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All-D amino acid-containing channel-forming antibiotic peptides

(D enantiomeric peptides/antimalarial peptides/cecropin/magainin/melittin)

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ABSTRACT **The D enantiomers of three naturally occurring antibiotics—cecropin A, magainin 2 amide, and melittin—were synthesized. In addition, the D enantiomers of two synthetic chimeric cecropin–melittin hybrid peptides were prepared. Each D isomer was shown by circular dichroism to be a mirror image of the corresponding L isomer in several solvent mixtures. In 20% hexafluoro-2-propanol the peptides contained 43–75% α-helix. The all-D peptides were resistant to enzymatic degradation. The peptides produced single-channel conductances in planar lipid bilayers, and the D and L enantiomers caused equivalent amounts of electrical conductivity. All of the peptides were potent antibacterial agents against representative Gram-negative and Gram-positive species. The D and L enantiomers of each peptide pair were equally active, within experimental error. Sheep erythrocytes were lysed by both D- and L-melittin but not by either isomer of cecropin A, magainin 2 amide, or the hybrids cecropin A-(1–13)-melittin-(1–13)-NH_2 or cecropin A-(1–8)-melittin-(1–18)-NH_2. The infectivity of the bloodstream form of the malaria parasite *Plasmodium falciparum* was also inhibited by the D and L hybrids. It is suggested that the mode of action of these peptides on the membranes of bacteria, erythrocytes, plasmodia, and artificial lipid bilayers may be similar and involves the formation of ion-channel pores spanning the membranes, but without specific interaction with chiral receptors or enzymes.**

The cecropins (1, 2) and several other antibiotic peptides of the animal kingdom, including defensins (3), magainins (4), and the bee venom toxin melittin (5), are thought to function through the formation of ion channels in lipid membranes. This idea has been based on recent studies of electrical conductivity in artificial lipid bilayers (3, 6–8), where activity is a function of the structure of the peptide and the composition of the membrane lipids. The bilayer lipids and cell membranes are chiral and contain many asymmetric centers. It has been generally assumed that the chirality of the membrane would require a specific chirality of the peptide for it to be active, in much the same way that peptide hormones are required to fit with the conformation of their natural receptors or for a substrate and enzyme to form a tight stereospecific complex. However, we have suggested that these peptide antibiotics can exert their effect without requiring a specific target receptor on the cell membrane (7, 9).

The purpose of the present study was to test this assumption by the synthesis of the all-D enantiomers of several natural, all-L peptide antibiotics and some of their active analogs. These D stereoisomers would be expected to assume equivalent, but mirror image, conformations when placed in the same environment as the all-L peptides. If a close molecular contact with the chiral components of the cell membrane is required, the D enantiomers would be expected to be inactive. However, if the interaction of the peptide with the membrane is only between achiral components, or if the requirement is simply for an appropriate hydrophobic environment, the D antibiotics might be expected to form active ion channels and cause the lysis and killing of cells. In addition to throwing light on its mechanism of action, such an active all-D peptide would have certain practical advantages as an antibiotic.

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MATERIALS AND METHODS

Synthesis and Purification of the Peptides. All of the peptides were synthesized by the solid-phase peptide synthesis technique (10, 11). They were prepared on a benzhydrylamine resin (0.65–0.9 mmol N per g), either manually by our most effective methods (12) or on an Applied Biosystems model 430A synthesizer. The couplings, with dicyclohexylcarbodiimide in dichloromethane, or symmetrical anhydrides in dimethylformamide, were monitored by the quantitative ninhydrin method (13). The N^{α}-*tert*-butoxycarbonyl (Boc) group was removed from the completed peptide-resin and the peptide was deprotected and cleaved with low/high HF. It was purified on Sephadex G-25 and then on a 2.2 × 25 cm column of Vydac C_{18} (218TPB1520) by elution with a linear gradient of 15–60% (vol/vol) acetonitrile in aqueous 0.05% F_3CCOOH. The center cut of the main peak was assessed for homogeneity by analytical HPLC, and molecular weights were determined by ^{252}Cf fission fragment time-of-flight mass spectrometry.

