# Promotion of Peptide Antimicrobial Activity by Fatty Acid Conjugation

Alexander F. Chu-Kung,[†] Kristen N. Bozzelli,[†] Nathan A. Lockwood,[‡] Judith R. Haseman,[§] Kevin H. Mayo,[§] and Matthew V. Tirrell*[,†]

Department of Chemical Engineering, University of California, Santa Barbara, Santa Barbara, California 93106, Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, Minnesota 55455, and Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota 55455. Received September 3, 2003; Revised Manuscript Received February 3, 2004

Three peptides, YGAA[KKAAKAA]$_2$ (AKK), KLFKRHLKWKII (SC4), and YG[AKAKAAKA]$_2$ (KAK), were conjugated with lauric acid and tested for the effect on their structure, antibacterial activity, and eukaryotic cell toxicity. The conjugated AKK and SC4 peptides showed increased antimicrobial activity relative to unconjugated peptides, but the conjugated KAK peptide did not. The circular dichroism spectrum of AKK showed a significantly larger increase in its α-helical content in the conjugated form than peptide KAK in a solution containing phosphatidylethanolamine/phosphotidylglycerol vesicles, which mimics bacterial membranes. The KAK and AKK peptides and their corresponding fatty acid conjugates showed little change in their structure in the presence of phosphatidylcholine vesicles, which mimic the cell membrane of eukaryotic cells. The hemolytic activity of the KAK and AKK peptides and conjugates was low. However, the SC4 fatty acid conjugate showed a large increase in hemolytic activity and a corresponding increase in helical content in the presence of phosphatidylcholine vesicles. These results support the model of antimicrobial peptide hemolytic and antimicrobial activity being linked to changes in secondary structure as the peptides interact with lipid membranes. Fatty acid conjugation may improve the usefulness of peptides as antimicrobial agents by enhancing their ability to form secondary structures upon interacting with the bacterial membranes.

## INTRODUCTION

Multicellular organisms produce a wide variety of antimicrobial peptides, suggesting that they play a crucial role in innate immunity (*1*). Antimicrobial peptides are secreted constantly at low levels to prevent infection, but local production can be increased in response to an infection (*1*). The peptides are cationic, which allows them to preferentially target the negatively charged cell membranes of prokaryotes such as bacteria. Eukaryotic cells have a different lipid composition in the outer leaflet of their cell membranes, resulting in a zwitterionic surface that is less attractive to the peptides. This difference between eukaryotic and prokaryotic membranes can be utilized effectively to target many pathogens, since major changes to their membrane composition would be necessary to develop resistance (*1*). As a result, efforts are being made to develop antimicrobial peptides into drugs that will retain their effectiveness against pathogens, especially against those strains of bacteria that have already become multiple drug-resistant.

* Corresponding author: Matthew Tirrell, Department of Chemical Engineering, University of California Santa Barbara, Santa Barbara, CA 93105. Phone: (805) 893-3141. E-mail: tirrell@engineering.ucsb.edu.

[†] University of California.

[‡] Department of Chemical Engineering and Materials Science, University of Minnesota.

[§] Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota.

Antimicrobial peptides share two common features: an overall positive charge and the ability to assume a three-dimensional structure with distinct hydrophobic and hydrophilic faces, called an amphipathic structure (*2*). Despite these common features there is very little sequence conservation between antimicrobial peptides. One of the most common classes of antimicrobial peptides are linear peptides that adopt an α-helical amphipathic structure (*2*). The mode of action of this class of peptides, which includes magainins (*3*, *4*), cecropins (*5*, *6*), and mellitins (*4*, *7*) have been extensively studied. The currently accepted model for the mechanism of action of helical antimicrobial peptides involves peptides that are typically unfolded in solution but fold in the presence of membranes to form an amphipathic structure (*8*, *9*). The formation of an amphipathic structure seems to be required for membrane binding and lytic activity (*2*, *10*). When the concentration of peptide bound to the membrane reaches a critical concentration, the peptides either insert into the membrane to form pores, or destabilize the membrane by disrupting the membrane curvature (*11*).

Although they are effective antimicrobial agents in vitro, antimicrobial peptides require high doses to be effective when administered systemically, so much so that toxicity to the host becomes an issue (*12*). Clinical use of antimicrobial peptides requires improving efficacy without also increasing their toxicity to eukaryotic cells.

