ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1997, p. 2394–2398 Vol. 41, No. 11
0066-4804/97/$04.00+0

Novel Antimicrobial Peptides Derived from Human Immunodeficiency Virus Type 1 and Other Lentivirus Transmembrane Proteins

SARAH BURROUGHS TENCZA, JOHN P. DOUGLASS, DONALD J. CREIGHTON, JR., RONALD C. MONTELARO,* AND TIMOTHY A. MIETZNER

Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Received 4 April 1997/Returned for modification 16 June 1997/Accepted 14 August 1997

We have previously described a conserved set of peptides derived from lentiviral envelope transmembrane proteins that are similar to the natural antimicrobial peptides cecropins and magainins in overall structure but bear no sequence homology to them or other members of their class. We describe here an evaluation of the antimicrobial properties of these virally derived peptides, designated lentivirus lytic peptides (LLPs). The results of this study demonstrate that they are potent and selective antibacterial peptides: the prototype sequence, LLP1, is bactericidal to both gram-positive and gram-negative organisms at micromolar concentrations in 10 mM phosphate buffer. Furthermore, LLP1 kills bacteria quite rapidly, causing a 1,000-fold reduction in viable organisms within 50 s. Peptides corresponding to sequences from three lentivirus envelope proteins were synthesized and characterized. Several of these peptides are selective, killing bacteria at concentrations 50- to 100-fold lower than those required to lyse erythrocytes. Development of antimicrobial agents based on these peptides may lead to improved therapeutics for the management of a variety of infectious diseases.

The emergence of multiple-drug-resistant bacterial strains requires a constant development of new strategies for the management of microbial infectious disease. The last decade has seen the discovery of a new class of antibiotic compound, the antimicrobial peptide. The cecropins were the first such peptides identified; they are found in insect hemolymph and function as nonspecific microbicides (33, 36). Since then, many peptides with antimicrobial properties have been identified (reviewed in references 3 and 15). These include magainins on frog epithelia (38), cecropins in pigs (19), and defensins in many mammals (8, 31). These molecules, collectively termed host defense peptides, are thought to provide a rapid response to protect the host from potential microbial threats. Many of these peptides are being studied with the goal of developing new antimicrobial therapies.

Antimicrobial peptides possess various structural characteristics (3, 26), including amphipathic alpha helices and beta sheets; in addition, they are usually cationic. Amphipathic alpha helices typically possess a high hydrophobic moment and are often classified as membrane-associated or surface-seeking (9) sequences. These motifs are found in the context of whole proteins, where they are associated with both membrane- and calmodulin-binding activities (7, 17). Molecular modeling studies of protein sequences (10) identified two such positively charged, highly amphipathic amino acid segments in the cytoplasmic tail of the transmembrane (TM) envelope protein of human immunodeficiency virus type 1 (HIV-1). These peptide sequences (amino acids 828 to 856 and 768 to 788 of HIV-1 strain HXB2R Env) resemble magainins and cecropins in overall secondary structural properties, namely, high positive charge, amphipathicity, and high hydrophobic moment. However, they possess no obvious amino acid sequence homology to the known antimicrobial peptides. The identification of these classic membrane-binding motifs in the TM protein (10) led us to hypothesize that synthetic lentivirus lytic peptides (LLPs) based on these sequences (LLP1 and LLP2, respectively) would have functional properties similar to those of known antimicrobial and cytolytic peptides (22). Previous studies in this laboratory confirmed that the LLP1 synthetic peptide is cytolytic (21, 22) and binds calmodulin (23, 34). Studies with similar LLPs also show interactions with synthetic membranes (5, 12, 13, 32). Although it was originally reported that LLP1 lysed prokaryotic and eukaryotic cells with equal potency (22), more recent data on its hemolytic activity (32, 35) suggested that it may be more selective than previously thought and therefore may be useful as an antimicrobial agent. We have since undertaken an evaluation of the potency and selectivity of LLP1 and related virus-derived peptides by assessing their ability to kill bacteria, lyse erythrocytes (RBC), and kill eukaryotic cells in culture. The data reported here demonstrate that these peptides are selective, broad-spectrum antimicrobial agents which have potential as new weapons for the cure or management of diseases caused by a wide variety of microbial pathogens.

MATERIALS AND METHODS

Structural analysis of peptide sequences. Viral envelope protein sequences of HIV-1, simian immunodeficiency virus (SIV), and equine infectious anemia virus (EIAV) were analyzed for predicted secondary structure (6), hydrophobic moment (9), and amphipathicity by using the Genetics Computer Group (Madison, Wis.) package of programs accessed through the Pittsburgh Super Computing Center. Peptides based on protein sequences which had the potential to form basic amphipathic alpha helices were chosen for synthesis.