Circular Dichroism. Spectra were recorded on an Aviv 62DS CD spectropolarimeter. Peptide solutions contained 25 μM peptide and 0–20% (vol/vol) hexafluoro-2-propanol (HFP) in 2.5 mM sodium phosphate, pH 7.4. Measurements were in a 1-mm cell from 270 to 190 nm at 1-nm intervals, and three scans were averaged. The percentages of α-helix, β-sheet, turns, and random coil were calculated by the Prosec program, which is based on the data of Chen and Yang (14).

Enzymatic Degradation. Stock solutions of enzymes containing trypsin at 5 mg/ml or immune inhibitor A (InA) (15) at 0.1 mg/ml were diluted and mixed with an equal volume of solution containing the peptide (D-cecropin A or L-cecropin A) at 1.3 μg/μl (325 μM). All samples were incubated for 20 min at 36°C. After the incubation, 3 μl was placed in wells on plates seeded with *Escherichia coli* D21 to determine the amount of antibiotic remaining. The diameter of the inhibition zone was compared with a previously prepared standard curve.

Electrical Conductivity Measurements. The equipment and procedures were similar to those described previously (7, 16). Bilayers of the Mueller–Rudin type (17) were formed from 15

Abbreviations: Boc, *tert*-butoxycarbonyl; CA, cecropin A fragment; HFP, hexafluoro-2-propanol; InA, immune inhibitor A; LC, lethal concentration; M, melittin fragment; [Phy_2]PtdCho, diphytanoyl phosphatidylcholine; PtdSer, phosphatidylserine.

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Cecropin A: K W K L F K K I E K V G Q N I R D G I I K A G P A V A V V G Q A T Q I A K-NH_2
Melittin: G I G A V L K V L T T G L P A L I S W I K R K R Q Q-NH_2
Magainin 2 amide: G I G K F L H S A K K F G K A F V G E I M N S-NH_2
CA(1-13)M(1-13)NH_2: K W K L F K K I E K V G Q G I G A V L K V L T T G L-NH_2
CA(1-8)M(1-18)NH_2: K W K L F K K I G I G A V L K V L T T G L P A L I S-NH_2

FIG. 1. Amino acid sequences of some ion channel-forming antibacterial peptides. The last two are hybrids, with CA indicating cecropin A and M indicating melittin. The residue numbers (shown in parentheses) are those of the parent peptides.

mg/ml solutions of lipids in decane on a 1-mm-diameter hole in a Teflon septum separating two 1.5-ml cells. The rear (trans) compartment was held at ground and voltage was applied to the front (cis) compartment. The peptides were dissolved in 0.1 M NaCl/10 mM Hepes, pH 7.2, buffer and added to the cis compartment, final concentrations 40–4000 ng/ml.

Inhibition Zone Assay. Thin agarose plates were prepared as described previously (18) from 6 ml of rich medium containing 1–4 × 10^5 logarithmic-phase cells of a test organism. Serially diluted peptide samples (3 μl) were placed in 3-mm-diameter wells in the plates. After incubation for 18 hr at 30°C the diameters of the zones of inhibition around the wells were measured. The square of the diameter was plotted against the logarithm of the molar concentration of peptide, and from the slope and intercept the lethal concentration, LC, of the peptide was calculated according to ref. 19.

Erythrocyte Lysis. The antibacterial plate assay was adapted to an erythrocyte lysis assay. The plates contained 6 ml of 1% agarose, 0.9% NaCl, and 10% sheep erythrocytes suspended in Alsevers solution. The dilution series of peptide was placed in the wells. After incubation at 30°C for 24 hr, the diameters of the clear zones were recorded and LC values were calculated as before.

Antiparasitic Assay. The bloodstream form of the malaria parasite *Plasmodium falciparum* was assayed by measuring the inhibition of the reinvasion of human erythrocytes (20). Strain F32 (Tanzania) was incubated for 20 hr at 37°C in a tissue culture medium with different concentrations of the peptide. After acridine orange staining, the percentage of newly infected erythrocytes was scored in a fluorescence microscope.