Some natural antimicrobial peptides, such as the polymyxin (*13*) and lipopetaibols (*14*) are coupled to fatty acid tails. Removal of the fatty acid tail decreases the

10.1021/bc0341573 CCC: $27.50 
Published on Web 05/04/2004

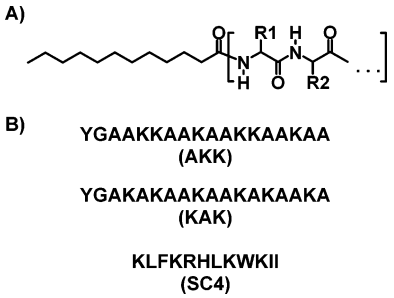


**Figure 1.** (A) Chemical structure of a fatty acid-conjugated peptide. The portion within the square brackets represents the two N-terminal amino acids of the peptide. (B) Sequence of the AKK, KAK, and SC4 peptides starting with the N-terminus. SC4 has an amidated C-terminus.

antimicrobial activity of polymyxin (*15*), demonstrating that it was intimately linked to the peptide activity. This has inspired attempts to increase antimicrobial activity by conjugating naturally occurring antimicrobial peptides with fatty acid tails (*16*−*18*). The effect of fatty acid tails on antifungal activity (*19*) and antiprotozoal activity (*20*) has also been studied. Work in our group (*21*−*23*), and in a group of our collaborators (*24*), has shown that addition of hydrocarbon tails to short peptides, to form peptide amphiphiles, can promote the formation of secondary structure. Since formation of secondary structure appears to be requisite for activity in antimicrobial peptides, we conjecture that fatty acid conjugation will increase activity by facilitating the formation of secondary structure.

We have conjugated three peptides to lauric acid to study the effect of fatty acid tail addition on peptide secondary structure as well as the correlation between structure and antimicrobial activity (Figure 1). Peptide AKK is based on the sequence of a previously studied synthetic leucine-lysine antimicrobial peptide (*25*). This peptide segregates its hydrophobic and hydrophilic amino acids into distinct regions on the helical wheel diagram (Figure 2). Peptide KAK is a reordering of the peptide AKK with the same amino acid composition and is based on a sequence shown to form nonamphipathic helices (*26*). Peptide SC4 is a naturally derived, α-helical antimicrobial peptide that has antimicrobial activity in the micromolar range (*27*). The helical wheel diagram of SC4 suggests a distinct hydrophilic face and a strongly hydrophobic face. All three peptides were coupled with lauric acid to form peptide amphiphiles, C12-AKK, C12-KAK, and C12-SC4. The structure of these peptides and peptide amphiphiles was studied in aqueous buffer, in the presence of vesicles composed of phosphatidylcholine (zwitterionic) or in the presence of vesicles composed of phosphatidylethanolamine mixed with phosphatidylglycerol (anionic). The peptide and peptide amphiphiles were further tested for their ability to kill bacteria, destabilize the inner membrane of Gram-negative bacteria, and lyse human red blood cells. We also observed the aggregation state of peptide amphiphiles in solution.

## MATERIALS AND METHODS

**Materials.** Peptides AKK and KAK were synthesized by Synpep Corporation. Peptide SC4 was prepared by the Microchemical Facility at the University of Minnesota. *N*-Hydrobenzotriazole (HOBt) and 2-(1*H*-benzotriazole-1yl)1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Nova Biochemem. 1,2-Dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dilauroyl-*sn*-glycero-3-phospoethanolamine (DLPE), and 1,2-dilauroyl-*sn*-glycero-3-phospoglycerol (DLPG) were purchased from Avanti Polar Lipids. All other chemical reagents were purchased through Sigma-Aldrich. Bacteria strains used to determine antimicrobial activity are *Escherichia coli* DH5α and ML-35 and *Staphylococcus epidermidis* (ATCC#12228). *E. coli* ML-35 was also used to determine cell membrane permeability.

**Peptide Amphiphile Synthesis.** Resin-bound peptide was received from either the Microchemical Facility at University of Minnesota or Synpep Corporation. Resin-bound peptide was deprotected with 20 vol % piperidine in dimethyl formaldehyde (DMF) for 2 h. Following deprotection, the resin-bound peptide was washed three times with DMF and then coupled to lauric acid using a 4-fold molar excess of HTBU, HOBt, *N*,*N*′-diisopropylethylamine, and lauric acid. The peptide amphiphile was then cleaved, purified, and characterized according to the method below.

**Peptide Cleavage and Purification.** AKK, KAK, C12-AKK, and C12-KAK were cleaved using a mixture of 95/5 vol % trifloroacetic acid (TFA)/water solution and precipitated in cold methyl *tert*-butyl ether. SC4 and C12-SC4 were cleaved a mixture of 82.5 vol % TFA, 5 vol % thioanisole, 5 vol % phenol, 5 vol % water, and 2.5 vol % ethanedithiol and then precipitated in cold methyl-*tert*-butyl-ether. Peptides were purified by reverse phase HPLC on a Vydac C4 column using 99.9/0.1 vol % water/TFA and 99.9/0.1 vol % acetonitrile/TFA as the mobile phases. The samples were lyophilized and then analyzed for molecular mass using a Thermo Bioanalysis MALDI-TOF system.

