expected to decrease hydrophobic interactions, had little effect. In parallel with the HA results, binding by gingipain K was

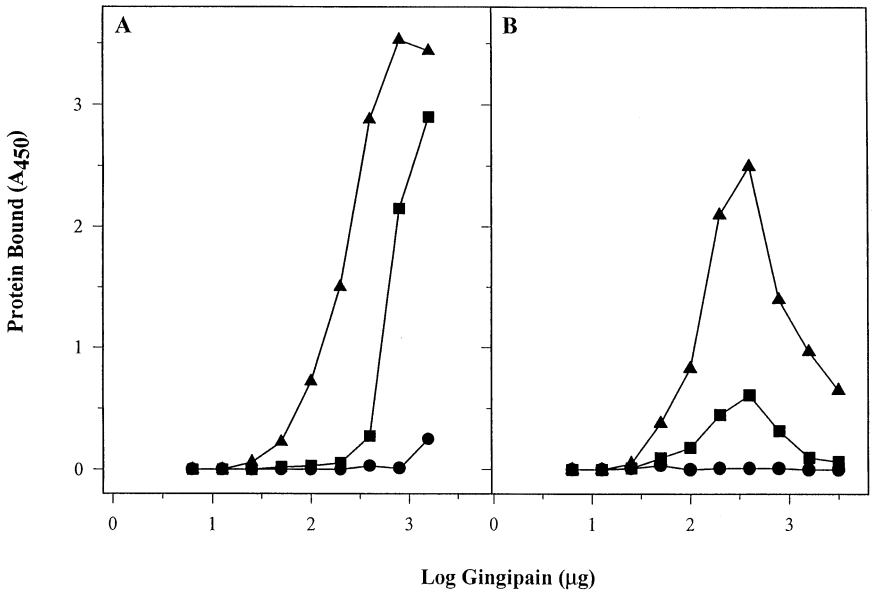

FIG. 2. Binding of gingipain R and gingipain K to plasmic fragments of Fb. Fb (▲) and its plasmic fragments D (■) and E (●) were coated at 5 μg/ml, and the ELISA was carried out using gingipain R (A) and gingipain K (B) at the concentrations shown, as described in Materials and Methods.

unaffected by up to 100 mM cysteine, but gingipain R adhesion was markedly inhibited by concentrations of cysteine above 1 mM. The binding of either proteinase to Fb was abolished by boiling or incubation in 8 M urea, suggesting that protein conformation was important, although this effect may be limited, since SDS at 1% caused an only 40% reduction in affinity. Two important results supported the interaction between gingipains and Fb as being due to protein-protein adherence: (i) polymyxin B at 1 to 100 μg/ml had no effect, indicating that lipopolysaccharide was not playing a role, and (ii) mild periodate treatment of each of the proteins was ineffective, ruling out any contribution by carbohydrate residues.

The final characterization of the association of the gingipains with Fb involved a determination of the K_d for this interaction. By a Scatchard plot analysis of competition ELISA data, as recommended by Friguet et al. (7), it was found (Fig. 3) that the K_d for binding by gingipain R was 8.5 nM and that for binding by gingipain K was 4 nM. This indicates a strong interaction for either enzyme with Fb, gingipain K being marginally better.