RESULTS

Physical and Chemical Properties of the Synthetic Peptides. The amino acid sequences of the peptides reported here are given in Fig. 1. The peptides are considered to be pure and of correct structure on the basis of analytical HPLC, mass spectrometry, and amino acid analysis.

The solution conformation was measured by circular dichroism as a function of solvent composition between 0% and 20% (vol/vol) HFP (Table 1). In aqueous solution the peptides were largely random coils or β structures, with no observable α-helix. Helicity increased with increasing HFP. Cecropin A reached 75% helix in 16% HFP, melittin was ≈60% helical in 4–8% HFP, while magainin 2 amide was only 44% helix in 20% HFP. CA-(1–13)-M-(1–13)NH_2 was strongly helical (up to 90%) above 8% HFP and CA-(1–8)-M-(1–18)NH_2 reached 63% helix at 20% HFP. The circular dichroism curves (θ vs λ) for every peptide pair were exact mirror images, with ellipticities equivalent but of opposite sign (Fig. 2).

The resistance of L- and D-cecropin A to enzymatic cleavage by the enzymes trypsin and InA (15) is illustrated in Fig. 3 *Upper* and *Lower*, respectively. The all-L peptide was hydrolyzed and inactivated rapidly by trypsin (50% in 20 min at a peptide-to-enzyme weight ratio of 2500:1) or by InA (50% in 20 min at a ratio of 200:1). In sharp contrast, the all-D peptide was completely stable to both enzymes, up to a concentration of trypsin 2000 times higher than needed for 50% inactivation of the L peptide. In addition, experiments in rabbit serum showed that L-cecropin A was 50% degraded in 2 hr, whereas the D enantiomer was much more stable (half-time for loss of activity, 30 hr).

Antibacterial Activity. The antibacterial activity of the peptides was determined by an inhibition zone assay (18) on agarose plates. Table 2 gives the LC assay data for a representative set of test organisms, which includes two Gram-negative species, *Escherichia coli* D21 and *Pseudomonas aeruginosa* OT97, and three Gram-positive species, *Bacillus subtilis* Bs11, *Staphylococcus aureus* Cowan 1, and *Streptococcus pyogenes*. Although the sensitivity of the organisms to these antibacterial peptides varied considerably, the bacteria were generally lysed by micromolar concentrations. The least sensitive of this group to cecropin A or magainin 2 amide was *Staph. aureus*, which required >300 μM peptide. However, as reported recently (18), the hybrid analog CA-(1–13)-M-(1–13)NH_2 was about two orders of magnitude more effective, and this is true also for CA-(1–8)-M-(1–18)NH_2. Melittin is also much more active, but is not useful as an antibiotic because it is highly toxic to many cells, including sheep erythrocytes (last column, Table 2). In contrast, the other compounds did not significantly lyse these representative eukaryotic cells.

Table 1. Conformer composition of the synthetic peptide antibiotics calculated from circular dichroism measurements

	% α-helix						% β structures						% coil					
Peptide	0% HFP	4% HFP	8% HFP	12% HFP	16% HFP	20% HFP	0% HFP	4% HFP	8% HFP	12% HFP	16% HFP	20% HFP	0% HFP	4% HFP	8% HFP	12% HFP	16% HFP	20% HFP
L-Cecropin A	0	10	59	25	75	75	45	43	29	56	0	0	55	47	12	19	25	25
D-Cecropin A	0	0	52	35	72	75	38	53	33	42	0	0	63	47	15	23	28	25
L-[CA-(1–13)-M-(1–13)NH_2]	0	29	71	50	90	55	47	60	29	32	0	19	54	12	0	18	11	27
D-[CA-(1–13)-M-(1–13)NH_2]	0	27	59	72	89	71	44	73	37	0	0	0	56	0	4	28	11	29
L-[CA-(1–8)-M-(1–18)NH_2]	0	40	42	35	56	63	55	52	49	51	14	0	45	8	9	14	30	36
D-[CA-(1–8)-M-(1–18)NH_2]	0	—	46	43	56	63	49	—	30	25	5	0	51	—	23	32	38	37
L-Magainin-NH_2	0	0	42	38	38	44	68	68	47	44	41	31	32	32	11	19	21	25
D-Magainin-NH_2	0	0	42	30	39	43	69	69	40	52	35	31	31	31	19	19	26	26
L-Melittin	0	74	61	75	75	75	50	13	17	0	0	0	50	13	22	25	25	25
D-Melittin	0	59	54	69	72	75	48	22	16	0	0	0	52	19	27	31	28	25