**Minimum Bactericidal Concentration (MBC) Determination.** Overnight cultures of test bacteria, either Gram-negative *E. coli* (strains DH5α or ML-35) or Gram-positive *S. epidermidis* (ATCC#12228), were grown at 37 °C in tryptic soy broth (TSB) for at least 18 h. The following day, subcultures were prepared by diluting the overnight cultures 1:100 in TSB. The subcultures were grown to log phase ($OD_{600}$ of 0.5) and then diluted to a concentration of $1 \times 10^5$ to $1 \times 10^6$ colony forming units (CFU)/mL. A stock solution of test peptide was prepared at a concentration of 1 mg/mL in Millipore water to avoid aggregation. The stock peptide concentration was verified by amino acid analysis performed at the molecular structure facility at University of California—Davis. Test

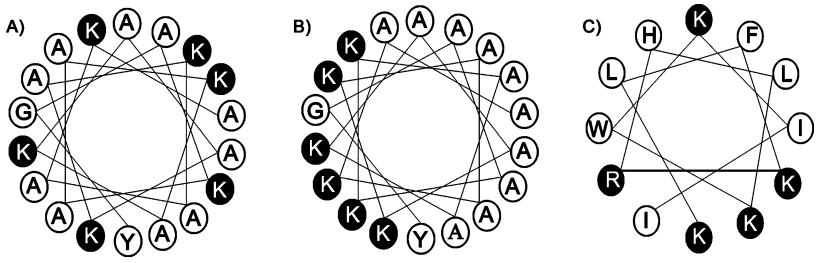


**Figure 2.** Helical wheel diagram of peptides (A) KAK (B) AKK and (C) SC4. The black filled circles are charged amino acids.

peptide solutions were added to polypropylene tubes and diluted with TSB to the desired concentration. The sample tubes were then inoculated with a bacterial stock solution to a final bacteria concentration of $1 \times 10^4$ to $1 \times 10^5$ CFU/mL. After incubation for 18 h at 37 °C, 50 $\mu$L aliquots were plated out and grown overnight. The MBC was taken as the lowest concentration where no colonies grew.

**Hemolysis.** Fresh human red blood cells were centrifuged and washed three times with phosphate-buffered saline (PBS). The red blood cells were diluted to a concentration of 0.4 vol % in PBS and placed in Eppendorf tubes. Peptide or peptide amphiphile was added at various concentrations, and the samples were incubated for 1 h at 37 °C. The samples were then centrifuged to sediment the intact red blood cells. Hemolytic activity was determined by measuring the UV absorbance at 414 nm. One hundred percent hemolysis was determined by addition of 1 vol % Triton-X100. The percentage of hemolysis was calculated as follows:

$$\% \text{ hemolysis} = 100(A_{414} \text{ with peptide} - A_{414} \text{ in plain PBS})/(A_{414} \text{ in Triton-X100} - A_{414} \text{ in plain PBS})$$

**Vesicle Preparation.** A chloroform solution of lipids, either 70/30 wt % DLPE/DLPG or DLPC, was dried under nitrogen until a thin film was formed. The film was then further dried under vacuum for at least 12 h. The lipids were dispersed in 10 mM sodium phosphate buffer, pH 7.4, to a concentration of 2.4 mM. The solution was then extruded through a 100 nm Millipore polycarbonate filter five times. Vesicle size was confirmed to be approximately 100 nm using dynamic light scattering. Henceforth, vesicles prepared with phosphatidylcholine are referred to as PC, and vesicles prepared with phosphatidylethanolamine and phosphatidylglycerol mixture are referred to as PE/PG.

**Circular Dichroism.** Circular dichroism spectra were recorded on an Olis-RSM CD with a 5 mm quartz cell. Data reported are an average of three scans. Spectra were recorded at 37 °C in 10 mM sodium phosphate buffer, pH 7.4, with a peptide concentration of 0.02 mM. Spectra were also recorded in the presence of either PE/PG or PC vesicles at a vesicle concentration of 0.3 mM. To estimate secondary structure contribution, a least-squares fit was performed using a linear combination of standard α-helix and random coil spectra (*28*).

**Membrane Permeability.** *E. coli* inner membrane permeability was determined by the ability of the peptide or peptide amphiphile to unmask $\beta$-galactosidase activity using ONPG (*o*-nitrophenyl-$\beta$-D-galactopyraniside) as a substrate (*29*). *E. coli* ML-35 produces the enzyme $\beta$-galactosidase, which hydrolyzes the colorless substrate ONPG to yellow *o*-nitrophenol. ONPG cannot cross an intact bacterial membrane, so the rate of hydrolysis of ONPG can be related to the rate of permeabilization of the bacterial inner membrane. Logarithmic phase *E. coli* ML-35 was centrifuged, and the resulting pellet was washed three times in 10 mM sodium phosphate buffer, pH 7.4, containing 100 mM NaCl and resuspended to a concentration of $1 \times 10^8$ CFU/mL in the same buffer. Solutions of peptide and ONPG were prepared in the same buffer, and bacteria solution was added to a final concentration of 0.02 mM peptide, 1.5 mM ONPG, and $1 \times 10^7$ CFU/mL *E. coli*. The production of *o*-nitrophenol was measured over time by UV absorbance at 420 nm.

