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Bactericidal Activities of Synthetic Human Leukocyte Cathepsin G-Derived Antibiotic Peptides and Congeners against *Actinobacillus actinomycetemcomitans* and *Capnocytophaga sputigena*

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***Actinobacillus actinomycetemcomitans* and *Capnocytophaga* spp. are gram-negative bacteria implicated in the etiology of periodontal disease (particularly in individuals with neutrophil defects) and life-threatening systemic infections. They are resistant to many antibiotics of microbial origin but are sensitive to the nonoxidative microbicidal action of neutrophils. These organisms are susceptible to the microbicidal effect of cathepsin G but are killed by two distinct mechanisms. The purpose of this study was to assess their sensitivity to the antibiotic effects of IIGGR and HPQYNQR, antimicrobial peptides derived from human neutrophil cathepsin G. The efficacies of the synthetic peptides IIGGR and HPQYNQR were tested by single-dose screening, dose-response, and kinetic assays against three representative strains (each) of *A. actinomycetemcomitans* and *Capnocytophaga* spp. and one strain of *Eikenella corrodens*. Strains of *A. actinomycetemcomitans* were sensitive to IIGGR and HPQYNQR at equal concentrations (wt/vol), whereas strains of *Capnocytophaga* and *E. corrodens* were more sensitive to IIGGR than to HPQYNQR. These differential antibiotic effects occurred over both time and dose ranges too narrow to be of therapeutic significance but are consistent with the premise that cathepsin G kills these oral bacteria by two distinct mechanisms. Except for IVGGR, congeners of IIGGR, including AIGGR, IAGGR, IIAGR, IIGAR, IIGGA, IQGGR, ILGGR, and I-norleucyl-GGR (InLGGR), were microbicidal at 500 μg/ml. IIGGR-amide exhibited no antibiotic activity. The D-enantiomer of IIGGR, DIDIGGDR, was as potent as IIGGR itself. APQYNQR exhibited antibiotic activity but somewhat less than HPQYNQR. We conclude that charge distribution, but not chirality or net charge, is an important determinant in the antibiotic efficacy of IIGGR. Moreover, peptide antibiotics derived from cathepsin G may have therapeutic value against periodontal gram-negative, facultative bacteria.**

Actinobacillus actinomycetemcomitans and *Capnocytophaga* spp. are gram-negative, facultative bacteria indigenous to dental plaque. *A. actinomycetemcomitans* has been implicated in severe, possibly invasive forms of periodontal infection, bacterial endocarditis, and focal infections involving various other major organs (10, 25). The periodontal infections have been associated with dysfunctional neutrophil killing and chemotaxis (9). Sepsis by oral *Capnocytophaga* spp. has been documented for the immunocompromised individual (14) and as a sequela of granulocytopenia (6). Both organisms are resistant to a number of antibiotics of microbial origin. *A. actinomycetemcomitans* exhibits substantial resistance to the antimicrobial effects of vancomycin, spiramycin, and bacitracin; variable resistance to clindamycin, erythromycin, kanamycin, streptomycin, and penicillin G; and sensitivity to chloramphenicol, doxycycline, minocycline, tetracycline, gentamicin, and ciprofloxacin (4, 21). *Capnocytophaga* spp. are resistant to colistin, kanamycin, nalidixic acid, gentamicin, and tobramycin; show modest or variable resistance to cephalothin, cefazolin, vancomycin, and oxacillin; and are sensitive to penicillin G, erythromycin, cefoxitin, clindamycin, and chloramphenicol (7).

Antibiotics within the animal kingdom may take the form of peptides (2). We have observed that *A. actinomycetemcomitans* is highly sensitive to the bactericidal effects of several natural, host-derived antibiotics, including the rabbit neutrophil defensin peptide, NP-1 (but not to the human defensins at the concentrations tested), and the human neutrophil protein, cathepsin G, in vitro (11, 12). *Capnocytophaga* spp. are sensitive to cathepsin G and human defensins. Two small antibiotic peptide domains, Ile-Ile-Gly-Gly-Arg and His-Pro-Gln-Tyr-Asn-Gln-Arg (IIGGR and HPQYNQR, respectively) have been isolated from a clostripain digest of cathepsin G (1, 18). Recently, other antibiotic peptide domains from both cathepsin G and the enzymatically inactive azurocidin/CAP37 (15, 16, 19) have been described.

