INFECTION AND IMMUNITY, Sept. 1991, p. 3015–3020
0019-9567/91/093015-06$02.00/0

Vol. 59, No. 9

In Vitro Killing of *Actinobacillus actinomycetemcomitans* and *Capnocytophaga* spp. by Human Neutrophil Cathepsin G and Elastase

KENNETH T. MIYASAKI* AND AMY L. BODEAU

Section of Oral Biology and Dental Research Institute, UCLA School of Dentistry, Center for the Health Sciences, Los Angeles, California 90024

Received 21 March 1991/Accepted 19 June 1991

The purpose of this study was to compare the killing of *Actinobacillus actinomycetemcomitans* with that of *Capnocytophaga* spp. by purified cathepsin G and elastase in vitro. Both were sensitive to killing by purified cathepsin G, but only the *Capnocytophaga* spp. were killed by elastase. Killing by cathepsin G exhibited logarithmic kinetics, was enhanced slightly by alkaline pH, and was enhanced greatly under hypotonic conditions. Treatment of cathepsin G with diisopropyl fluorophosphate significantly reduced its bactericidal activity against *Capnocytophaga* spp. but not against *Escherichia coli* or *A. actinomycetemcomitans*. The bactericidal effects of cathepsin G against *Capnocytophaga sputigena* and *A. actinomycetemcomitans* were inhibited by α-1-antichymotrypsin, α-1-antitrypsin, and α-2-macroglobulin but not by bovine serum albumin. We conclude that (i) cathepsin G kills *Capnocytophaga* spp. and *A. actinomycetemcomitans*, (ii) elastase kills *Capnocytophaga* spp., (iii) the bactericidal activity of cathepsin G is enzyme dependent against *Capnocytophaga* spp. and enzyme independent against *A. actinomycetemcomitans*, and (iv) natural plasma antiproteases may control both enzyme-dependent and enzyme-independent bactericidal activities of cathepsin G.

Cathepsin G and leukocyte elastase are members of the neutrophil neutral serine protease family. Cathepsin G is a chymotrypsinlike cationic protein encoded on human chromosome 14 that exhibits at least three 24- to 29-kDa glycoprotein isoforms and accounts for 1 to 2% of the total protein of neutrophils (5, 8, 27). Cathepsin G kills certain bacteria and fungi independent of enzymatic activity (10, 15). It has recently been observed that two small oligopeptide fragments of cathepsin G kill *Staphylococcus aureus* and *Neisseria gonorrhoeae*, demonstrating both the presence of distinct microbicidal domains and a possible mechanism of enzyme-independent killing (2).

The biologic function of the enzymatic activity of cathepsin G is enigmatic. Some possible biologic functions of cathepsin G which may be dependent upon enzymatic activity include enhancement of phagocytosis, activation and degradation of complement, stimulation of granulocyte chemotactic activity, increases in vascular permeability, and aggregation of platelets (25). We have observed that the killing of *Capnocytophaga sputigena*, but not of *Escherichia coli*, requires enzymatically active cathepsin G; thus, cathepsin G kills certain bacteria by another enzyme-dependent pathway (11).

Leukocyte elastase consists of at least three elastolytic, cationic, 24- to 29-kDa glycoprotein isoforms encoded on chromosome 11 (22, 27). Leukocyte elastase kills the *Acinetobacter* spp., which are gram negative, and produces lysis of *S. aureus* (24). Elastase, but not cathepsin G, produces marked proteolysis of the *Acinetobacter* outer membrane proteins. Nevertheless, the microbicidal potential of elastase is generally considered to be low (6).

Actinobacillus actinomycetemcomitans and *Capnocytophaga* spp. are gram-negative, facultative bacteria which have been associated with periodontal and systemic infections in humans (18, 28, 29). Neutrophils kill *A. actinomycetemcomitans* and *Capnocytophaga* spp. under aerobic and anaerobic conditions, suggesting that nonoxidative mechanisms play a significant role (1, 14, 23). The identities of the nonoxidative mechanisms remain to be ascertained. We have found that *A. actinomycetemcomitans* and *Capnocytophaga* spp. are differentially sensitive to certain nonoxidative neutrophil bactericidal mechanisms. Whereas both *Capnocytophaga* spp. and *A. actinomycetemcomitans* are sensitive to the rabbit defensin NP-1, only *Capnocytophaga* spp. are sensitive to the bactericidal effects of purified human neutrophil defensins in vitro (12, 13). The purposes of this report are to compare the in vitro sensitivities of *A. actinomycetemcomitans* and *Capnocytophaga* spp. to the bactericidal properties of purified human neutrophil cathepsin G and elastase, to determine the effects of the enzymatic activities of these compounds, and to determine whether plasma antiproteases modulate both enzyme-dependent and enzyme-independent bactericidal activity.