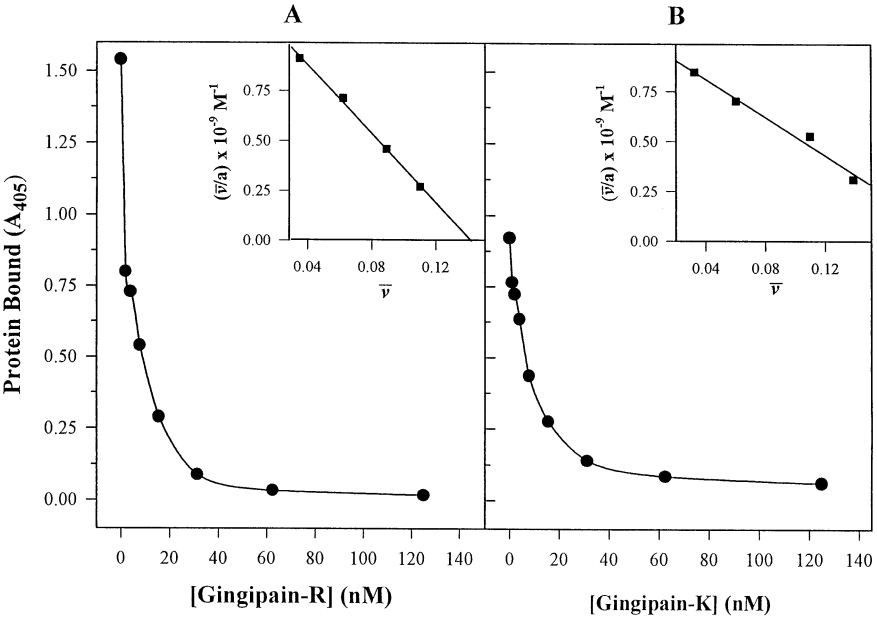

FIG. 3. ELISA to determine the K_d of binding of gingipains to Fb. Gingipain R (A) and gingipain K (B) were coated at 5 nM and then incubated with 2.5 nM biotinylated Fb, together with increasing amounts of competing gingipain R or K as shown in the main graphs. These data were used to generate the graphs shown in the insets, which in turn allowed the calculation of the K_d values as described previously (7).

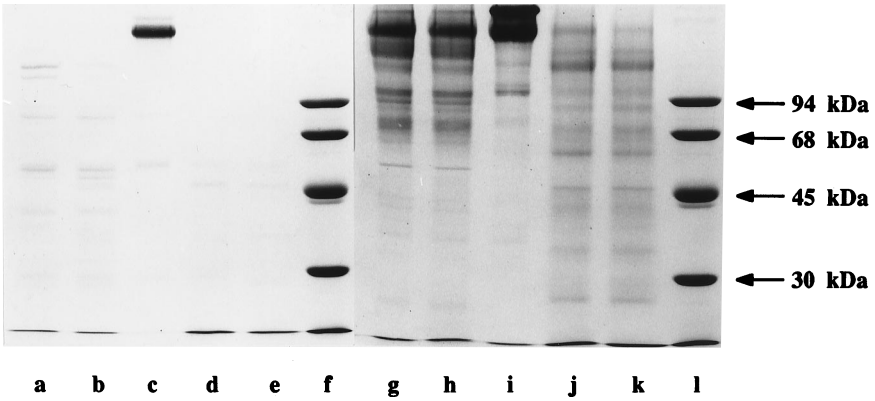

FIG. 4. Degradation of Fn and laminin by gingipain K and gingipain R. Fn (lanes a to e) and laminin (lanes g to k) at 1 mg/ml were incubated with 25 nM gingipain K for 1 h (lanes a and g) and 4 h (lanes b and h) or 25 nM gingipain R for 1 h (lanes d and j) and 4 h (lanes e and k). Enzyme/substrate ratios were 1:90 for Fn and 1:47 for laminin. Lanes c and i contain control proteins, while lanes f and l contain marker proteins with molecular masses shown. Ten micrograms of each protein sample was electrophoresed on Laemmli SDS–7.5% PAGE gels.

Degradation of target proteins by proteinase-adhesin complexes. The patterns of degradation of target proteins by the gingipains was determined in order to support our hypothesis that the apparent detachment of the active proteinase-adhesin complexes from their target proteins over time, versus stable binding by inhibited forms, was due to protein degradation. The results showed that Fb, Fn, and laminin were all susceptible to digestion by the gingipains. Gingipain R readily degraded Fn and laminin, although the former was significantly more sensitive (Fig. 4). At the enzyme/substrate ratios (molar concentrations) used (1:90 for Fn; 1:47 for laminin), digestion of these proteins by gingipain R was essentially complete after 1 h, with gingipain K being somewhat less effective. The degradation of the Fb Aα chain was very rapid with either enzyme at low enzyme/substrate ratios (1:1,760) (Fig. 5). Gingipain K was more effective in proteolysis of the Bβ chain than was gingipain R, however, and both enzymes appeared to slowly degrade the γ subunit.