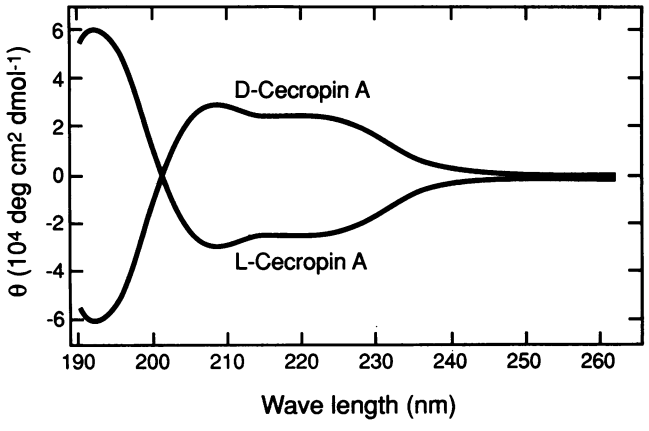

FIG. 2. Mean residue ellipticity spectra of the L and D enantiomers of cecropin A. Solvent, 20% (vol/vol) HFP in 2.5 mM sodium phosphate, pH 7.4; peptide concentration, 25 μM; temperature, 22°C.

In all cases the activity of the D enantiomer was quantitatively equivalent to that of the L enantiomer within the limits of these experiments (approximately a factor of 2). For example, the LC ratios of the L and D isomers of cecropin A were 0.7, 1.3, 1, (1), and 2.5 for *E. coli*, *P. aeruginosa*, *B. sublilis*, *Staph. aureus*, and *Strep. pyogenes*, respectively, and neither lysed erythrocytes. For CA-(1–8)-M-(1–18)NH_2 the corresponding ratios were 1, 1.2, 2, 3.3, and 2.5, with no erythrocyte lysis. For melittin the ratios were 0.8, 1.5, 0.5, 2, and 0.6, and all-D-melittin lysed erythrocytes essentially as well as the L enantiomer.

Antimalarial Activity. Measurements of the activity of the peptides against the bloodstream form of the malaria parasite *Plasmodium falciparum* showed that the D enantiomers of CA-(1–13)-M-(1–13)NH_2 and CA-(1–8)-M-(1–18)NH_2 were 20% as active as the corresponding L isomers (50% inactivation at 10 μM vs. 50 μM). The two enantiomers of magainin 2 amide were approximately one order of magnitude less active than the hybrids, and cecropin A was almost completely inactive. Melittin could not be tested because it lysed the erythrocytes.

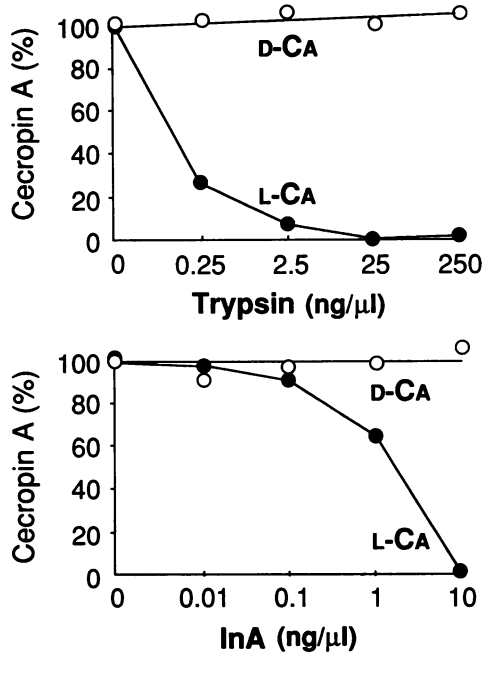

FIG. 3. Inactivation of the L and D enantiomers of cecropin A by the enzymes trypsin (*Upper*) and InA (*Lower*). Cecropin A remaining after a 20-min incubation at 36°C with enzyme was assessed by the inhibition assay on agarose plates of *E. coli* and is expressed as percent of control containing peptide but no enzyme.