Peptide synthesis. Peptides were synthesized as C-terminal amides as described previously (11, 23), using either an Advanced Chemtech 200 (Advanced Chemtech, Louisville, Ky.) or a Millipore 9050+ (Millipore, Bedford, Mass.) automated peptide synthesizer with standard 9-fluorenylmethoxycarbonyl synthesis protocols. After cleavage and deprotection, synthetic peptides were characterized and purified by reverse-phase high-pressure liquid chromatography (HPLC) on Vydac C18 or C4 columns (The Separations Group, Hesperia, Calif.). The identity of each peptide was confirmed by mass spectrometry. Magainin 2 was purchased from Calbiochem (La Jolla, Calif.) and used without further purification. The sequences of the peptides used in this study are listed in Table 1. Viral envelope source designations employ the numbering schemes of Myers et al. (25) for HIV-1 and SIV and Rushlow et al. (28) for EIAV.

Peptide quantitation. Peptides were dissolved in Milli-Q water or 0.01% acetic acid at 3 to 10 mg/ml, and the molar concentrations were determined by quan-

* Corresponding author. Mailing address: Department of Molecular Genetics and Biochemistry, W1144 Biomedical Science Tower, Pittsburgh, PA 15261. Phone: (412) 648-8869. Fax: (412) 383-8859.

TABLE 1. Sequences of the synthetic peptides used in this study

Peptide name	Sequence	Viral envelope source[a]
LLP1	RVIEVVQGACRAIRHIPRRIR QGLERIL	HIV-1 HXB2R 828–855
SLP1	DLWETLRRGGRWILAIPRRI RQGLELTL	SIV MM239 852–879
ELP	RIAGYGLRGLAVIIRICIRGL NLIFEIIR	EIAV 808–836
LLP2	YHRLRDLLLIVTRIVELLGRR	HIV-1 HXB2R 768–788
SLP2A	FLIRQLIRLLTWLFSNCRTLL SRVY	SIV MM239 771–795
SLP2B	LLSRVYQILQPILQRLSATLQ RIREVLR	SIV MM239 790–817
Magainin 2	GIGKFLHSAKKFGKAFVGE IMNS	Reference 38

[a] Viral isolate designations and sequences of the family of LLPs derived from the HIV-1 and SIV Env proteins are consistent with the nomenclature of Myers et al. (25). The ELP peptide sequence is taken from EIAV (28).

titative ninhydrin assay. Ninhydrin reagents A, B, and C (190 μl per sample; purchased from Dupont or made as described by Sarin et al. [29]) were added to borosilicate tubes (10 by 75 mm; VWR Scientific, Pittsburgh, Pa.) containing approximately 20 to 60 nmol of peptide (or free amino groups) in 60 μl; standards consisting of 0 to 60 nmol of leucine in the same volume were prepared in parallel and used to create a standard curve. The purple product formed upon incubation of the tubes at 100°C for 10 min was quantitated by dilution in 1:1 isopropanol-water (1.0 ml/tube), transfer to triplicate wells of a 96-well flat-bottom plate (Sarstedt, Newton, N.C.), and measurement of the A_{570} using a microwell plate reader (Dynatech, Chantilly, Va.). The results were confirmed by routine amino acid composition analysis of both the leucine standard and peptide solutions.

Bacterial test strains. The panel of bacterial isolates used for this study consisted of both gram-positive and gram-negative strains, as well as two antibiotic-resistant clinical isolates. The strains of *Pseudomonas aeruginosa* and methicillin-susceptible *Staphylococcus aureus* used were laboratory-passaged clinical isolates, whereas the methicillin-resistant *S. aureus*, vancomycin-resistant and -susceptible *Enterococcus faecalis*, and *Serratia marcescens* cultures were all recently obtained patient isolates.

Bacterial killing assay. Bacterial lysis assays were conducted in a manner similar to that described previously (20, 22). Bacterial suspensions were cultured in Luria broth to mid-log phase and washed by several cycles of centrifugation and resuspension in an equal volume of 10 mM potassium phosphate buffer (PB), pH 7.2. The A_{600} of the suspension was adjusted with PB such that, upon dilution, 0.5×10^6 to 1×10^6 CFU/ml would be treated in the assay (27). The bacteria were incubated with twofold dilutions of peptides (32 to 0.16 μM) in 96-well plates in PB for 1 h at 37°C. Serial 10-fold dilutions of both control and test wells were performed to a dilution of 1:1,000; a 100-μl aliquot from each dilution was plated on tryptic soy agar (Difco, Detroit, Mich.), and the plates were incubated overnight at 37°C. Colonies of surviving bacteria were counted and compared to non-peptide-treated controls to determine the amount of peptide-induced killing under each condition. Log killing is defined as the log of the ratio of the numbers of CFU present with and without peptide treatment. If zero colonies per 100-μl aliquot of an undiluted bacterium-peptide mixture remain, complete killing is noted. From the above experiment, both the MBC and the concentration required to kill 50% of the bacterial culture (MBC_{50}) were calculated. MBC, the concentration of peptide required to cause 99.9% or 3-log killing was determined by comparing colony counts of the controls (1:1,000 dilution) to those on the undiluted test plates and is the concentration at which the number of colonies on the undiluted test plate was less than the number on the 1:1,000 dilution control plate. The MBC_{50} was determined in the same trial as the MBC by comparing colony counts in the 1:1,000 dilutions of both the control and treated plates and is the lowest peptide concentration that resulted in fewer than half of the number of colonies of the controls. The colony counts on the serial 10-fold dilutions indicated that antibiotic carryover was not occurring; additionally, identical results were obtained whether the plate inoculum was 10 or 100 μl, so the larger volume was used. Each assay was performed at least three times, and the range of concentrations is reported.