MATERIALS AND METHODS

Bacterial growth conditions. Bacteria, including *C. sputigena* ATCC 33123; *Capnocytophaga gingivalis* ATCC 33124; *Capnocytophaga ochracea* ATCC 27872; *A. actinomycetemcomitans* ATCC 29523 (serotype a), FDC Y4 (serotype b), and NCTC 9709 (serotype c); and *E. coli* ML-35 were grown as described previously (11–13). The bacterial cell concentration was determined turbidometrically (an optical density at 540 nm of 0.1 to 0.3 was equivalent to 10^9 cells per ml).

Reagents. Purified human neutrophil cathepsin G and elastase were obtained from a commercial supplier (Biodesign International, Kennebunkport, Maine). The purity and integrity of these reagents were verified by cationic polyacrylamide gel electrophoresis (PAGE) performed at pH 5.2 with a 20% acrylamide resolving gel and a 3% acrylamide

* Corresponding author.

TABLE 1. Amino acid composition of cathepsin G and elastase used in this study compared with anticipated composition based on sequence information

Amino acid	No. of residues[a]			
	Cathepsin G[b]		Elastase[c]	
	This study	Reference 16	This study	Reference 19
Isoleucine	10	13	8	10
Leucine	15	19	20	21
Valine	14	16	23	27
Threonine	15	12	7	6
Alanine	14	12	24	22
Glycine	28	21	27	24
Histidine	6	5	6	5
Phenylalanine	5	7	9	9
Tyrosine	4	5	2	2
Tryptophan	ND[d]	2	ND	3
Proline	15	13	11	9
Cysteine	5	6	7	8
Serine	17	15	14	12
Methionine	2	5	2	4
Aspartate or asparagine	19	18	22	21
Glutamate or glutamine	23	23	16	15
Arginine	41	34	23	19
Lysine	2	6	0	0
Total	230	232	215	218

[a] Data for cathepsin G based upon 232 residues, and data for elastase based upon 218 residues, with subtraction for tryptophan.
[b] Extrapolated from cloned DNA sequence.
[c] Determined by complete amino acid sequence.
[d] ND, not determined.

photopolymerized sample gel, both containing 0.5% Triton X-100 and 0.01% digitonin (Sigma) (5, 21), and sodium dodecyl sulfate-PAGE performed as described by Laemmli (9) with a 12% acrylamide resolving gel. The commercial elastase contained no detectable cathepsin G, and the commercial cathepsin G contained no detectable elastase. Neither was contaminated with other neutral serine proteases, including proteinase 3 (p29b) and azurocidin. The amino acid compositions of both elastase and cathepsin G were consistent with compositions inferred from sequence information (Table 1). For microbicidal assays, cathepsin G and elastase were suspended in distilled water and stored at −85°C until use. Plasma α-1-antitrypsin (AT; Sigma Chemical Co., St. Louis, Mo.), α-1-antichymotrypsin (AC; ICN Biomedical Inc., Costa Mesa, Calif.), and α-2-macroglobulin (A2M; Sigma) were dissolved in sterile distilled water and/or desalted against water by ultrafiltration using Centriflo cones (Amicon Corp., Danvers, Mass.). Protein concentrations were verified by the dye-binding method of Bradford (3) using bovine serum albumin (BSA) as a standard. Plasma antiproteases were preincubated with cathepsin G for 2 h prior to testing for the inhibition of microbicidal or enzymatic activities.

Inactivation of cathepsin G with DFP and PMSF. Cathepsin G was inactivated with 20 mM diisopropyl fluorophosphate (DFP; Sigma), in 50 mM phosphate (pH 7.0) for 2 h on ice. Residual DFP was eliminated by microdialysis (1.25×10^5 final dialysis ratio) against three exchanges of distilled water for 6 h at 4°C, and the inactivated enzyme was concentrated to 1 mg/ml with a SpeedVac (Savant Instruments, Farmingdale, N.Y.). A stock solution of phenylmethylsulfonyl fluoride (PMSF; Sigma) was prepared at 20 mg/ml in dimethyl sulfoxide and was diluted in 10 mM sodium phosphate (pH 7.0) to provide final concentrations of 50 μg/ml to inactivate enzymes for electrophoretic analysis and 10 μg/ml to use in the bactericidal assay. Controls for 0.05% dimethyl sulfoxide were used in bactericidal assays when appropriate.