Electrical Conductivity in Lipid Bilayers. Planar lipid bilayers, made of a 3:1 (vol/vol) mixture of diphytanoyl phosphatidylcholine ([Phy_2]PtdCho) and phosphatidylserine (PtdSer) were tested for their conductivity as a function of voltage gradient and composition of added peptide antibiotic under conditions similar to those reported previously for natural cecropins and several of their all-L analogs (7). The D enantiomers behaved in exactly the same way as their L enantiomer controls. For example, D-cecropin A and L-cecropin A developed single-channel conductances of 67 ± 8 pS and 70 ± 13 pS, respectively, at −60 mV when added to the cis side of the lipid bilayer (Fig. 4), and the D and L isomers of CA-(1–13)-M-(1–13)NH_2 also gave equal conductances (130 ± 30 pS at −60 mV). The macroscopic conductances were voltage dependent but linear with peptide concentration (7). For cecropin A the conductance was 0.1 nS/μM. The details of these and similar experiments on other D and L peptide pairs will be reported elsewhere.

DISCUSSION

An enantiomer is a molecule possessing a three-dimensional shape that is the mirror image of the parent compound. A peptide only partially substituted with stereoisomers of the component amino acids is a diastereomer but not an enantiomer. When it is composed of all-D amino acids, in the same sequence, the peptide is the D enantiomer of the all-L peptide, and it is subject to the same conformational constraints as the L enantiomer. In such a D peptide structure the normal right-handed helical segments found in the parent L peptide will be present as left-handed helices and the entire folded chains are expected to be mirror images.

There are several examples of synthetic all-D peptide hormones—e.g., bradykinin (21), oxytocin (22), and angiotensin (23). Because these hormones must interact with chiral receptors on the susceptible cell surfaces, they have, in all cases, been found to be inactive. β-Endorphin containing D residues in the C-terminal region, but L residues in the specific 1–5 recognition region, retained opiate activity (24). Only a few examples of synthetic enantiomers of naturally occurring peptide antibiotics are known. The earliest, to our knowledge, was enantio-enniatin B (25). However, this is a very special case, in which the high redundancy of structure of the cyclic depsipeptide allows the two isomers to become topologically superimposable simply by rotating one of the molecules by 60°. The isomer could then be recognized by the presumed chiral receptor. This work led to the idea of retroenantio isomers (26), which can give peptides that are topologically superimposable with the parent isomers, but with the direction of the peptide bonds reversed, and they can retain biological activity. In these cases the objective is to make a new compound which will have the same topology as the natural derivative and will react with the same receptor. In the present work, the D isomer and the natural L isomer will not have the same topology and would not be expected to interact with a natural chiral receptor.

Anthelvencin is a recent example of an enantiomer of an antibiotic, containing a single asymmetric center, that retains the ability of the parent natural isomer to bind to the minor groove of duplex calf thymus DNA, although in an altered alignment (27). An enantiomeric peptide pair with two asymmetric centers, dihydroxybis(netropsin)succinamide, showed 10-fold differences in binding affinity in the DNA minor groove (28).

We have synthesized the L and D enantiomeric pairs of three naturally occurring peptide antibiotics—cecropin A,

Table 2. LC values for bacteria and erythrocytes

Peptide	LC, μM					LC ratio for L/D isomers*	LC for erythrocytes, μM
	E. coli	*P. aeruginosa*	*B. subtilis*	*Staph. aureus*	*Strep. pyogenes*		
L-Cecropin A	0.2	1	3	>300	5	1.3 ± 0.4	>200
D-Cecropin A	0.3	0.8	3	>300	2		>300
L-Magainin-NH_2	4	30	3	300	4	2.0 ± 0.9	300
D-Magainin-NH_2	1	30	3	100	3		>400
L-Melittin	0.8	3	0.2	0.2	0.5	1.1 ± 0.5	4
D-Melittin	1	2	0.4	0.1	0.9		2
L-[CA-(1–13)-M-(1–13)NH_2]	0.5	1	0.7	2	1	0.7 ± 0.2	>200
D-[CA-(1–13)-M-(1–13)NH_2]	0.8	2	1	8	0.8		500
L-[CA-(1–8)-M-(1–18)NH_2]	0.3	0.7	0.4	1	2	2.0 ± 0.5	>600
D-[CA-(1–8)-M-(1–18)NH_2]	0.3	0.6	0.2	0.3	0.8		>400
					Mean	1.4 ± 0.5	