On the basis of the resistance of *A. actinomycetemcomitans* and *Capnocytophaga* spp. to antibiotics of microbial origin and their sensitivity to human leukocyte cathepsin G, we believe that naturally occurring animal antibiotics may be of therapeutic value. For example, such antibiotics may be used topically prior to periodontal surgery, as part of an oral rinse prior to dental manipulation to reduce the risk of systemic infection, or in a timed-release delivery system. Theoretically, small peptide antibiotics may be less toxic and less allergenic than antibiotics of microbial origin. On a molar basis, synthetic peptide homologs of those derived by clostripain digestion of cathepsin G are more effective than tetracycline, streptomycin, kanamycin, and the defensins

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TABLE 1. Formulae, formula weights, theoretical reversed-phase chromatography-HPLC index, and theoretical pI of synthetic peptides used in this study

Peptide formula[a]	Formula weight	HPLC index	Theoretical pI
IIGGR	515	13.8	11.3
AIGGR	473	14.5	11.3
IAGGR	473	14.5	11.3
IIAGR	529	22.3	11.3
IIGAR	529	22.3	11.3
IIGGA	430	24.7	8.2
IVGGR	501	10.7	11.3
ILGGR	515	27.2	11.3
InLGGR[b]	514		11.3
DIDIGGDR[c]	515	13.8	11.3
IIGGR-amide	515		
HPQYNQR	943	5.6	10.5
APQYNQR	876	15.0	10.5

[a] Single-letter amino acid code: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.
[b] nL, norleucine.
[c] D-enantiomer of IIGGR.

against nonoral pathogens such as *Neisseria gonorrhoeae* (1). Recently, it was reported that synthetic IIGGR killed a number of oral bacteria, including *Actinomyces viscosus*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Veillonella* sp., and *Streptococcus sanguis* (3). The purpose of this study was to examine the efficacies of synthetic IIGGR and HPQYNQR (and certain congeners) against bacterial pathogens of periodontal origin, especially *A. actinomycetemcomitans*.

MATERIALS AND METHODS

Bacteria. Bacteria, including *A. actinomycetemcomitans* ATCC 25923, FDC-Y4, and NCTC 9709, *Capnocytophaga sputigena* ATCC 33123, *Capnocytophaga gingivalis* ATCC 33124, *Capnocytophaga ochracea* ATCC 27872, *Eikenella corrodens* ATCC 23834, *Staphylococcus aureus* 8325-4, and *Escherichia coli* ML-35 were grown to early log growth phase in Trypticase soy broth (TSB) (BBL Microbiology, Cockeysville, Md.) containing 0.1% sodium bicarbonate, 0.05% equine hemin III (Sigma Chemical Co., St. Louis, Mo.), 0.0001% menadione (Sigma), and 0.1% yeast extract (Difco Laboratories, Detroit, Mich.). These organisms and the growth conditions have been previously described (12, 17). The bacteria were adjusted turbidometrically so that the final concentration was 10^6 cells per ml in a solution containing 0.325% (vol/vol) TSB (BBL Microbiology), 0.035% $NaHCO_3$, and 1% (vol/vol) Hank's balanced salt solution (HBSS) (Sigma), pH 7.0.

Synthetic peptides. Peptides (Table 1) were synthesized by solid-phase synthesis on an Applied Biosystems, Inc., model 430A peptide synthesizer (Foster City, Calif.) as previously described (18). Peptides were purified by reversed-phase high-performance liquid chromatography (HPLC) on an Aquapore OD-300 C_{18} silica column (1 by 10 cm; Applied Biosystems) or on an MRPH-Gel RP polystyrene column (1 by 10 cm; The Nest Group, Southborough, Mass.) with an acetonitrile gradient in the presence of 0.1% aqueous trifluoroacetic acid (18). The purity and structural integrity of the peptides were confirmed by analytical reversed-phase HPLC, amino acid composition analysis, and fast atom bombardment mass spectrometry. The purified peptides were lyophilized and stored at −20°C in the form of trifluoroacetate salts. Stock solutions were prepared in 1% HBSS. Molar and/or weight concentrations are nominal; i.e., they are not corrected for contents of water and trifluoracetate present in the lyophilized material. Control human neutrophil cathepsin G was obtained from a commercial supplier (Biodesign International, Inc., Kennebunkport, Maine) and was characterized with respect to purity and antimicrobial activity in a prior study (11).