**Aggregation Assay.** The aggregation state of the peptide amphiphiles was determined according to the pyrene solubilization method presented by Kim and coworkers (*30*). Pyrene is a polycyclic aromatic hydrocarbon with low solubility in aqueous solutions. Pyrene can be solubilized within the hydrophobic core of micelles providing a method to test for the presence of micelles. A test peptide dissolved in 10 mM sodium phosphate buffer, pH 7.4, containing 100 mM NaCl was added to an excess of pyrene at various concentrations. The suspension was sonicated for at least 8 h and then centrifuged. The supernatant was added to a 50 mM solution of sodium dodecyl sulfate, and the absorbance at 336 nm was recorded.

**Table 1. Bactericidal Activities of AKK, KAK, SC4, C12-AKK, C12-KAK, and C12-SC4. All MBC Values Are ± 2 $\mu$M**

| | MBC ($\mu$M) | | |
|---|---|---|---|
| | *E. coli* DH5α | *E. coli* ML-35 | *S. epidermidis* ATCC 12228 |
| AKK | >65 | >65 | >65 |
| KAK | >65 | >65 | >65 |
| SC4 | 17 | 34 | 48 |
| C12-AKK | 11 | 11 | 30 |
| C12-KAK | >65 | >65 | >65 |
| C12−SC4 | 1 | 4 | 2 |

## RESULTS

Bactericidal activities of peptides AKK, KAK, SC4 and the peptide amphiphiles C12-AKK, C12-KAK, and C12-SC4 were measured against two strains of the Gram-negative bacteria *E. coli* (ML-35 and DH5α) and one strain of the Gram-positive bacteria S. epidermidis (ATCC#12228) (Table 1). Among the unmodified peptides only SC4 had an MBC below 65 $\mu$M, which was the highest concentration studied. The MBC of C12-SC4 was between 9- and 20-fold lower than that of SC4, indicating that fatty acid conjugation increased antimicrobial activity. C12-AKK was moderately antimicrobial. Neither KAK nor C12-KAK showed bactericidal activity in this assay.

The ability of the peptides and peptide amphiphiles to permeabilize the cytoplasmic membranes of *E. coli* ML-35 was assessed. As expected, both C12-SC4 and C12-AKK rapidly destabilized the target membrane (Figure 3A). SC4, which had moderate antimicrobial activity as measured by MBC tests, showed low membrane permeabilization activity. This result implies that SC4 may kill bacteria by another mechanism in addition to destabilization of bacterial membranes. AKK and C12-KAK showed moderate rates of ONPG hydrolysis, whereas KAK showed the lowest rate.

The toxicities of the peptides and peptide amphiphiles to eukaryotic cells were tested by their ability to lyse human red blood cells (Figure 3B). C12-KAK had no detectable hemolytic activity within the concentrations studied. AKK, KAK, and C12-AKK showed no hemolytic activity up to concentrations of 500 $\mu$M, and only exhibited mild (<15%) hemolysis at 500 $\mu$M. SC4 showed mild (<15%) hemolytic activity at 3 $\mu$M that increased slightly over the range of concentrations tested. The hemolytic activity of C12-SC4 was low at 0.3 $\mu$M but rapidly increased to over 90% hemolysis at concentrations of 18 $\mu$M. However, when tested against endothelial cells neither SC4 nor C12-SC4 demonstrate lytic activity up to 1 mM concentrations (data not shown).

Circular dichroism was used to assess the structure of the peptide and peptide amphiphiles in various environments (Figure 4). The resulting spectra were fit to evaluate the helical content in each environment (Table