*Data are presented as mean ± mean deviation from the mean.

melittin, and magainin 2 amide—and two of their hybrid analogs. The peptides were all purified to apparent homogeneity and they all showed the expected amino acid composition and molecular weight by mass spectrometry. Circular dichroism measurements in solvents containing HFP showed increasing proportions of helical structure as the organic component increased. Cecropin A, for example, increased to 75% helix, which is quite close to the amount expected for a globular protein of that composition according to Chou–Fasman parameters (29). The other peptides reached 43–90% helix. The ellipticities of the D enantiomers, in every instance, were essentially equivalent to their all-L counterparts, but with opposite sign (Fig. 2). The data support the expectation that the D enantiomers do form mirror images of the L peptides.

Cecropin A was very sensitive to degradation by trypsin because of the seven L-lysine and one L-arginine residues. It was also sensitive to InA, an enzyme isolated from *Bacillus thuringiensis*, which is known to cleave cecropin A in several places (15), and furthermore it was inactivated by serum. In sharp contrast, all-D-cecropin A was very resistant to degradation by trypsin, InA, and serum. We believe this stability to be an important feature of these D antibiotics because their half-lives *in vivo* should be much longer than those of the natural peptides.

It was interesting to find that the D enantiomers of all five of the antibiotic peptides studied induced the same kinds of electrical conductivity in artificial planar lipid membranes as their L enantiomers. Single-channel conductances were measured and there were no significant differences between the two isomers of each pair.

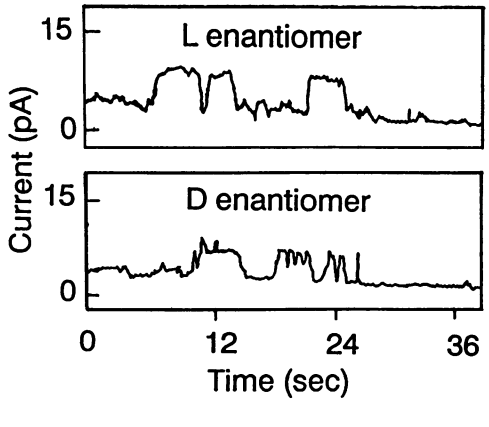

FIG. 4. Demonstration of single-channel conductance activity of the L and D enantiomers of cecropin A in lipid bilayers. Peptide concentration, 90 nM in pH 7.2 buffer, on the cis side of PtdSer/[Phy_2]PtdCho membrane. Transmembrane potential, −60 mV. Start of the recordings was 30 min after addition of peptide and application of voltage gradient.

It has been shown (7) that the behavior of the natural cecropins on lipid bilayer membranes is well explained by the formation of electrically conducting ion channels, or pores. The pores are thought to be composed of mobile aggregates of six or more peptide chains, the largest reaching approximately 40 Å in diameter. Under these conditions, the peptides are helical and amphipathic at the N terminus and helical and hydrophobic at the C terminus (12, 30, 31). Other data (32) indicate that a flexible region is necessary near the center of peptide. We have suggested (7) that the peptides first associate with the membrane by electrostatic forces and then the helical molecules span the lipid bilayer. Association of several such peptides would form a water-filled pore, lined with many positively charged lysine residues, which would serve as an ion-conducting, anion-selective, channel.