Microscale bactericidal assay. Bacteria were incubated with peptides or cathepsin G in a total volume of 24 μl in 1% HBSS and maintained at 37°C (in a temperature block), unless otherwise indicated. The bactericidal reaction was terminated by 1:10 dilution in 1% HBSS and immediate distribution of the resultant suspension on laked blood agar plates by using a Spiral plater (Spiral Systems, Bethesda, Md.). Because the peptides (and cathepsin G) exhibit relatively sharp dose-response characteristics, this termination procedure has been found to be adequate, and even at the highest concentrations of antibiotic peptide, microbial growth could be observed at the center of the spiral where carryover peptide concentrations should be the highest. CFU were enumerated after 48 to 72 h of incubation. Bactericidal activity was quantified as the $\log_{10}$ reduction in CFU and calculated by the formula δ ($\log_{10}$ killing) = $\log_{10} n_0 - \log_{10} n_t$, where n is the bacterial CFU per milliliter at time zero or time t. The n_0 was adjusted to 2 to 5 × 10^5 CFU/ml in all bactericidal assays. Variance of δ is described, where applicable, as a maximum estimate of the standard deviation, by the formula $SD_\delta = \log_{10} n_t - \log_{10} (n_t - SD)$, where SD is the standard deviation of the CFU per milliliter.

RESULTS

Screening of peptides against periodontal bacteria. Consistent patterns of bactericidal activity were observed for the panel of peptides at 500 μg/ml (Table 2). In general, strains representative of *A. actinomycetemcomitans* were sensitive to the bactericidal activities of both IIGGR and HPQYNQR, and exhibited about 3 to 4 $\log_{10}$ orders of death over a 2-h period when exposed to either compound. Strains representative of the genus *Capnocytophaga* and one strain representative of *E. corrodens* were sensitive to IIGGR (2 to 3 $\log_{10}$ orders of death over 2 h) but relatively less sensitive to HPQYNQR (<1 $\log_{10}$ order of death). Nonoral bacteria exhibited less sensitivity to the panel of peptides than the periodontal bacteria. Nonoral bacteria included a strain of gram-negative *E. coli* and a strain of gram-positive *S. aureus*. *E. coli* ML-35 was moderately sensitive to both IIGGR and HPQYNQR. *S. aureus* 8325-4 was moderately sensitive to IIGGR and weakly affected by HPQYNQR. All bacteria were killed by the positive control, cathepsin G, at 100 μg/ml. These screening results prompted us to examine the effects of concentration and time on the microbicidal activities of the peptides in order to ascertain whether the antibiotics were truly selective in their effects.

Concentration dependency. We assessed the concentration dependency of killing by the peptides IIGGR, IIGGA, IVGGR, HPQYNQR, and APQYNQR against the three strains of *A. actinomycetemcomitans* and against *C. sputigena* ATCC 33123 (Table 3). Both IIGGR and IIGGA exerted antimicrobial effects, but usually, IIGGR was slightly more effective on a weight (and molar) basis. The 90% lethal dose for IIGGR ranged from 200 to 500 μg/ml,

TABLE 2. Screening periodontopathic bacteria for sensitivity to synthetic IIGGR, IIGGA, IVGGR, HPQYNQR, and APQYNQR[a]

Bacterial strain	Sensitivity to[b]:					
	CatG	IIGGR (742)	IIGGA (543)	IVGGR (728)	HPQYNQR (1,284)	APQYNQR (1,103)
A. actinomycetemcomitans ATCC 29523	±	++++	++	−	++++	++
A. actinomycetemcomitans FDC Y4	++	+++±	++	−	++±	±
A. actinomycetemcomitans NCTC 9709	++	+++	++	−	+++	+++
C. sputigena ATCC 33123	++++	++	+	−	+	−
C. gingivalis ATCC 33124	++++	+++	±	−	+	−
C. ochracea ATCC 27872	+++	++	±	−	−	−
E. corrodens ATCC 23834	+	+++	+++	−	±	−
E. coli ML-35	++	+±	+±	−	+±	±
S. aureus ATCC 8325-4	+++	++	ND[c]	ND	±	±