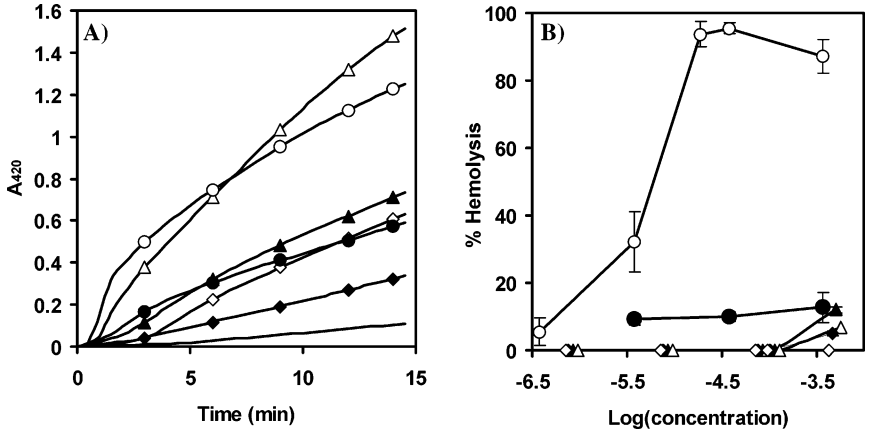


**Figure 3.** (A) Rate of ONPG hydrolysis of peptides SC4 (●), AKK (▲), and KAK (◆) as well as peptide amphiphiles C12-SC4 (○), C12-AKK (△), and C12-KAK (◇). The unmarked line is the baseline (no peptide added). (B) Hemolytic activity as a function of concentration for the same peptide and peptide amphiphiles (the same symbols are used).

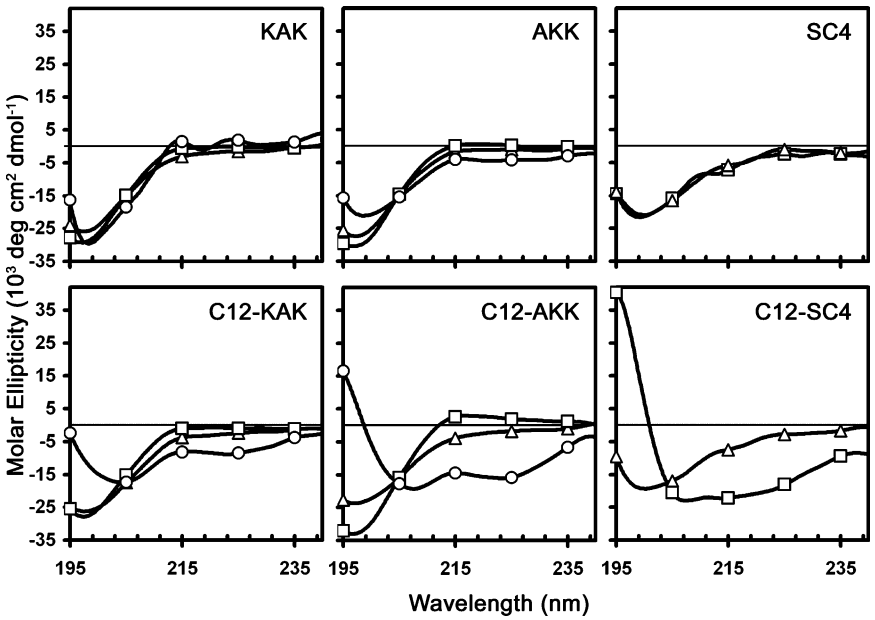


**Figure 4.** CD traces of peptides KAK, AKK, SC4, and peptide amphiphiles C12-KAK, C12-AKK, and C12-SC4. Experiments are conducted in sodium phosphate buffer (△) with the addition of DLPC vesicles (□) or DLPE/DLPG vesicles (○) (symbols are not data points but are solely to differentiate curves).

**Table 2. Degree of Helix Formation in Sodium Phosphate Buffer Alone, with the Addition of PC Vesicles or PE/PG Vesicles**

| | buffer: % α-helix | PC: % α-helix | PE/PG: % α-helix |
|---|---|---|---|
| AKK | 12 | 5 | 26 |
| KAK | 15 | 8 | 17 |
| SC4 | 26 | 27 | – |
| C12-AKK | 17 | 0 | 73 |
| C12-KAK | 18 | 11 | 44 |
| C12-SC4 | 32 | 100 | – |

2). AKK and C12-AKK had little α-helical character in benign sodium phosphate buffer solution or PC environments. In the presence of PE/PG vesicles, α-helical structure developed. The increase in structure with the addition of PE/PG vesicles was four times greater for C12-AKK than AKK. KAK and C12-KAK showed a similar relationship; neither had α-helical structure in sodium phosphate buffer or PC, but both showed an increase in structure in PE/PG. Again, the change in structure between the environments was greater in the peptide amphiphile than the unmodified peptide, but by less than 3-fold. KAK formed a precipitate with PE/PG vesicles, although we were still able to obtain a spectrum. Both SC4 and C12-SC4 formed precipitate in the presence of PE/PG, which prevented the collection of useable data. C12-SC4 did, however, show a significant increase in structure in PC, when compared to SC4 in PC or either sample in $NaPO_4$.