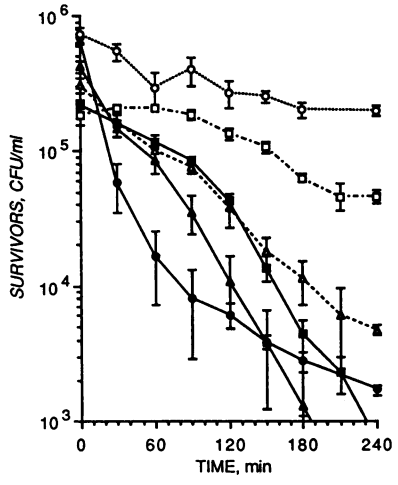

FIG. 1. Bactericidal activity of cathepsin G (100 μg/ml) against *A. actinomycetemcomitans* ATCC 29523, FDC Y4, and NCTC 9709 as a function of time. The assay was performed with 10 mM sodium phosphate–1% TSB (pH 8.0) over 4 h at 37°C. Points and vertical lines represent the mean and standard deviation of four trials. Symbols: ●, ATCC 29523 plus cathepsin G; ○, ATCC 29523 alone; ▲, FDC Y4 plus cathepsin G; △, FDC Y4 alone; ■, NCTC 9709 plus cathepsin G; □, NCTC 9709 alone.

Microenzyme assays. End point enzyme assays for cathepsin G and elastase were performed in microtiter format with the specific esterase substrates *N*-benzoyl-D,L-phenylalanyl-2-naphthyl ester (BPNE; Sigma) (50 μg/ml) for cathepsin G and *N*-benzyloxycarbonyl-L-alanyl-2-naphthyl ester (ZANE; Bachem Inc., Torrance, Calif.) (50 μg/ml) for elastase (11, 20).

Bactericidal assay. Bactericidal activity was quantified as described elsewhere (11), except that the bactericidal assays were performed with 10 mM phosphate (pH 8.0) containing 1% (vol/vol) Trypticase soy broth (TSB) at 37°C and the reactions were terminated by 1:10 dilution in 10 mM sodium phosphate (pH 8.0) unless otherwise specified. Survivors were enumerated as CFU per milliliter, and when appropriate, bactericidal activity was quantified as the $\log_{10}$ reduction in CFU ($\delta[\log_{10}$ killing]).

RESULTS

Bactericidal activity of cathepsin G. *A. actinomycetemcomitans* ATCC 29523, FDC Y4, and NCTC 9709 were used to examine the bactericidal kinetics of cathepsin G. Microbicidal activity against all three organisms was observed (Fig. 1). Although *A. actinomycetemcomitans* FDC Y4 died in buffer alone, the rate of death was increased by the presence of cathepsin G at a concentration of 100 μg/ml. A reduction in CFU of 1 to 2 orders of magnitude ($\delta[\log_{10}$ killing] = 1 to 2) was observed over 2 h.

Comparing killing by cathepsin G and elastase. *A. actinomycetemcomitans* ATCC 29523, FDC Y4, and NCTC 9709 were used to compare the bactericidal potency of cathepsin G and elastase. Bactericidal activity against *A. actinomycetemcomitans* was observed at concentrations of cathepsin G