[a] Bactericidal assays were performed in 1% HBSS for 2 h at 37°C in quadruplicate.
[b] The concentration of the peptides was 500 μg/ml. Molecular weights for the peptide-trifluoroacetate salts in 1% HBSS (control values) are given in parentheses. +, 1 $\log_{10}$ order of killing over 2 h; ±, 1/2 $\log_{10}$ order of killing over 2 h; −, no death or bacterial growth. CatG, cathepsin G, 100 μg/ml.
[c] ND, not done.

with *C. sputigena* being modestly more resistant than strains representative of *A. actinomycetemcomitans*. The 90% lethal doses for IIGGA ranged from 500 to 800 μg/ml. Strains representative of *A. actinomycetemcomitans* appeared to be as resistant as *C. sputigena* ATCC 33123 to IIGGA. Synthetic IVGGR, a homolog of the leukocyte elastase N-terminal sequence (20), exhibited no antimicrobial effects at any of the tested concentrations. HPQYNQR and APQYNQR both killed the three strains of *A. actinomycetemcomitans* and *C. sputigena* ATCC 33123, with 90% lethal doses between 200 and 600 μg/ml. HPQYNQR was slightly more effective than APQYNQR on a weight-per-volume basis against two of the three strains of *A. actinomycetemcomitans* and *C. sputigena* ATCC 33123. Considering that the molecular weight of HPQYNQR is 941.5 and that of APQYNQR is 875.4, the differences in efficacy on a molar basis would be greater.

Kinetics. We evaluated the bactericidal kinetics of IIGGR, IIGGA, IVGGR, HPQYNQR, and APQYNQR against three strains of *A. actinomycetemcomitans* and *C. sputigena* ATCC 33123 at four concentrations—100, 200, 500, and 1,000 μg/ml—over a 2-h period. Whereas neither 100 nor 200 μg of IIGGR, IIGGA, or IVGGR per ml exerted any effect against the test organisms, both 500 and 1,000 μg of IIGGR per ml produced over 3 $\log_{10}$ orders of killing within the 2-h assay period (Fig. 1). IIGGA (500 and 1,000 μg/ml) exhibited less killing, apparently because of a lag period as well as the killing rates. IVGGR produced no killing over 2 h at either 500 or 1,000 μg/ml.

Very little difference was observed between the rates of killing for the four test organisms at 1,000 μg/ml (Fig. 2),

TABLE 3. Concentration dependency of the microbicidal activities of synthetic IIGGR, IIGGA, IVGGR, HPQYNQR, and APQYNQR[a]

Bacterial strain	90% Lethal dose (μg/ml)				
	IIGGR	IIGGA	IVGGR	HPQYNQR	APQYNQR
A. actinomycetemcomitans					
ATCC 29523	200	500	>2,000	200	600
FDC Y4	300	500	>2,000	300	500
NCTC 9709	200	800	>2,000	600	600
C. sputigena ATCC 33123	500	500	>2,000	500	600

[a] Bactericidal assays were performed with 1% HBSS for 2 h at 37°C in quadruplicate.