Aggregation of C12-AKK, C12-AKA, and C12-SC4 was probed by their ability to solublize the hydrophobic molecule pyrene. All the peptide amphiphiles were tested at concentrations from 1 $\mu$M to 250 $\mu$M in 10 $\mu$M sodium phosphate, 100 $\mu$M sodium chloride solutions. None of the molecules showed the ability to solubilize pyrene, implying the molecules are not aggregated below 250 $\mu$M (data not shown).

## DISCUSSION

Membrane binding and membrane insertion, processes involved in the initial steps of cell lysis, are influenced by both hydrophobic and electrostatic interactions of antimicrobial peptides. Conjugation of lauric acid to antimicrobial peptides shifts the balance of electrostatic and hydrophobic forces. Increasing the hydrophobicity of the peptide can stabilize the membrane-bound secondary

structures requisite for antimicrobial activity. One potential drawback to fatty acid conjugation is that it may promote peptide folding in solution, which was previously shown to inhibit membrane binding (*31*). A second potential drawback is peptide self-assembly in solution which was previously shown to increase the potency of peptides toward zwitterionic membranes (*32*).

Fatty acid conjugation affects the structure and activity of peptides in an environment-specific manner. The high positive charge density on peptides and peptide amphiphiles tends to destabilize helical secondary structures. All the studied peptides are unstructured in solution; addition of a fatty acid tail is insufficient to overcome charge repulsion in that environment. Previous studies on peptide amphiphiles have shown stabilization of helical secondary structures in solution at concentrations above the critical micelle concentration, implying that conjugation of peptides to hydrocarbon tails facilitates cooperative folding in solution (*23*, *24*). Although all three peptide amphiphiles clearly show greater ability to fold, adhere, and destabilize bacterial membranes than their parent peptides, our pyrene solution tests indicate that the peptide amphiphiles have not formed micelles at these concentrations. These results are consistent with the low α-helical content of the samples in solution; one would expect to see more α-helical structure if the peptide amphiphiles were micellized. It is possible that some sort of peptide amphiphile self-organization takes place on the target membranes, where the local concentration of peptide amphiphile will be greater than in free solution. It is also believed that the insertion of peptides into a membrane is a cooperative process (*9*). The peptide−peptide interaction of peptide amphiphiles may be higher in the membrane-bound state, which would help the cooperative insertion into the membrane.

Activity, hemolytic or bactericidal, appears to be correlated with the degree of structure induced in the peptide by the presence of the appropriate lipids. Results of circular dichroism, MBC assays, and ONPG hydrolysis assays indicate that the interaction between the peptide and PE/PG membranes is enhanced through the addition of hydrocarbon tails. Thus, the hydrophobic attraction between lipid membranes and the antimicrobial peptides serves to promote folding of the peptide and binding to membranes independent of membrane charge. In fact, increasing the hydrophobicity of the antimicrobial peptides decreases their selectivity for bacterial membranes (*33*). Our findings are consistent with these trends. Peptide amphiphiles are more hydrophobic than their parent peptides and thus have increased interaction with membranes. This may account for the observed improved bactericidal activity for all the peptide amphiphiles compared to their simple peptide counterparts.

KAK is neither very hydrophobic, nor does it have a strong amphipathic character; thus, it is expected to be the least bactericidal of all three peptides. The addition of a lipid tail increases the hydrophobicity sufficiently that C12-KAK forms a partial helix in the presence of a bacterial membrane. Although the antimicrobial activity of C12-KAK was not detectable by MBC test, fatty acid conjugation improved the ability of KAK to permeablize *E. coli* membrane. While it is generally believed that amphipathic structure is required for wide-spectrum antimicrobial activity, studies show that some nonamphipathic peptides have mild bactericidal activity against Gram-negative bacteria (*34*). It is possible that such peptides fold into structures other than α-helices in order to maximize their amphipathicity and allow them to be mildly antimicrobial (*35*). This may also be the case with C12-KAK; we speculate that it may form a helical structure with either greater or fewer amino acids per turn than an α-helix.

AKK also lacks strongly hydrophobic amino acids, which accounts for its low antimicrobial activity. However, the hydrophobic residues create a wide hydrophobic face when the peptide is folded into an α-helix, giving AKK a greater potential for activity than KAK. We conjecture that the lipid tail of C12-AKK increases both its ability to fold and its attraction to membranes. The strong antimicrobial activity of the C12-AKK peptide amphiphile supports this conclusion. It is interesting that even the addition of the hydrophobic fatty acid tail does not cause this peptide to have significant hemolytic activity.

SC4 is the most hydrophobic of the peptides tested here and is the only peptide with measurable hemolytic activity. It has been observed that highly hydrophobic peptides tend to be more hemolytic (*36*). C12-SC4 shows a much higher degree of hemolytic activity than the SC4 peptide, which can be attributed to its even higher hydrophobicity.