Kinetics of bacterial killing. The time course of bactericidal activity was determined by a modification of the above procedure in which bacteria (10^6 CFU/ml) were treated with 4 μM peptide in PB. Aliquots of this mixture were removed every 10 s and immediately diluted 1:10 into Luria broth, which substantially inhibits the killing process; 100 μl of each resulting suspension was then plated on agar and incubated overnight. The colonies were counted the next day, and the number of colonies was plotted as a function of peptide exposure time.

Erythrocyte lysis assay. Peptides were screened for hemolytic activity as previously described (35), by treating a 1% suspension of freshly isolated human RBC with various concentrations of peptides, centrifuging the samples to pellet intact cells, and measuring the amount of hemoglobin in the supernatant to determine the extent of RBC lysis under each condition. The method used is similar to that described by Srinivas et al. (32), with the following modifications: peptide concentrations of 0.1 to 100 μM were tested, the incubation period was 1 h at 37°C, and the A_{570} was measured in duplicate wells of a microwell plate as described above. A calibration curve was constructed by treating various amounts of cells (0, 20, 40, 60, 80, and 100% of the volume of RBC used in test samples) with Milli-Q water, which completely lysed the cells. The standard curve was used to calculate the percent lysis at each peptide concentration. The concentration of peptide resulting in 50% hemoglobin release (50% lytic dose or LD_{50}) was measured graphically, and the average of two to four independent trials was determined.

RESULTS

To determine its relative antimicrobial activity, LLP1 was compared on a molar basis with magainin 2 in a bacterial killing assay (20). Various concentrations of each peptide were incubated with the test bacteria in 10 mM PB for 1 h; this treatment resulted in a dose-dependent reduction of the num-

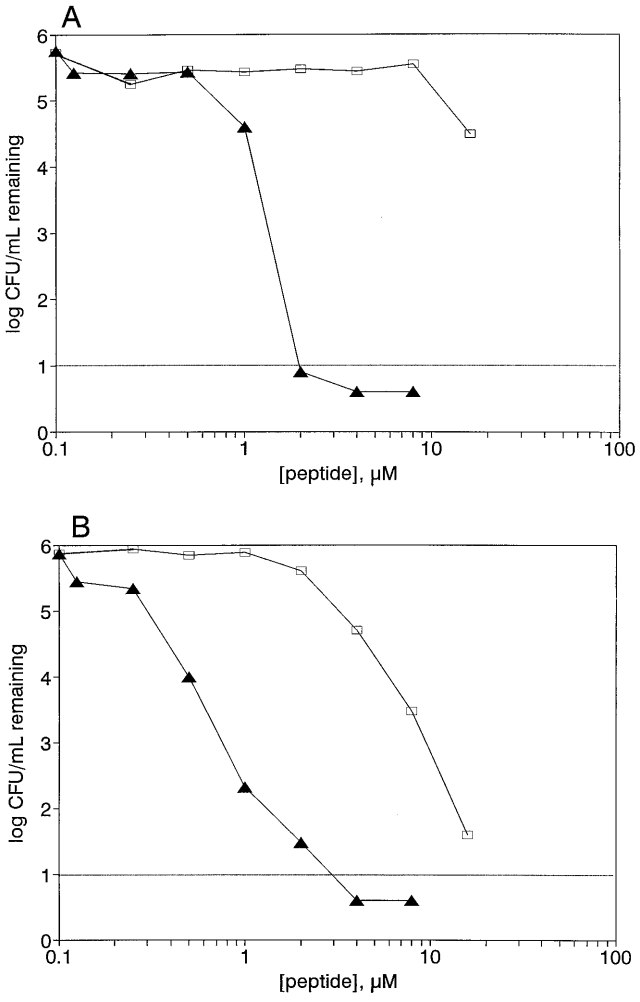

FIG. 1. Dose-dependent killing of bacteria by LLP1. Bacterial cultures (0.5×10^6 to 1×10^6 CFU/ml) were treated with twofold dilutions of the peptide in PB; the log of the number of CFU per milliliter remaining upon treatment is plotted as a function of the peptide concentration. The antibiotic activity of magainin 2 is shown for comparison. Points below the dotted line represent less than one colony in 100 μl (<10 CFU/ml). A, *S. aureus* versus LLP1 (▲) and magainin 2 (□); B, *P. aeruginosa* versus LLP1 (▲) and magainin 2 (□).