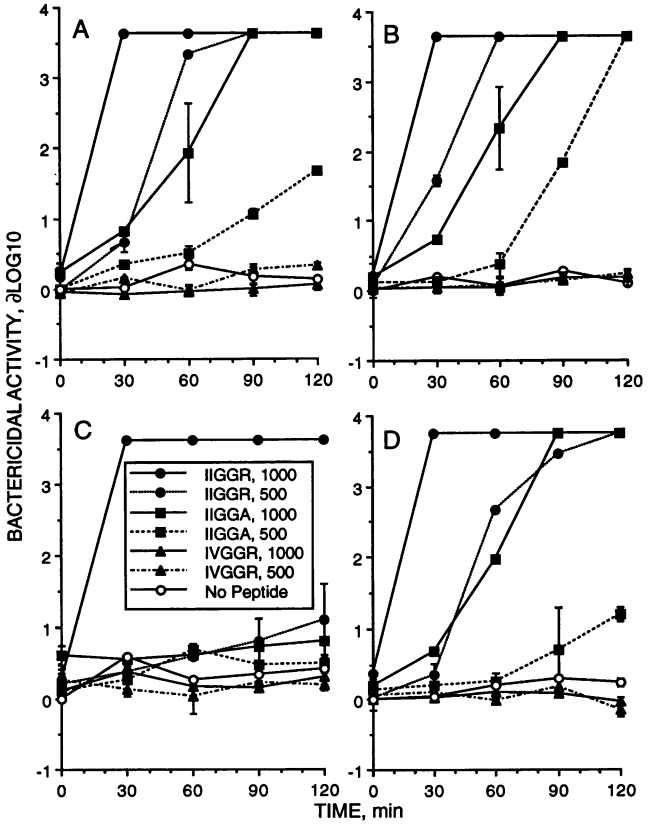

FIG. 1. Kinetics of killing of *A. actinomycetemcomitans* and *C. sputigena* by IIGGR, IIGGA, and IVGGR at 500 and 1,000 μg/ml. (A) *A. actinomycetemcomitans* ATCC 29523. (B) *A. actinomycetemcomitans* FDC-Y4. (C) *A. actinomycetemcomitans* NCTC 9709. (D) *C. sputigena* ATCC 33123. The assays were performed with 10^6 cells per ml in 1% HBSS–1% TSB at 37°C in quadruplicate. Points represent the means, and vertical lines represent the standard deviations of quadruplicate assays.

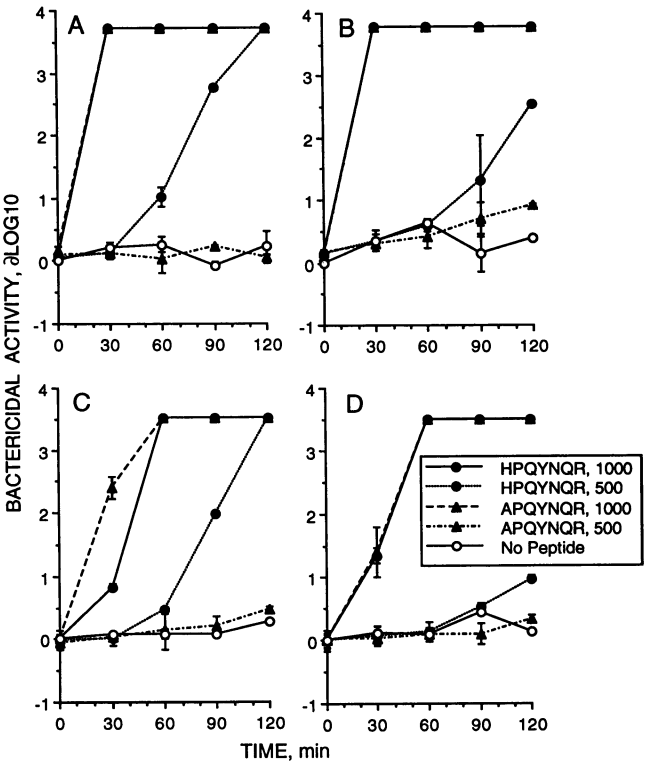

FIG. 2. Kinetics of killing of *A. actinomycetemcomitans* and *C. sputigena* by HPQYNQR and APQYNQR at 500 and 1,000 μg/ml. (A) *A. actinomycetemcomitans* ATCC 29523. (B) *A. actinomycetemcomitans* FDC-Y4. (C) *A. actinomycetemcomitans* NCTC 9709. (D) *C. sputigena* ATCC 33123. The assays were performed with 10^6 cells per ml in 1% HBSS–1% TSB at 37°C in quadruplicate. Points represent the means, and vertical lines represent the standard deviations of quadruplicate assays.