In conclusion, our results indicate that peptide amphiphiles have increased formation of secondary structures in the presence of vesicles that mimic bacterial membranes. The increase in bactericidal activity accompanying coupling of the fatty acid tail clearly establishes a structure−activity relationship. Thus, the addition of fatty acid tails to form peptide amphiphiles may be a viable strategy to improve the antimicrobial activity of certain peptides. It is interesting that the improvement of bactericidal activity for C12-AKK is not accompanied by a concomitant increase in hemolytic activity as with C12-SC4. It is important to further explore the underlying reasons for the differences in the selectivity of these two antimicrobial peptides. It may elucidate other features that are important to the design of effective antimicrobial peptides with minimal toxicity to eukaryotes.

## ACKNOWLEDGMENT

We would like to thank the group of Robert Hancock at the University of British Columbia for kindly providing us with *E. coli* ML-35. This work made use of MRL Central Facilities supported by the MRSEC Program of the National Science Foundation under award No. DMR00-80034. This work was supported by the National Institute of Health (award #HL62427).

## LITERATURE CITED

(1) Zasloff, M. (2002) Antimicrobial peptides of multicellular organisms. *Nature 415*, 389−395.
(2) Giangaspero, A., Sandri, L., and Tossi, A. (2001) Amphipathic alpha helical antimicrobial peptides − A systematic study of the effects of structural and physical properties on biological activity. *Eur. J. Biochem. 268*, 5589−5600.
(3) Bevins, C. L., and Zasloff, M. (1990) Peptides from Frog-Skin. *Annu. Rev. Biochem. 59*, 395−414.
(4) Sansom, M. S. P. (1991) The Biophysics of Peptide Models of Ion Channels. *Prog. Biophys. Mol. Biol. 55*, 139−235.
(5) Fink, J., Boman, A., Boman, H. G., and Merrifield, R. B. (1989) Design, Synthesis and Antibacterial Activity of Cecropin-Like Model Peptides. *Int. J. Pept. Protein. Res. 33*, 412−421.
(6) Hultmark, D., Steiner, H., Rasmuson, T., and Boman, H. G. (1980) Insect Immunity − Purification and Properties of 3 Inducible Bactericidal Proteins from Hemolymph of Immunized Pupae of Hyalophora-Cecropia. *Eur. J. Biochem. 106*, 7−16.
(7) Haberman, E. (1972) Bee Wasp Venoms. *Science 177*, 314−&.