TABLE 2. MBCs of LLP1 and magainin 2 against various bacterial isolates

Bacterial strain	Phenotype	MBC (μM)		MBC (μg/ml)	
		LLP1	Magainin 2	LLP1	Magainin 2
S. aureus	Gram positive	2–4	>16	6.6–13	>40
S. aureus	Methicillin resistant	2	>16	6.6	>40
E. faecalis	Gram positive	2.5–5	>10	8.3–17	>25
E. faecalis	Vancomycin resistant	1.3–2.5	>10	4.1–8.3	>25
P. aeruginosa	Gram negative	1–2	8–16	3.3–6.6	20–40
S. marcescens	Gram negative	0.5–2	>16	1.7–6.6	>40

ber of bacterial CFU per milliliter (Fig. 1). LLP1 was effective against both gram-positive and gram-negative bacteria. At and above 2 μM LLP1, complete killing of *S. aureus* was observed (Fig. 1A); that is, there was less than 1 CFU remaining in 100 μl of the bacterium-peptide mixture, which corresponds to a reduction of at least 10^5 CFU/ml (99.999%) by this peptide concentration. Magainin 2, on the other hand, resulted in a reduction of less than 99% of *S. aureus* CFU per milliliter at 16 μM. At a peptide concentration of 2 μM, LLP1 reduced the viable *S. aureus* by at least 4 orders of magnitude more than did magainin 2. Similarly, complete killing of *P. aeruginosa* was achieved with 4 to 8 μM LLP1 in PB (Fig. 1B), whereas magainin 2 killed about 99% of these organisms at these concentrations. These data suggest that LLP1 is more potent than magainin 2 against both gram-positive and gram-negative bacteria under the present conditions.

To quantitate antimicrobial activity, the MBCs of LLP1 and magainin 2 against each strain were determined in PB. The potency and spectrum of antimicrobial activity of LLP1 compared very favorably to those of magainin 2 (see Table 2). LLP1 was bactericidal at low concentrations to the entire panel of test bacteria, demonstrating MBCs of 0.5 to 4 μM against both gram-positive and gram-negative organisms. Magainin 2, however, was bactericidal to only one species (*P. aeruginosa*) at the concentrations tested. LLP1 was particularly effective against *S. marcescens*, as evidenced by its low MBC (0.5 to 2 μM). The consistently low MBCs among the panel indicate that LLP1 has broad-spectrum antibacterial activity.

Pairs of clinical isolates of *S. aureus* and *E. faecalis* that are resistant or susceptible to methicillin and vancomycin, respectively, were also tested to determine their susceptibility to LLP1. The resistant pathogens had equal or greater sensitivity to LLP1 exposure than the corresponding susceptible strains (Table 2). Similar MBCs were obtained for the two strains of *S. aureus* tested; however, LLP1 was slightly more potent against a vancomycin-resistant isolate of *E. faecalis* than the vancomycin-susceptible isolate of this microbe. In addition, LLP1 exhibited low MBCs against several *S. marcescens* clinical isolates having various antibiotic resistance patterns (data not shown). These findings indicate that the antibiotic resistance mechanisms employed by these organisms do not greatly alter their susceptibility to LLP1.

In the previous assays, bacteria were exposed to peptide for 1 h; to determine the time course of bacterial killing, a kinetic assay was performed with LLP1 against both *S. aureus* and *S. marcescens*. As can be seen in Fig. 2, a 100-fold reduction in the number of CFU per milliliter was observed within 20 to 30 s of exposure of either type of bacteria to 4 μM LLP1. Killing of *S. aureus* was slightly more rapid; approximately 10-fold fewer viable bacteria remained after 10 s compared to *S. marcescens*. Reduction of bacteria to undetectable levels (less than 100 CFU/ml) was achieved within a few minutes for both cultures. These largely similar results indicate that the peptide's antibacterial activity is little affected by the membrane characteristics that distinguish gram-positive and gram-negative bacteria.

Examination of TM protein sequences among several lentiviruses has identified a family of LLP regions (22). Like HIV-1, SIV and EIAV contain positively charged amphipathic helical segments, designated SLP1 and ELP, respectively, at the C termini of their TM proteins. Similar regions were not found in other lentiviruses. Eisenberg and Wesson (10) described a second LLP region with similar structural properties in the HIV-1 TM protein. By using secondary-structure predictions for amphipathicity and hydrophobic moment, we identified similar sequences existing in the SIV (but not the EIAV) TM protein. These regions of SIV and HIV-1, collectively termed LLP2s, have much less amino acid variation than seen in LLP1 sequences (25); the LLP2 regions are also generally more hydrophobic than their LLP1 counterparts. The SIV TM protein is characterized by an unusually long LLP2 region (amino acids 771 to 817 of SIV strain MM239); overlapping 25-amino-acid peptides, SLP2A and SLP2B, corresponding to portions of this region were synthesized. Table 1 describes the LLP1 and LLP2 synthetic peptides of the study panel; each is based on a natural sequence from a lentivirus TM protein. The striking feature of these sequences is their apparent lack of homology to known host defense peptides (e.g., cecropins and magainins); however, like these and other antimicrobial peptides, each of the LLPs is rich in basic residues and is predicted to form an amphipathic helix. The helical structure has been confirmed by circular dichroism for SLP1 (37), LLP1, and LLP2 (32, 37a). Therefore, although the primary structures of the host defense and LLP types of peptides vary greatly, the secondary structures are largely conserved; as shown below, this structure imparts interesting membrane-interactive properties to the LLPs.