Our interpretation of the data presented here is that the D enantiomers of these antibiotics also form ion channels, or pores, in the lipid bilayers. The results indicate that the chiral L and D isomer pairs are mirror images of one another and the helical segments may be either right-handed as in the natural all-L peptides or left-handed as in the D enantiomers. In addition, both D and L enantiomers are expected to form higher-order structures that are comparable but of opposite chirality, each maintaining an amphipathic character in which the hydrophilic charged surfaces of each helix face the interior of the water-filled pore and the hydrophobic surfaces face the lipids of the membrane. Since the behavior of the five stereoisomer pairs shows no measurable chiral selectivity, it appears that the peptides are not influenced by close interactions with any stereo centers of the membranes while exerting their effects, requiring only a hydrophobic environment to assume their opposite but equivalent final quaternary structures. Consequently, the D and L enantiomers form the same kind and number of ion channels with the same electrical conductances.

The cecropins are quite active against a wide range of Gram-negative and Gram-positive bacteria, but they do not lyse eukaryotic cells such as sheep erythrocytes and Chang liver cells (2). For many organisms they are active in the micromolar range, although some, such as *Staph. aureus*, are resistant to >300 μM peptide. Magainin 2, a 23-residue peptide recently isolated from frog skin, has a rather similar antibacterial spectrum (4), although for some species it is severalfold less potent than cecropin A (Table 2). At high concentration magainin is measurably hemolytic, whereas cecropins are not. Melittin, the major peptide component of bee venom, is known mainly as a toxin, but it is an even more

potent antibacterial peptide than cecropin A, especially toward Gram-positive bacteria. Unfortunately, the toxicity of melittin prevents its use as an antibiotic. Several of our chimeric hybrid peptides containing segments of cecropin A combined with segments of melittin were recently reported (18) to overcome the resistance of Gram-positive species such as *Staph. aureus*, and therefore they represent broader-spectrum antibiotics, which, in addition, do not lyse erythrocytes.

The striking result from our antibacterial assays was the fact that in all five isomeric pairs of peptides the activity of the D enantiomer was quantitatively equivalent to that of the L enantiomer against each of the five test organisms. The ratios of the lethal concentrations for the L and D peptides averaged 1.4 ± 0.5. Thus, there was no chiral selectivity in the lysis and killing of the bacteria. Fortunately, the great improvement in the sensitivity of *Staph. aureus* to the L hybrids, L-[CA-(1–13)-M-(1–13)NH_2] and L-[CA-(1–8)-M-(1–18)NH_2], was maintained by the two D analogs. Furthermore, the D enantiomers did not have increased ability to lyse erythrocytes.

It has already been shown (18) that some of these antibacterial peptides have activity against the bloodstream form of the malaria parasite *Plasmodium falciparum*. Cecropin A is only weakly active, while cecropin B and magainin 2 amide caused a 50% inhibition of the reinvasion of erythrocytes by these organisms at 100 μM and the hybrid L-[CA-(1–13)-M-(1–13)NH_2] had the same effect at only 10 μM. Melittin itself could not be tested because it is strongly hemolytic. The D enantiomers, D-[CA-(1–13)-M-(1–13)NH_2] and D-[CA-(1–8)-M-(1–18)NH_2] have now been tested and were also found to be potent antibiotics against *Plasmodium falciparum* although, for this protozoan, the D enantiomers were less active than the L isomers. Presumably, these results reflect differences in the cell membranes and indicate that more than one type of cell inactivation mechanism may come into play in this complex system, one chiral and one achiral.

One of our goals has been to develop peptide antibiotics with sufficient potency and range of susceptible organisms to be of practical utility, and this has, to some extent, been achieved. The best peptides studied here, both D and L, are very active against a range of Gram-positive and Gram-negative bacteria and are much more active than either cecropin or magainin for certain species. In addition, the D enantiomers are resistant to enzymatic degradation and inactivation. Their half-lives *in vivo* should be much longer than those of the natural L forms and, as a consequence, they may be effective orally.

From all of the data obtained to date, we find that the L and D enantiomeric pairs of cecropin A, melittin, magainin 2, and two cecropin A–melittin hybrids behave the same in electrical conductance measurements in planar lipid bilayers and in antibacterial and erythrocyte assays. This suggests to us that the mechanism of action in all three systems may be similar. The simple ion-channel mechanism for the antibacterial effect of these peptides seems to fit the facts that we now have, and it is clear from the D enantiomer data that the mechanism does not involve a stereoselective interaction with a chiral enzyme or lipid or protein receptor.

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