DISCUSSION

The initial finding that hemagglutinins and proteinases from *P. gingivalis* were associated (29) has now been confirmed at the genetic level (31). The results presented here indicate that, in the purified proteinase-hemagglutinin complexes, the two domains are independent of each other, since inhibition of proteinase activity has little or no effect on HA. This is confirmed by the fact that a truncated form of gingipain R (50 kDa) (3) does not possess any hemagglutinating activity but has proteolytic activity identical to that of the 95-kDa form (29).

Significantly, high concentrations of gingipain R and gingipain K were found to be associated with outer membranes (30), and we assume that these molecules are primarily responsible for the hemagglutinating activity of vesicles. However, in terms of inhibition of this activity by proteinase inhibitors, arginine, and some human plasma or tissue proteins, there are considerable differences in the susceptibilities of bound versus free proteinases. This can readily be resolved through the realization that HA by soluble gingipains must involve two spatially independent binding sites to cross-link with erythrocytes. Obviously, affinities for these sites and their susceptibilities to various compounds can differ greatly, and blocking of one site will totally eliminate HA by such soluble proteins. In contrast, when many molecules of gingipain R and gingipain K are associated with vesicles, blocking of one erythrocyte binding site on a membrane-associated enzyme will have only a limited effect on hemagglutinating activity. This is clearly seen with arginine, where soluble gingipains are dramatically inhibited while little effect occurs with vesicles. The same reasoning can also be applied to explain the differences in susceptibility

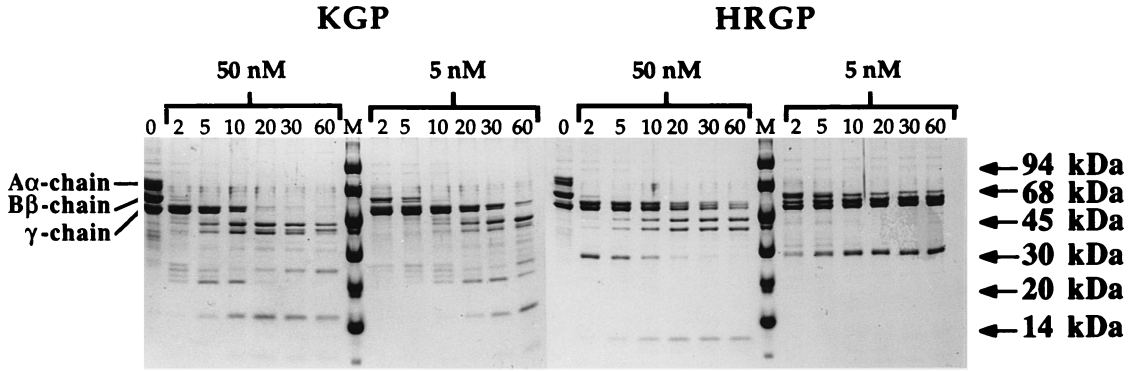

FIG. 5. Degradation of Fb (8.8 mM or 3 mg/ml) by gingipain K and gingipain R at various times (minutes) and enzyme concentrations. An enzyme concentration of 5 nM corresponds to an enzyme/substrate ratio of 1:1,760, and 50 nM corresponds to 1:176. The 0 min time point serves as a control, and M designates marker proteins with the molecular masses shown. Fifteen micrograms of Fb or Fb digest was loaded per lane and electrophoresed with 10% Tris-Tricine SDS-PAGE gels.

of HA activity of gingipains and vesicles by some plasma and tissue proteins. On the other hand, the discrepant effect of proteinase inhibitors on HA by gingipains and vesicles is most likely to be due to the fact that the proteinase active site in the membrane-bound gingipains is positioned in proximity to the HA domain. Therefore, inhibition of proteinase activity could affect erythrocyte binding because of steric hindrance.

The inhibition of HA by a variety of proteins indicated that many could readily interfere with this function. Significantly, the effect of such proteins was clearly obvious at concentrations far below those in body fluids, suggesting to us that HA was not a primary function of the noncatalytic domain of vesicle-bound gingipains. Rather, it seemed more plausible to assign an adhesion function, particularly since the most tightly bound proteins were Fb, Fn, and laminin, in that order.