The peptides derived from the LLP1 and LLP2 regions of

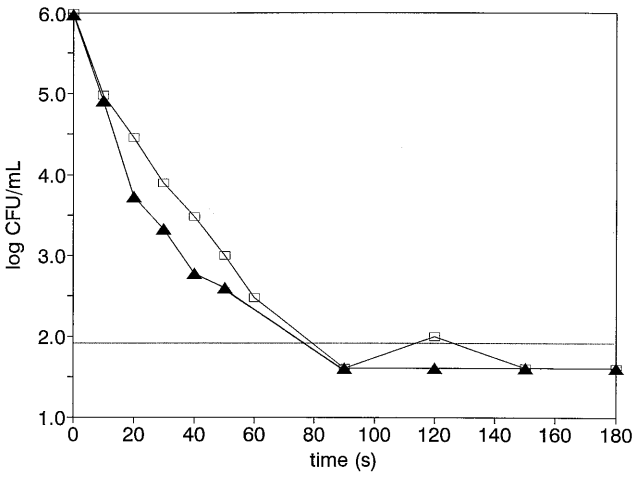

FIG. 2. Kinetics of bactericidal activity of LLP1. LLP1 (4 μM) was added to 10^6 CFU of bacteria per ml of PB; aliquots were taken every 10 s and immediately diluted 1:10 in Luria broth. This plot of the log of the number of CFU per milliliter remaining as a function of treatment time indicates that both gram-positive and gram-negative organisms are rapidly killed by LLP1. Points below the dotted line represent less than one colony in 10 μl (<100 CFU/ml). Symbols: ▲, *S. aureus* versus LLP1; □, *S. marcescens* versus LLP1.

TABLE 3. Antibacterial and hemolytic activities of LLPs

Peptide name	MBC (μM)[a]	MBC_{50} (μM)[b]	RBC LD_{50} (μM)[c]
LLP1	2–4	0.25–2	40
LLP2	4	1	7
SLP1	>16	4–16	40
SLP2A	>16	2–4	2
SLP2B	0.5–1	0.25–0.5	8
ELP	>10	>10	12

[a] Peptide concentration which is bactericidal to *S. aureus* in PB.
[b] Concentration of peptide in PB required to reduce the number of CFU of *S. aureus* per milliliter by 50%.
[c] Peptide concentration (as measured on a plot of A_{560} versus peptide concentration) required to cause 50% lysis of treated RBC.

both HIV and SIV were active in our antibacterial activity assay, as demonstrated by their MBCs against *S. aureus* (Table 3). Each of the peptides was able to kill at least 50% of treated bacteria at concentrations of 0.25 to 16 μM, and half of them were bactericidal under the conditions tested. The MBC of SLP2B was the lowest, making it the most potent antimicrobial of the group. Thus, most of the peptides displayed some antibacterial activity despite significant sequence variations. In contrast to the generally good antibacterial activity of the HIV-1- and SIV-based peptides, ELP, the peptide derived from EIAV, was inactive against *S. aureus* at concentrations as high as 10 μM.

Selectivity, or the killing of bacteria at concentrations not harmful to normal eukaryotic cells, is a highly desirable characteristic of an antimicrobial agent. To determine selectivity, each peptide was evaluated for its toxicity to RBC (LD_{50}) and other eukaryotic cells relative to its antimicrobial activity (MBC_{50}). LLP1 and LLP2 have been previously tested for activity in an erythrocyte lysis assay (32, 35). LLP1 was hemolytic only at high concentrations, with an LD_{50} about 2 orders of magnitude higher than the MBC_{50} for *S. aureus*. Below 10 μM, very little RBC lysis is observed (35), suggesting that this peptide is a selective antibacterial agent. Examination of the hemolytic properties of the present panel of LLPs (Table 3) revealed some variation in potency and selectivity among them. The LLP2 and SLP2 peptides were, on average, 10-fold more hemolytic than either of the LLP1-type peptides, LLP1 and SLP1. LLP1 and SLP1 are only 50% homologous yet possess similar hemolytic activities; likewise, SLP2A, SLP2B, and LLP2 have different primary structures but a small range of LD_{50}s. However, these two groups are alike in general amino acid composition, the latter being more hydrophobic than arginine-rich LLP1 and SLP1. Selectivity was high for LLP1 (about 100-fold) and good for SLP2B (30-fold) but low for SLP2A (equal activities against *S. aureus* and RBC). At the other end of the membrane activity spectrum was ELP, which was hemolytic but displayed no antibacterial activity at the concentrations used. Thus, both bactericidal and hemolytic properties varied among the panel, with LLP1 and SLP2B being the more selective peptides tested.