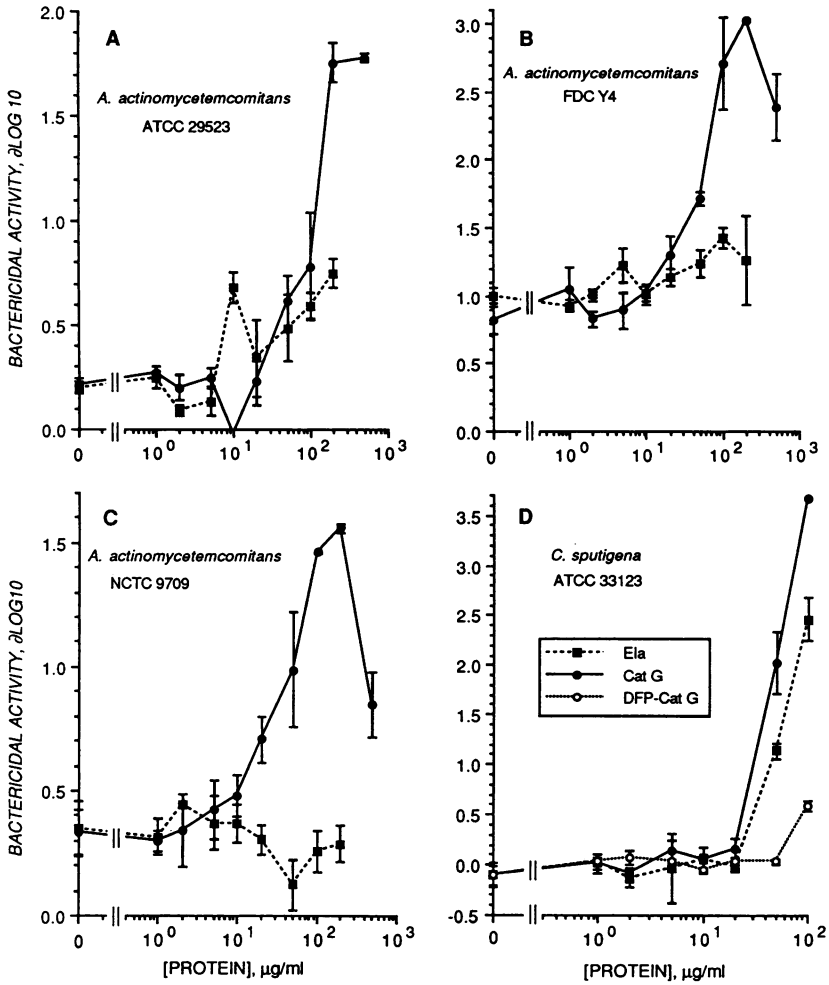

FIG. 2. Bactericidal activity of cathepsin G (Cat G) and elastase (Ela) against *A. actinomycetemcomitans* ATCC 29523 (A), FDC Y4 (B), and NCTC 9709 (C) and bactericidal activity of cathepsin G, DFP-inactivated cathepsin G (DFP-Cat G), and elastase against *C. sputigena* ATCC 33123 (D) as a function of concentration. The assay was performed with 10 mM sodium phosphate–1% TSB (pH 8.0) for 2 h at 37°C. Points and vertical lines represent the mean and standard deviation of four trials.

above 20 μg/ml (Fig. 2A to C). Elastase did not kill *A. actinomycetemcomitans* NCTC 9709 and was weakly bactericidal against *A. actinomycetemcomitans* ATCC 29523 and FDC Y4. In comparison, both elastase and cathepsin G exerted strong bactericidal effects against *C. sputigena* ATCC 33123 (Fig. 2D). DFP-inactivated cathepsin G was much less potent than either cathepsin G or elastase in killing *C. sputigena* ATCC 33123. Because we feared that the bactericidal activity of elastase (a highly proteolytic enzyme) may decrease rapidly under assay conditions, we examined the effects of preincubating fresh commercial elastase for 1 h at 37°C at pH 8.0. The bactericidal activity of elastase (100 μg/ml) against *C. sputigena* ATCC 33123 was stable at 37°C and pH 8.0 over the 1-h preincubation period ($\delta[\log_{10}$ killing] = 3.2 ± 0.0 without preincubation and 3.6 ± 0.0 after 1 h of preincubation [mean ± standard deviation]). Therefore, the ineffective killing activity that elastase exerted against *A. actinomycetemcomitans* was not likely to be due to enzymatic autolysis.

Inhibition by DFP and PMSF. Bactericidal activity of cathepsin G against *C. gingivalis* ATCC 33124 and *C. ochracea* ATCC 27872 was observed at concentrations above 50 μg/ml (data not shown), as observed for *C. sputigena* ATCC 33123 (Fig. 2D). Inactivation with DFP greatly reduced the bactericidal activity of cathepsin G against *Capnocytophaga* spp. but had only modest effects on the killing of *A. actinomycetemcomitans* (Fig. 3). DFP-inactivated cathepsin G exhibited microbicidal activity against *A. actinomycetemcomitans* strains and *E. coli* ML-35 which was virtually equal to killing by the native enzyme. Both DFP and PMSF inactivation reduce enzyme activity by >95% (11). Elastase (100 μg/ml) and PMSF-inactivated elastase both killed *C. sputigena* ATCC 33123, but killing by PMSF-inactivated elastase was relatively diminished ($\delta[\log_{10}$ killing] = 2.9 ± 0.3 and 1.2 ± 0.2, respectively, after 2 h at 37°C and pH 8.0).

Effects of pH and ionic strength on bactericidal activity. The effect of pH and ionic strength on the killing of *A. actinomycetemcomitans* was assessed with 10 mM sodium phosphate–1% TSB by using a 2-h incubation period at 37°C. Killing of *A. actinomycetemcomitans* ATCC 29523 by cathepsin G was decreased by lower pH (Fig. 4A) and higher ionic strengths (Fig. 4B). Elastase did not kill *A. actinomycetemcomitans* at any pH tested. Killing of *C. sputigena*