(8) Zhang, L. J., Rozek, A., and Hancock, R. E. W. (2001) Interaction of cationic antimicrobial peptides with model membranes. *J. Biol. Chem. 276*, 35714−35722.
(9) Hancock, R. E. W., and Rozek, A. (2002) Role of membranes in the activities of antimicrobial cationic peptides. *FEMS Microbiol. Lett. 206*, 143−149.
(10) Blondelle, S. E., and Houghten, R. A. (1992) Design of Model Amphipathic Peptides Having Potent Antimicrobial Activities. *Biochemistry 31*, 12688−12694.
(11) Shai, Y. (2002) Mode of action of membrane active antimicrobial peptides. *Biopolymers 66*, 236−248.
(12) Darveau, R. P., Cunningham, M. D., Seachord, C. L., Cassianoclough, L., Cosand, W. L., Blake, J., and Watkins, C. S. (1991) Beta-Lactam Antibiotics Potentiate Magainin-2 Antimicrobial Activity Invitro and Invivo. *Antimicrob. Agents Chemother. 35*, 1153−1159.
(13) Benedict, R. G., and Langlykke, A. F. (1947) Antibiotic Activity of Bacillus-Polymyxa. *J. Bacteriol. 54*, 24−25.
(14) Toniolo, C., Crisma, M., Formaggio, F., Peggion, C., Epand, R. F., and Epand, R. M. (2001) Lipopeptaibols, a novel family of membrane active, antimicrobial peptides. *Cell. Mol. Life Sci. 58*, 1179−1188.
(15) Tsubery, H., Ofek, I., Cohen, S., and Fridkin, M. (2001) *N*-terminal modifications of Polymyxin B nonapeptide and their effect on antibacterial activity. *Peptides 22*, 1675−1681.
(16) Mak, P., Pohl, J., Dubin, A., Reed, M. S., Bowers, S. E., Fallon, M. T., and Shafer, W. M. (2003) The increased bactericidal activity of a fatty acid-modified synthetic antimicrobial peptide of human cathepsin G correlates with its enhanced capacity to interact with model membranes. *Int. J. Antimicrob. Agents 21*, 13−19.
(17) Wakabayashi, H., Matsumoto, H., Hashimoto, K., Teraguchi, S., Takase, M., and Hayasawa, H. (1999) *N*-acylated and D enantiomer derivatives of a nonamer core peptide of lactoferricin B showing improved antimicrobial activity. *Antimicrob. Agents Chemother. 43*, 1267−1269.
(18) Majerle, A., Kidric, J., and Jerala, R. (2003) Enhancement of antibacterial and lipopolysaccharide binding activities of a human lactoferrin peptide fragment by the addition of acyl chain. *J. Antimicrob. Chemother. 51*, 1159−1165.
(19) Avrahami, D., and Shai, Y. (2002) Conjugation of a magainin analogue with lipophilic acids controls hydrophobicity, solution assembly, and cell selectivity. *Biochemistry 41*, 2254−2263.
(20) Chicharro, C., Granata, C., Lozano, R., Andreu, D., and Rivas, L. (2001) *N*-terminal fatty acid substitution increases the leishmanicidal activity of CA(1−7)M(2−9), a cecropin-melittin hybrid peptide. *Antimicrob. Agents Chemother. 45*, 2441−2449.
(21) Yu, Y. C., Tirrell, M., and Fields, G. B. (1998) Minimal lipidation stabilizes protein-like molecular architecture. *J. Am. Chem. Soc. 120*, 9979−9987.
(22) Yu, Y. C., Berndt, P., Tirrell, M., and Fields, G. B. (1996) Self-assembling amphiphiles for construction of protein molecular architecture. *J. Am. Chem. Soc. 118*, 12515−12520.
(23) Forns, P., Lauer-Fields, J. L., Gao, S., and Fields, G. B. (2000) Induction of protein-like molecular architecture by monoalkyl hydrocarbon chains. *Biopolymers 54*, 531−546.
(24) Fields, G. B., Lauer, J. L., Dori, Y., Forns, P., Yu, Y. C., and Tirrell, M. (1998) Proteinlike molecular architecture: Biomaterial applications for inducing cellular receptor binding and signal transduction. *Biopolymers 47*, 143−151.
(25) Haynie, S. L., Crum, G. A., and Doele, B. A. (1995) Antimicrobial Activities of Amphiphilic Peptides Covalently Bonded to a Water-Insoluble Resin. *Antimicrob. Agents Chemother. 39*, 301−307.
(26) Marqusee, S., Robbins, V. H., and Baldwin, R. L. (1989) Unusually Stable Helix Formation in Short Alanine-Based Peptides. *Proc. Natl. Acad. Sci. U.S.A. 86*, 5286−5290.
(27) Mayo, K. H., Haseman, J., Young, H. C., and Mayo, J. W. (2000) Structure−function relationships in novel peptide dodecamers with broad-spectrum bactericidal and endotoxin-neutralizing activities. *Biochem. J. 349*, 717−728.
(28) Reed, J., and Reed, T. A. (1997) A set of constructed type spectra for the practical estimation of peptide secondary structure from circular dichroism. *Anal. Biochem. 254*, 36−40.
(29) Lehrer, R. I., Barton, A., Daher, K. A., Harwig, S. S. L., Ganz, T., and Selsted, M. E. (1989) Interaction of Human Defensins with Escherichia-Coli − Mechanism of Bactericidal Activity. *J. Clin. Investig. 84*, 553−561.
(30) Kim, J. H., Domach, M. M., and Tilton, R. D. (1999) Pyrene micropartitioning and solubilization by sodium dodecyl sulfate complexes with poly(ethylene glycol). *J. Phys. Chem. B 103*, 10582−10590.
(31) Houston, M. E., Kondejewski, L. H., Karunaratne, D. N., Gough, M., Fidai, S., Hodges, R. S., and Hancock, R. E. W. (1998) Influence of preformed alpha-helix and alpha-helix induction on the activity of cationic antimicrobial peptides. *J. Pept. Res. 52*, 81−88.
(32) Sal-Man, N., Oren, Z., and Shai, Y. (2002) Preassembly of membrane-active peptides is an important factor in their selectivity toward target cells. *Biochemistry 41*, 11921−11930.
(33) Wieprecht, T., Dathe, M., Beyermann, M., Krause, E., Maloy, W. L., MacDonald, D. L., and Bienert, M. (1997) Peptide hydrophobicity controls the activity and selectivity of magainin 2 amide in interaction with membranes. *Biochemistry 36*, 6124−6132.
(34) Tossi, A., Sandri, L., and Giangaspero, A. (2000) Amphipathic, alpha-helical antimicrobial peptides. *Biopolymers 55*, 4−30.
(35) Oren, Z., Lerman, J. C., Gudmundsson, G. H., Agerberth, B., and Shai, Y. (1999) Structure and organization of the human antimicrobial peptide LL-37 in phospholipid membranes: relevance to the molecular basis for its noncell-selective activity. *Biochem. J. 341*, 501−513.
(36) Dathe, M., and Wieprecht, T. (1999) Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells. *Biochim. Biophys. Acta 1462*, 71−87.

BC0341573