The toxicity of the peptides to eukaryotic cells in culture was also examined. Viability of Jurkat human T cells after a 48-h exposure to peptide was measured by trypan blue vital staining (data not shown). These assays indicated that long-term culture in the presence of up to 2 μM peptide reduced live cell counts by less than 10%. These results and others (21, 32) demonstrate that most LLPs kill bacteria at much lower concentrations than those required to damage eukaryotic cells.

DISCUSSION

The problem of bacterial resistance to antimicrobial chemotherapy is nearly as old as the drugs themselves. The combination of increased use of and decreased research into new antibiotics (18) has resulted in a surge in the frequency of bacterial infections caused by organisms resistant to virtually all of the drugs currently available. Because of this, there must be new and better efforts to discover and develop novel therapies for the management of infectious disease.

LLP1 is bactericidal at micromolar concentrations to many medically important bacteria. The emergence of methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecalis* has greatly complicated the management of patients recovering from surgical procedures; like many cationic peptides, LLP1 is highly effective against these antibiotic-resistant microbial pathogens (Table 2). Moreover, LLP1 readily kills *S. marcescens*, an important organism of ophthalmologic concern which is generally resistant to cationic peptides (15). The speed with which LLP1 eliminates both gram-positive and gram-negative viable bacteria (Fig. 2) further demonstrates the truly broad spectrum of activity of this peptide.

The LLPs are members of a class of antimicrobial peptides that, like cecropins and other cationic amphipathic helices, function through membrane perturbation (21) and exhibit selectivity for microbial pathogens. However, the LLPs are unusual in that they are part of a naturally occurring sequence that exists in nature as a larger folded protein; there are a just a few other examples of this (3). Furthermore, the sequences of these peptides are rather different than those of other antimicrobial peptides; they are composed of a high percentage of arginine residues and are devoid of lysine residues. Other known arginine-rich peptides are either also proline rich (e.g., PR-39 [1] and apidaecins [4]) or folded into structures comprising beta sheets and intramolecular disulfide bonds, as found in defensins (16). The structure of the LLPs is different from all of these: they are arginine rich yet can form alpha helices, as shown by circular dichroism (32, 37).

The LLPs are also unique in their functional properties, exhibiting high antimicrobial and low hemolytic activities. Helical cationic peptides can be classified into two major functional types. Antibacterial peptides (magainins and cecropins, for example) specifically kill bacteria and play a part in host defense. Hemolytic peptides, on the other hand, are equally active against bacteria and RBC; melittin from bee venom is an example of such a peptide. As a group, the LLPs have functional properties resembling both antimicrobial and cytolytic peptides. For example, they do lyse RBC but, in general, only at relatively high concentrations (Table 3); in contrast, melittin is highly active against RBC and shows little selectivity (14). The unique structure of the LLPs seems to impart antibacterial activity to these peptides while, in most cases, maintaining selectivity. ELP, the outlier of the group, is hemolytic but not antibacterial; this lack of activity may be due to its predicted secondary structure, which is not as strongly helical as the others.

Antimicrobial agents act by one of several mechanisms, including inhibition of cell wall, protein, or DNA synthesis or permeabilization of the cell membrane. Cationic antimicrobial peptides are thought to target the latter and act by formation of channels or pores in the membrane. The mechanism by which they kill bacteria is unknown but is generally believed to involve loss of membrane potential upon pore formation and lysis. LLP1 has been shown to form pores which have a discrete size but little to no charge selectivity (5, 10, 21); studies are

under way to examine in more detail the LLPs' interactions with membranes.

The LLPs, derived from a variety of natural lentiviral envelope sequences, display a spectrum of potency and selectivity (Table 3). The experiments reported here show that the peptides are generally active at micromolar concentrations in 10 mM phosphate buffer; under these conditions, they may be applicable as topical antimicrobials. Engineered alterations in amino acid composition, or even the sequence of residues, have been shown to have profound effects on the peptides' membrane-perturbative properties (21, 35). These observations suggest that, like other antimicrobial peptides, LLPs may be engineered to improve their properties and better understand the contribution of each residue to the activity of the peptide. The structure-function relationships so elucidated may be exploited to develop more effective and selective antimicrobial peptides that function under a variety of buffer conditions. In addition to their bactericidal properties, several antimicrobial peptides have been shown to be effective against fungal (30) and tumor (2, 24) cells. To maximize their potency and utility for a variety of potential applications, efforts are under way to engineer the peptides for optimal length, sequence, and secondary-structural properties. Development of the LLPs as antimicrobial agents may provide the medical community with a new generation of chemotherapeutics to combat many diseases.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (AI36198 to R.C.M.) and from the Pittsburgh Supercomputing Center through the NIH National Center for Research Resources (2 P41 RR06009).