Progulske-Fox et al. (32) have previously speculated that hemagglutinins from *P. gingivalis* are the same as or have high homology to Fb-binding proteins from this organism. This was prompted by findings that antibodies to an Fb-binding protein reacted with a cloned hemagglutinin and also, interestingly, inhibited a cysteine proteinase. The results obtained here strongly support these conclusions. Fb- and Fn-binding proteins have previously been identified in studies of whole *P. gingivalis* cells (20, 21) in which two proteins of 120 and 150 kDa which bound and degraded Fb and Fn were studied. Recently (4), an enzyme designated porphypain was isolated, also as 120- and 150-kDa forms, and found to be a fibrinogenase with Arg-Lys-splitting activity. Unfortunately, this enzyme preparation, which probably represents a mixture of gingipain K and gingipain R, was not examined for its binding characteristics. It is clearly related to the purified gingipains used here, but this study appears to be the first to use purified molecules to (i) study the binding and degradation of target proteins and (ii) experimentally link the binding activities of purified hemagglutinins to adherence of proteins.

Characterization of the interaction between the purified proteinase-adhesin complexes and Fb reveals that it is specific, of a high affinity, and exclusively mediated by protein-to-protein recognition. At this stage it is possible to definitively state only that such binding does not take place through the active site of the proteinase. In addition, it can be hypothesized that, for gingipain R, the proteinase domain does not seem to be involved in adhesion, making it more likely that the associated, nonenzymatic fragments of initial polyprotein are the adhesins. For gingipain K, the situation is less clearly resolved, since the proteinase domain is not available, alone, for binding studies, as is the case for gingipain R. The high homology between the 44-, 27-, 17-, and 15-kDa proteins of the two gingipains (27a, 28), however, makes these components of the complexes the most attractive candidates for the adhesin in both molecules. It does, however, appear likely that gingipain K and gingipain R bind to different parts of the Fb molecule, since gingipain R displays a much higher relative binding to plasmic fragment D than does gingipain K. Different amino acid residues in each enzyme also appear to play a role in the binding, since gingipain R is much more sensitive to reducing agents than is gingipain K in terms of this activity. It is interesting to note that arginine, alone, is capable of inhibiting HA by the purified gingipains but does not affect Fb binding. This may be due to a much larger array of amino acid residues and contact points being involved in the high-affinity binding of gingipains to Fb than in its presumably lower-affinity interaction with receptors on erythrocytes.

Evidence in this report and others (20, 21) indicates that the active proteinase-adhesin complexes most likely degrade bound proteins, causing detachment of such complexes. This suggests a mechanism whereby the proteinase-adhesin complexes cause progressive attachment to, degradation of, and dissociation from host receptor molecules. The purpose of this process by proteinase-adhesin complexes from *P. gingivalis* is open to speculation. Certainly, the characteristics of the proteinase-adhesin complexes described here places them in the family of microbial surface components recognizing adhesive matrix molecules (27). This family of adhesins is thought to play a major role in the colonization of host tissues by bacteria, and in the case of the gingipains, their binding to Fb may be important since this protein is known to coat many extracellular biosurfaces (27). Similarly, the ability to bind Fn and laminin, albeit at a lower affinity, may also allow interactions of *P. gingivalis* with the extracellular matrix as a prelude to the invasion of the host, a known characteristic of the organism (35).

The binding and degradation of Fb will obviously have an effect on clotting systems in the host. Periodontal patients often have a propensity to bleed on probing with mechanical instruments, compared with healthy subjects (39), and breakdown of Fb by the gingipains may contribute to this symptom. From the results presented here, it is apparent that gingipain K is the major enzyme involved in this degradation, and it has been reported to be one of the most potent fibrinogenases known (33). We have now found that the enzyme does indeed significantly interrupt clotting mechanisms (16), and this may therefore represent another mechanism whereby *P. gingivalis* could disrupt host functions, similar to that found in the activation of the kallikrein-kinin system by gingipain R, which causes edema in the host (15). Assuming that these are the primary roles of gingipains K and R, the next logical steps would involve the development of inhibitors against the proteinase and/or adhesin activities. Such studies are currently under way.

ACKNOWLEDGMENTS

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