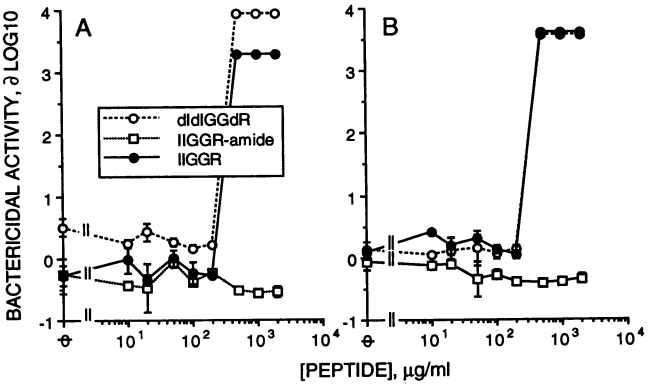

FIG. 3. Killing of *A. actinomycetemcomitans* FDC-Y4 (A) and *C. sputigena* ATCC 33123 (B) by IIGGR, DIDIGGDR, and IIGGR-amide. The assays were performed with 1% HBSS–1% TSB for 2 h at 37°C in quadruplicate. Points represent the means, and vertical lines represent the standard deviations of quadruplicate assays.

indicating rapid rates of killing relative to the sampling times. The lower peptide concentration (500 μg/ml) more clearly revealed the greater efficacy of HPQYNQR compared with that of APQYNQR. A clear shoulder (or lag) of killing was observed at 500 μg/ml. No killing was observed at the lower concentrations, 100 and 200 μg/ml.

Antimicrobial activities of DIDIGGDR, IIGGR-amide, and congeners. The D-enantiomer, DIDIGGDR, and IIGGR-amide were compared with IIGGR for antimicrobial activity against *A. actinomycetemcomitans* FDC-Y4 and *C. sputigena* ATCC 33123 (Fig. 3). The D-enantiomer, DIDIGGDR, was as effective as IIGGR against these two test strains. Both DIDIGGDR and IIGGR exerted antibiotic effects at concentrations above 500 μg/ml. In contrast, IIGGR-amide produced no antibiotic effects. Congeners of IIGGR, including AIGGR, IAGGR, IIAGR, IIGAR, IIGGA (previously discussed), ILGGR, I-norleucyl-GGR(InLGGR), and IQGGR, were all lethal at concentrations above 500 μg/ml but were less effective than IIGGR at lower concentrations (Fig. 4).

DISCUSSION

For technical reasons, antibiotics derived from the animal kingdom have not generated the same level of interest as those derived from the microbial kingdom. With the advent of solid-phase protein synthesis and protein microsequence analysis, it is now feasible to explore the vast world of animal peptides for antimicrobial activities. Recently, it was shown that cathepsin G, a mammalian neutral serine protease known for its antimicrobial activities, contained at least two small, antibiotic peptide domains, HPQYNQR and IIGGR, which could be obtained by digestion of cathepsin G with clostripain and the separation of the resultant digestion mixture by reversed-phase chromatography (1). The amino terminal IIGGX motif is a feature common to the granzyme serine protease family and is possessed by toxic molecules such as granzymes A to F, rat mast cell proteases I and II (chymases), human lymphocyte protease, and cathepsin G (8). The heptapeptide HPQYNQR is unique to cathepsin G but shares the HPXYN motif with a homologous antimicrobial peptide, HPAYNPK, from human granzyme B (18). Synthetic HPQYNQR and IIGGR are as active killing bac-