We thank Kazi Islam, Shaoqing Chen, and Harry Solimeo for technical assistance and W. Pasculle, R. Wadowsky, and S. Kominos for providing clinical isolates and their antibiotic susceptibility profiles. Mass spectrometry and amino acid composition analyses were performed by the Department of Molecular Genetics and Biochemistry Research Support Facilities (University of Pittsburgh).

REFERENCES

1. **Agerberth, B., J. Y. Lee, T. Bergman, M. Carlquist, H. G. Boman, V. Mutt, and H. Jornvall.** 1991. Amino acid sequence of PR-39. Isolation from pig intestine of a new member of the family of proline-arginine-rich antibacterial peptides. Eur. J. Biochem. **202:**849–854.
2. **Baker, M. A., W. L. Maloy, M. Zasloff, and L. S. Jacob.** 1993. Anticancer efficacy of magainin 2 and analogue peptides. Cancer Res. **53:**3052–3057.
3. **Boman, H. G.** 1995. Peptide antibiotics and their role in innate immunity. Annu. Rev. Immunol. **13:**61–92.
4. **Casteels, P., C. Ampe, F. Jacobs, M. Vaeck, and P. Tempst.** 1989. Apidaecins: antibacterial peptides from honeybees. EMBO J. **8:**2387–2391.
5. **Chernomordik, L., A. N. Chanturiya, E. Suss-Toby, E. Nora, and J. Zimmerberg.** 1994. An amphipathic peptide from the C-terminal region of the HIV envelope glycoprotein causes pore formation in membranes. J. Virol. **66:**7115–7123.
6. **Chou, Y. P., and G. D. Fasman.** 1974. Prediction of protein conformation. Biochemistry **13:**222–245.
7. **Cox, J. A., M. Comte, J. E. Fitton, and W. F. DeGrado.** 1985. The interaction of calmodulin with amphiphilic peptides. J. Biol. Chem. **260:**2527–2534.
8. **Diamond, G., M. Zasloff, H. Eck, M. Brasseur, W. L. Maloy, and C. L. Bevins.** 1991. Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: peptide isolation and cloning of a cDNA. Proc. Natl. Acad. Sci. USA **88:**3952–3956.
9. **Eisenberg, D., E. Schwarz, M. Komaromy, and R. Wall.** 1984. Analysis of membrane and surface protein sequences with the hydrophobic moment plot. J. Mol. Biol. **179:**125–142.
10. **Eisenberg, D., and M. Wesson.** 1990. The most highly amphiphilic alpha-helices include two amino acid segments in human immunodeficiency virus glycoprotein 41. Biopolymers **29:**171–177.
11. **Fontenot, J. D., J. M. Ball, M. A. Miller, C. M. David, and R. C. Montelaro.** 1991. A survey of potential problems and quality control in peptide synthesis by the fluorenylmethoxycarbonyl procedure. Peptide Res. **4:**19–25.
12. **Fujii, G., S. Horvath, S. Woodward, F. Eiserling, and D. Eisenberg.** 1992. A molecular model for membrane fusion based on solution studies of an amphiphilic peptide from HIV gp41. Protein Sci. **1:**1454–1464.
13. **Gawrisch, K., K. H. Han, J. S. Yang, L. D. Bergelson, and J. A. Ferretti.** 1993. Interaction of peptide fragment 828–848 of the envelope glycoprotein of human immunodeficiency virus type I with lipid bilayers. Biochemistry **32:**3112–3118.
14. **Habermann, E.** 1972. Bee and wasp venoms. Science **177:**314–322.
15. **Hancock, R. E. W.** 1997. Peptide antibiotics. Lancet **349:**418–422.
16. **Hill, C. P., J. Yee, M. E. Selsted, and D. Eisenberg.** 1991. Crystal structure of defensin HNP-3, an amphiphilic dimer—mechanisms of membrane permeabilization. Science **251:**1481–1485.
17. **Kaiser, E. T., F. J. Kezdy.** 1987. Peptides with affinity for membranes. Annu. Rev. Biophys. Chem. **16:**561–581.
18. **Kelley, K. J.** 1996. Using host defenses to fight infectious diseases. Nat. Biotechnol. **14:**587–590.
19. **Lee, J.-Y., A. Boman, S. Chuanxin, M. Andersson, H. Jornvall, V. Mutt, and H. G. Boman.** 1989. Antibacterial peptides from pig intestine: isolation of a mammalian cecropin. Proc. Natl. Acad. Sci. USA **86:**9159–9162.
20. **Lehrer, R. I., M. E. Selsted, D. Szklarek, and J. Fleischmann.** 1983. Antibacterial activity of microbicidal cationic proteins 1 and 2, natural peptide antibiotics of rabbit lung macrophages. Infect. Immun. **42:**10–14.