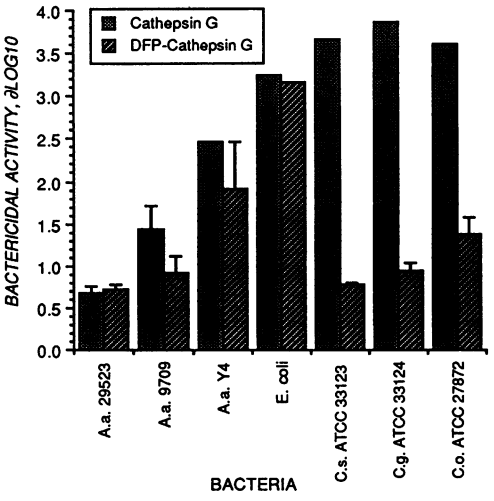

FIG. 3. Bactericidal activity of cathepsin G and cathepsin G inactivated with DFP (both at 100 μg/ml) against *C. sputigena* ATCC 33123; *C. gingivalis* ATCC 33124; *C. ochracea* ATCC 27872; *A. actinomycetemcomitans* ATCC 29523, FDC Y4, and NCTC 9709; and *E. coli* ML-35. The assay was performed with 10 mM sodium phosphate–1% TSB (pH 8.0) for 2 h at 37°C (*A. actinomycetemcomitans* strains were incubated for 4 h at 37°C). Bars and vertical lines represent the mean and standard deviation of four trials.

ATCC 33123 by elastase was diminished below pH 6.8 (Fig. 4C).

Effect of plasma antiproteases on the esterolytic activity of cathepsin G. The plasma antiproteases AT and AC inhibited the esterolytic activity of cathepsin G. Using 5 μg of cathepsin G per ml (0.2 nM) with 23.4 μM AT, or 2 μM AC produced 97% ± 3% or 84% ± 1%, inhibition, respectively. The normal plasma concentrations of AT and AC are 23.4 and 4.4 μM, respectively (7). Confirming other reports, A2M did not affect the esterolytic activity of cathepsin G against the small synthetic substrate (BPNE) used in our assay (7).

Effect of plasma antiproteases on killing. The plasma antiproteases AT, AC, and A2M are known to interfere with the enzymatic and bactericidal activities of cathepsin G (7, 26). It was therefore of interest to determine how effectively they inhibited the killing of *C. sputigena* ATCC 33123 (killed by an enzyme-dependent mechanism) and *A. actinomycetemcomitans* NCTC 9709 (killed by an enzyme-independent mechanism) by cathepsin G. The 2-h preincubation of cathepsin G with plasma antiproteases was compared with a sham (control) preincubation, which was found to have no effect on either the enzymatic activity, electrophoretic integrity, or bactericidal activity of cathepsin G (not shown). Killing of both *C. sputigena* ATCC 33123 and *A. actinomycetemcomitans* NCTC 9709 by cathepsin G was blocked at physiologic levels of AT, AC, and A2M (23.4, 4.4, and 0.5 μM, respectively) (Fig. 5A and B). BSA at identical micromolar concentrations did not appreciably alter the bactericidal activity of cathepsin G. Similarly, we observed that physiologic levels of AT, AC, and A2M (but not BSA at identical concentrations) blocked the killing of *E. coli* ML-35 (not shown), another organism which we have observed is killed by cathepsin G in an enzyme-independent manner. On a molar basis, A2M was the most efficacious, completely blocking killing at concentrations as low as 1 μM (Fig. 5C). AC and AT were almost equally effective, blocking killing at 2 and 10 μM, respectively.

DISCUSSION

In this study, we examined the microbicidal properties of purified human neutrophil cathepsin G and elastase against the potential periodontal pathogens *A. actinomycetemcomitans* and *Capnocytophaga* spp. Cathepsin G exhibited strong microbicidal effects against *Capnocytophaga* spp. and *A. actinomycetemcomitans*. Elastase was almost as effective as cathepsin G in killing *C. sputigena* ATCC 33123 but was not effective against *A. actinomycetemcomitans*.

Mildly alkaline conditions and low salt concentrations favored the killing of *A. actinomycetemcomitans* by cathepsin G; these results are similar to results reported for nonoral bacteria (17). It is known that human phagolysosomes alkalinize initially (4); however, the ionic composition of phago-