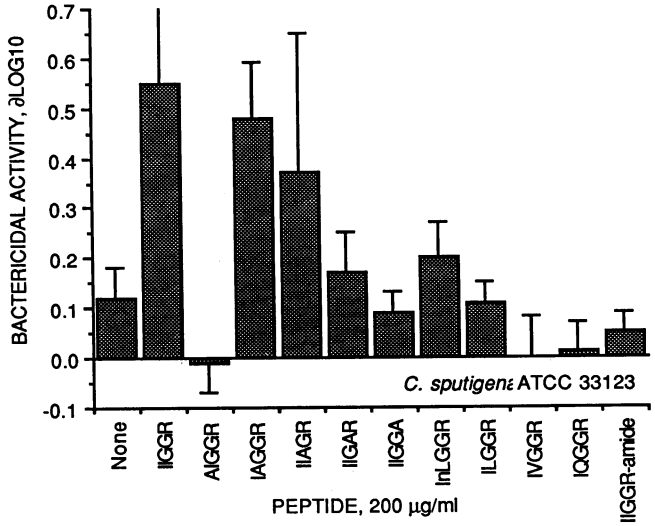

FIG. 4. Influence of single amino acid substitutions on the microbicidal effect of the pentapeptide. Peptides at concentrations above 500 μg/ml were microbicidal in all cases except IVGGR and IIGGR-amide. Modest differences among the peptides could be detected at lower concentrations (200 μg/ml), as shown in this figure. Bars represent the means, and vertical lines represent the standard deviations of quadruplicate assays.

teria as the naturally occurring peptides (1, 18). IVGGR represents a homolog of the N-terminal pentapeptide of human leukocyte elastase and azurocidin/CAP37/heparin-binding protein (5, 16, 20, 24).

In this study, we examined the antimicrobial effects of synthetic IIGGR, IIGGA, IVGGR, DIDIGGDR, IIGGR-amide, HPQYNQR, and APQYNQR against gram-negative, facultative bacteria implicated in certain forms of periodontal disease. We also compared the antimicrobial effects of these peptides with a number of congeners of IIGGR in order to obtain a complete alanine scan of IIGGR and to assess the impact of branching and chain length at position 2. We wanted to determine whether these peptides may have potential therapeutic value and, secondly, whether they could provide insight into the mechanism(s) whereby intact cathepsin G kills periodontal bacteria.

Initially, we observed that IIGGR was somewhat more effective than IIGGA on both a molar basis and a weight basis. Yet, both IIGGR and IIGGA killed the test bacteria at concentrations above 500 μg/ml. This indicates that the antimicrobial activity of the pentapeptide was not dependent upon the positively charged arginyl side chain, since an alanyl substitution for the arginine at position 5 still resulted in a potent antibiotic. Subsequently, we examined the effect of single alanine substitutions throughout the pentapeptide, including AIGGR, IAGGR, IIAGR, IIGAR, and IIGGA. All of these compounds were microbicidal against *C. sputigena* ATCC 33123 at concentrations above 500 μg/ml. At 200 μg/ml, minor differences among the compounds were detected and a rank order of microbicidal effects was observed: IIGGR > IAGGR > IIAGR > IIGAR > AIGGR > IIGGA. Therefore, although all of these compounds were microbicidal, IIGGR was the most potent and any substitution appeared to decrease potency to some extent.

Initially, we observed that the N-terminal pentapeptide of leukocyte elastase, IVGGR, showed very little antibiotic activity. This indicated that the arginine at position 5 was not sufficient to confer antimicrobial activity. The substitution of valine for isoleucine at position 2 in IVGGR represents a single methylene addition in branched side-chain structure and is a relatively neutral change with respect to charge, hydrophobicity, and molecular mass. Yet, IVGGR was far less potent (inactive) than IIGGR and IIGGA in its ability to kill the bacteria in our test panel. Because of this, we investigated the impact of substitutions at position 2, with particular emphasis on the effect of branching and charge. Interestingly, ILGGR, InLGGR, and IQGGR were all active at 500 μg/nl. However, at lower concentrations (200 μg/ml), minor differences among the compounds were detected and the microbicidal potencies of the congeners could be ranked as follows: IIGGR > InLGGR > ILGGR > IQGGR > IVGGR. This series of peptides illustrates that not only was the amino-terminal pentapeptide of cathepsin G optimized for antimicrobial activity but also the amino-terminal pentapeptide of elastase and azurocidin (IVGGR) was optimized for nontoxicity.

Surprisingly, IIGGR-amide was also inactive. Inasmuch as IIGGR-amide has a net charge of 2+ and IIGGR has a net charge of 1+, it seems most likely that the main effect of amidation was altering the charge distribution rather than the net charge. That is, the carboxy terminus may be neutral (IIGGR) or negative (IIGGA) but not positive (IIGGR-amide). The inactivity of IIGGR-amide raises another question: that is, can IIGGR function when it is an amino-terminal extension of a larger peptide? In other studies, we have observed that a 20-amino-acid synthetic peptide containing IIGGR and representing the amino terminus of cathepsin G was bactericidal against the oral bacteria described herein (13) and *Pseudomonas aeruginosa* (20).