21. **Miller, M. A., M. W. Cloyd, J. Liebmann, C. R. Rinaldo, Jr., K. R. Islam, S. Z. Wang, T. A. Mietzner, and R. C. Montelaro.** 1993. Alterations in cell membrane permeability by the lentivirus lytic peptide (LLP-1) of HIV-1 transmembrane protein. Virology **196:**89–100.
22. **Miller, M. A., R. F. Garry, J. M. Jaynes, and R. C. Montelaro.** 1991. A structural correlation between lentivirus transmembrane proteins and natural cytolytic peptides. AIDS Res. Hum. Retroviruses **7:**511–519.
23. **Miller, M. A., T. A. Mietzner, M. W. Cloyd, W. G. Robey, and R. C. Montelaro.** 1993. Identification of a calmodulin-binding and inhibitory peptide domain in the HIV-1 transmembrane glycoprotein. AIDS Res. Hum. Retroviruses **9:**1057–1066.
24. **Moore, A. J., D. A. Devine, and M. C. Bibby.** 1994. Preliminary experimental anticancer activity of cecropins. Peptide Res. **7:**165–269.
25. **Myers, G., B. Korber, B. H. Hahn, K.-T. Jeang, J. W. Mellors, F. E. McCutchan, L. E. Henderson, and G. N. Pavlakis (ed.).** 1995. Human retroviruses and AIDS 1995. Los Alamos National Laboratory, Los Alamos, N.Mex.
26. **Nicolas, P., and A. Mor.** 1995. Peptides as weapons against microorganisms in the chemical defense system of vertebrates. Annu. Rev. Microbiol. **49:**277–304.
27. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentration. Antimicrob. Agents Chemother. **18:**699–708.
28. **Rushlow, K., K. Olsen, G. Steigler, S. L. Payne, R. C. Montelaro, and C. J. Issel.** 1986. Lentivirus genomic organization: the complete nucleotide sequence of the env gene region of equine infectious anemia virus. Virology **155:**309–321.
29. **Sarin, V. K., S. B. H. Kent, J. P. Tam, and R. B. Merrifield.** 1981. Quantitative monitoring of solid-phase peptide synthesis by the ninhydrin reaction. Anal. Biochem. **117:**147–157.
30. **Schonwetter, B. S., E. D. Stolzenberg, and M. A. Zasloff.** 1995. Epithelial antibiotics induced at sites of inflammation. Science **267:**1645–1648.
31. **Selsted, M. E., D. M. Brown, R. J. DeLange, and R. I. Lehrer.** 1983. Primary structures of MCP-1 and MCP-2, natural peptide antibiotics of rabbit lung macrophages. J. Biol. Chem. **258:**14485–14489.
32. **Srinivas, S. K., R. V. Srinivas, G. M. Anantharamaiah, J. P. Segrest, and R. W. Compans.** 1992. Membrane interactions of synthetic peptides corresponding to amphipathic helical segments of the human immunodeficiency virus type-1 envelope glycoprotein. J. Biol. Chem. **267:**7121–7127.
33. **Steiner, H., D. Hultmark, A. Engstrom, H. Bennich, and H. G. Boman.** 1981. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature **292:**246–248.
34. **Tencza, S. B., T. A. Mietzner, and R. C. Montelaro.** 1997. Calmodulin-binding function of LLP segments from the HIV-1 transmembrane protein is conserved among natural sequence variants. AIDS Res. Hum. Retroviruses **13:**263–269.
35. **Tencza, S. B., M. A. Miller, K. Islam, T. A. Mietzner, and R. C. Montelaro.** 1995. Effect of amino acid substitutions on calmodulin binding and cytolytic properties of the LLP-1 peptide segment of human immunodeficiency virus type 1 transmembrane protein. J. Virol. **69:**5199–5202.
36. **von Hofsten, P., I. Faye, K. Kockum, J. Y. Lee, K. G. Xanthopoulos, I. A. Boman, H. G. Boman, A. Engstrom, D. Andreu, and R. B. Merrifield.** 1985. Molecular cloning, cDNA sequencing, and chemical synthesis of cecropin B from Hyalophora cecropia. Proc. Natl. Acad. Sci. USA **82:**2240–2243.
37. **Yuan, T., T. A. Mietzner, R. C. Montelaro, and H. J. Vogel.** 1995. Characterization of the calmodulin-binding domain of SIV transmembrane glycoprotein by NMR and CD spectroscopy. Biochemistry **34:**10690–10696.

37a. **Yuan, T., and H. Vogel.** Personal communication.

38. **Zasloff, M.** 1987. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc. Natl. Acad. Sci. USA **84:**5449–5453.