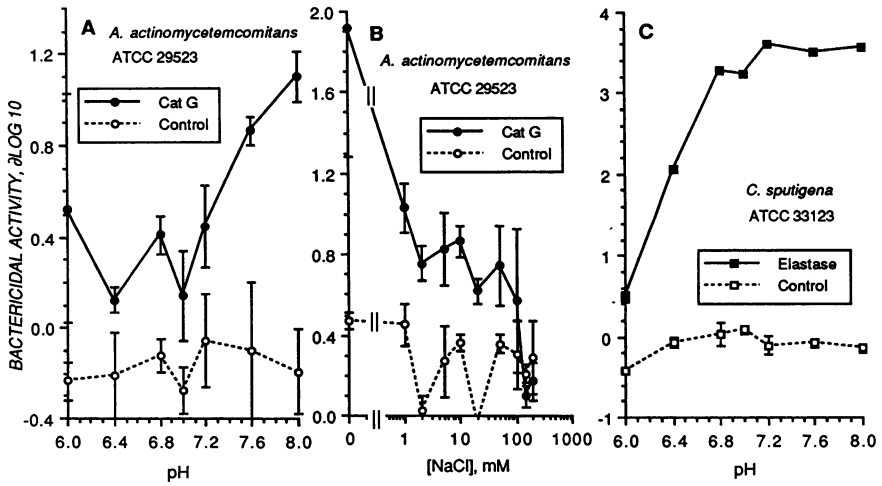

FIG. 4. Effect of pH (A) and salt concentration (B) on the killing of *A. actinomycetemcomitans* ATCC 29523 by cathepsin G (100 μg/ml). The effect of pH was determined with 10 mM sodium phosphate–1% TSB for 4 h at 37°C. The effect of salt was determined with 10 mM sodium phosphate–1% TSB (pH 8.0) for 4 h at 37°C. (C) Effect of pH on the bactericidal activity of elastase (100 μg/ml) against *C. sputigena* ATCC 33123 (2 h, 37°C). Points and vertical lines represent the mean and standard deviation of four trials.

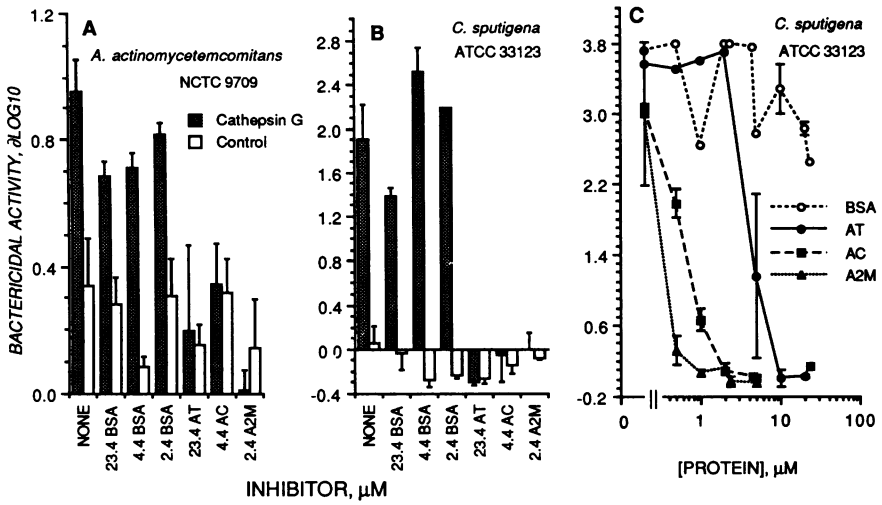

FIG. 5. Inhibition of killing of *A. actinomycetemcomitans* NCTC 9709 (A) and *C. sputigena* ATCC 33123 (B) by plasma antiproteases. The plasma antiproteases used were AT, AC, and A2M. BSA was used as a control. Numbers preceding the abbreviations for the antiproteases represent the micromolar concentrations. The assay was performed with 10 mM sodium phosphate–1% TSB (pH 8.0) for 2 h at 37°C. Bars and vertical lines represent the mean and standard deviation of four trials. (C) Dose-response curve of plasma antiproteases in inhibiting the killing of *C. sputigena* ATCC 33123 by cathepsin G. Points and thin vertical lines represent the mean and standard deviation of four trials.

lysosomes is unknown. Nevertheless, this suggests an important role for cathepsin G in the early phases of killing within phagolysosomes. The killing of *C. sputigena* ATCC 33123 by elastase was also favored by these conditions; however, the effect of pH on killing of *C. sputigena* ATCC 33123 by cathepsin G is not as great (11).

There are two mechanisms whereby cathepsin G kills microorganisms. One mechanism is enzyme independent, and the other is enzyme dependent (11). The killing of *Capnocytophaga* spp. but not *A. actinomycetemcomitans* by cathepsin G required an intact enzyme. It can be concluded that both mechanisms function against oral microorganisms. Enzymatic activity may play some role in the killing of *C. sputigena* ATCC 33123 by elastase, which was reduced by PMSF.