Chirality does not appear to be important to the antibiotic effects of IIGGR. Both the D- and L-enantiomers of IIGGR were effective antimicrobial agents against our test panel. These observations echo previous findings that the D-enantiomers of natural, intact peptide antibiotics from frogs and insects, including cecropin A, magainin 2, and melittin, were antibiotics as potent as the L-enantiomer (22). The present observations strongly suggest that the peptides do not exert antimicrobial effects by specific interaction with receptors or enzymes and support a hypothesis that part of the mechanism of antimicrobial action involves disruption of the target cell membrane (22).

HPQYNQR exerted intense microbicidal activity against *A. actinomycetemcomitans* but was fairly ineffective against *Capnocytophaga* spp. Previously, we observed that a congener, APQYNQR, has little bactericidal activity against *N. gonorrhoeae* or *S. aureus* (18). This study shows that APQYNQR is toxic to *A. actinomycetemcomitans* and *C. sputigena* ATCC 33123 but less toxic than HPQYNQR.

HPQYNQR was relatively less active than IIGGR and IIGGA, on both a weight-per-volume basis and a molar basis when tested against *Capnocytophaga* spp. and *E. corrodens*. However, on a weight-per-volume basis, HPQYNQR was as potent as IIGGR against *A. actinomycetemcomitans*. On a molar basis, HPQYNQR was about twice as potent as IIGGR. These results indicate that cathepsin G has two (or more) domains capable of killing these periodontal bacteria, as we previously hypothesized on the basis of observations that strains of *A. actinomycetemcomitans* are killed by cathepsin G in an enzyme-independent manner and *Capnocytophaga* spp. are killed by cathepsin G in an enzyme-dependent manner (11). Similarly, others have observed that nonoral bacteria are killed by cathepsin G by both enzyme-dependent and enzyme-independent pathways (23). It is probable that HPQYNQR is exposed and IIGGR is buried in the intact cathepsin G molecule (18). We suspect that enzyme dependency reflects either (i) a need for the enzyme active site to bind a critical bacterial target (23) or (ii) a requirement for proteolysis of the cathepsin G molecule by a host and/or microbial protease(s) to expose highly toxic buried domains such as IIGGR. Sensitivity to the exposed domain, HPQYNQR, may predict that an organism can be killed by inactivated cathepsin G. Although it is possible that the antibiotic activity of cathepsin G is unrelated to the antibiotic effects of these and other recently described domains, most current information would strongly support the concept that microbicidal activities derived from the animal kingdom result from membrane-disruptive peptides (2).

In conclusion, synthetic peptide antibiotics derived from sequences present in human neutrophil cathepsin G can kill *A. actinomycetemcomitans* and *Capnocytophaga* spp. We found that *A. actinomycetemcomitans* strains were sensitive to both IIGGR and HPQYNQR, whereas *Capnocytophaga* spp. were sensitive to IIGGR and relatively resistant to HPQYNQR. These results indicate that these two human peptide antibiotics kill periodontal bacteria differentially but the differential killing was observed over a range of concentrations which was too narrow to be selective if used therapeutically. From a therapeutic standpoint, DIDIGGDR may be useful as a protease-resistant antibiotic (22). From a pharmacologic perspective, it would appear that neither charge, hydrophobicity, nor chirality is an useful predictor

of the antibiotic activities of these peptides. Charge distribution appears to play an important role in the microbicidal activity of IIGGR, since IQGGR and IIGGR-amide both exhibited relatively low microbicidal effects. The therapeutic significance and the full range of microbicidal and cytocidal activities (both cytotoxicity and tumoricidal activities) of these peptide antibiotics remain to be determined. The results presented herein suggest that they may be valuable therapeutic agents in the treatment of periodontal infections. They also provide a hint that the biochemical explanation for the different mechanisms of killing bacteria resides in the differential sensitivities of bacteria to antibiotic peptide domains.

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