The molecular interactions involved in enzyme-dependent killing are unknown; however, the bactericidal activity against *Capnocytophaga* spp. by neutral serine proteases may require partial enzyme autodegradation. Two clostripain-generated peptide fragments of cathepsin G are microbicidal against *S. aureus* and *N. gonorrhoeae*. One fragment is an externally oriented heptapeptide (HPQYNQR), and the other is a buried N-terminal pentapeptide (IIGGR) (2). Enzyme-dependent bactericidal activity may be due to insensitivity to HPQYNQR and sensitivity to IIGGR. Killing by the IIGGR domain may require autolytic exposure of that site.

Plasma antiproteases, particularly A2M, can inhibit the bactericidal activity of cathepsin G against nonoral microbes (26). We found that plasma antiproteases blocked killing of periodontal bacteria by cathepsin G at physiologic concentrations. This was a fairly specific effect and was not observed when BSA was used at comparable molar concentrations. We observed that AT and AC (but not A2M) blocked the esterolytic activity of cathepsin G. A2M entraps neutral serine proteases without inactivation of the enzymatic site; thus, it inhibits the proteolytic activity but not the esterolytic activity of cathepsin G against small substrates (7). We have also found that the optimum concentration of AC against the esterolytic activity of cathepsin G was 2 μM, providing an inhibitor-to-enzyme ratio of 1,000:1. This coincided with the lowest concentration of AC that completely inhibited the killing of *C. sputigena* ATCC 33123. Higher concentrations (4.4 μM [normal plasma concentration]) virtually abolished detectable enzyme inhibition but not killing inhibition. Regardless, inhibition of enzymatic activity is not important with respect to blocking the microbicidal activity of cathepsin G. In this study, plasma antiproteases were shown to specifically inhibit both enzyme dependent and enzyme independent microbicidal activities.

In conclusion, (i) cathepsin G kills both *Capnocytophaga* spp. and *A. actinomycetemcomitans*; (ii) killing of *Capnocytophaga* spp., but not *A. actinomycetemcomitans*, by cathepsin G required enzymatic activity; (iii) elastase kills *Capnocytophaga* spp. but not *A. actinomycetemcomitans*; and (iv) serum antiproteases block both enzyme-dependent and enzyme-independent killing activities of cathepsin G. More studies will be required to determine the molecular mechanisms involved in the apparently enzyme-dependent killing of these microorganisms and to ascertain the effects of plasma antiproteases on the bactericidal activities of other members of the neutral serine protease family.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant DE-08161 and Research Career Development Award DE 00282 from the National Institute for Dental Research, National Institutes of Health.

We gratefully acknowledge the help of Sylvia Harwig in preparing DFP-inactivated cathepsin G and Audree V. Fowler and Richard Stevens of the UCLA Protein Microsequencing Service for the amino acid compositional analyses.

REFERENCES

1. **Baker, P. J., and M. E. Wilson.** 1988. Effect of clindamycin on neutrophil killing of gram-negative periodontal bacteria. Antimicrob. Agents Chemother. **32**:1521–1527.
2. **Bangalore, N., J. Travis, V. C. Onunka, J. Pohl, and W. M. Shafer.** 1990. Identification of the primary antimicrobial domains in human neutrophil cathepsin G. J. Biol Chem. **265**: 13584–13588.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the

quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **7**:248–254.
4. **Cech, P., and R. I. Lehrer.** 1984. Phagolysosomal pH of human neutrophils. Blood **63**:88–95.
5. **Dewald, B., R. Rindler-Ludwig, U. Bretz, and M. Baggiolini.** 1975. Subcellular localization and heterogeneity of neutral proteases in neutrophilic polymorphonuclear leukocytes. J. Exp. Med. **141**:709–723.
6. **Elsbach, P., and J. Weiss.** 1988. Phagocytic cells: oxygen-independent antimicrobial systems, p. 445–470. *In* J. I. Gallin, I. M. Goldstein, and R. Snyderman (ed.), Inflammation: basic principles and clinical correlates. Raven Press, Ltd., New York.
7. **Feinstein, G.** 1978. Polymorphonuclear leukocyte proteases and their effects on complement components and neutrophil function, p. 179–190. *In* K. Havemann and A. Janoff (ed.), Neutral proteases of human polymorphonuclear leukocytes. Urban and Schwarzenberg, Inc., Baltimore.
8. **Hohn, P. A., N. C. Popescu, R. D. Hanson, G. Salvesen, and T. J. Ley.** 1989. Genomic organization and chromosomal localization of the human cathepsin G gene. J. Biol. Chem. **264**:13412–13419.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
10. **Lehrer, R. I., K. M. Ladra, and R. B. Hake.** 1975. Nonoxidative fungicidal mechanisms of mammalian granulocytes: demonstration of components with candidacidal activity in human, rabbit, and guinea pig leukocytes. Infect. Immun. **11**:1226–1234.
11. **Miyasaki, K. T., and A. L. Bodeau.** 1991. In vitro killing of *Capnocytophaga* by granule fractions of human neutrophils is associated with cathepsin G activity. J. Clin. Invest. **87**:1585–1593.
12. **Miyasaki, K. T., A. L. Bodeau, T. Ganz, M. E. Selsted, and R. I. Lehrer.** 1990. Sensitivity of oral, gram-negative, facultative bacteria to the bactericidal activity of human neutrophil defensins. Infect. Immun. **58**:3934–3940.
13. **Miyasaki, K. T., A. L. Bodeau, M. E. Selsted, T. Ganz, and R. I. Lehrer.** 1990. Killing of oral, Gram-negative, facultative bacteria by the rabbit defensin, NP-1. Oral Microbiol. Immunol. **5**:315–319.
14. **Miyasaki, K. T., M. E. Wilson, A. J. Brunetti, and R. J. Genco.** 1986. Oxidative and nonoxidative killing of *Actinobacillus actinomycetemcomitans* by human neutrophils. Infect. Immun. **53**:154–160.
15. **Odeberg, H., and I. Olsson.** 1975. Antibacterial activity of cationic proteins of human granulocytes. Infect. Immun. **25**: 597–602.
16. **Salvesen, G., D. Farley, J. Shuman, A. Przybyla, C. Reilly, and J. Travis.** 1987. Molecular cloning of human cathepsin G: structural similarity to mast cell chymase and cytotoxic T lymphocyte proteinases. Biochemistry **26**:2289–2293.
17. **Shafer, W. M., and V. C. Onunka.** 1989. Mechanism of staphylococcal resistance to non-oxidative antimicrobial action of neutrophils: importance of pH and ionic strength in determining the bactericidal action of cathepsin G. J. Gen. Microbiol. **135**:825–830.
18. **Shurin, S. B., S. S. Socransky, E. Sweeney, and T. P. Stossal.** 1979. A neutrophil disorder induced by Capnocytophaga, a dental microorganism. N. Engl. J. Med. **301**:849–854.
19. **Sinha, S., W. Watorek, S. Karr, J. Giles, W. Bode, and J. Travis.** 1987. Primary structure of human neutrophil elastase. Proc. Natl. Acad. Sci. USA **84**:2228–2232.
20. **Starkey, P. M., and A. J. Barrett.** 1976. Neutral proteinases of human spleen. Purification and criteria for homogeneity of elastase and cathepsin G. Biochem. J. **155**:255–263.
21. **Sweetman, F., and L. Ornstein.** 1974. Electrophoresis of elastase-like esterases from human neutrophils. J. Histochem. Cytochem. **22**:327–339.
22. **Takahashi, H., T. Nukiwa, K. Yoshimura, C. D. Quick, D. J. States, M. D. Holmes, J. Whang-Peng, T. Knutsen, and R. G. Crystal.** 1988. Structure of the human neutrophil elastase gene. J. Biol. Chem. **263**:14739–14747.
23. **Thompson, H. L., and J. M. A. Wilton.** 1991. Effects of anaerobiosis and aerobiosis on interactions of human polymorphonuclear leukocytes with the dental plaque bacteria *Streptococcus mutans*, *Capnocytophaga ochracea*, and *Bacteroides gingivalis*. Infect. Immun. **59**:932–940.
24. **Thorne, K. J. I., R. C. Oliver, and A. J. Barrett.** 1976. Lysis and killing of bacteria by lysosomal proteinases. Infect. Immun. **14**:555–563.
25. **Venge, P.** 1978. Polymorphonuclear leukocyte proteases and their effects on complement components and neutrophil function, p. 264–275. *In* K. Havemann and A. Janoff (ed.), Neutral proteases of human polymorphonuclear leukocytes. Urban and Schwarzenberg, Inc., Baltimore.
26. **Venge, P., I. Olsson, and H. Odeberg.** 1975. Cationic proteins of human granulocytes. V. Interaction with plasma protease inhibitors. Scand. J. Clin. Lab. Invest. **35**:737–744.
27. **Watorek, W., D. Farley, G. Salvesen, and J. Travis.** 1988. Neutrophil elastase and cathepsin G: structure, function, and biological control. Adv. Exp. Med. Biol. **240**:23–31.
28. **Wilson, M. E., J. T. Jonak-Urbanczyk, P. M. Bronson, K. C. Dudas, M. A. Apicella, and R. J. Genco.** 1987. *Capnocytophaga* species: increased resistance of clinical isolates to serum bactericidal action. J. Infect. Dis. **156**:99–106.
29. **Zambon, J. J.** 1985. *Actinobacillus actinomycetemcomitans* in human periodontal disease. J. Clin. Periodontol. **12**:1–20.