Cationic Bactericidal Peptides

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1. INTRODUCTION

Over the past decade many naturally occurring (poly) cationic peptides from a variety of species have been isolated and studied, with respect to their activity, structure and genetic organization. These peptides possess antimicrobial activity against many species including bacteria (Gram-positive and -negative), fungi, and viruses and, in the case of the more potent peptides,

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can have a lytic activity on mammalian cells. The main thrust of research in this area has been to understand the relationship between structure and the biological activity that these peptides exert. These analyses have been substantiated by the design and characterization of synthetic chemical peptides based on studies of these naturally occurring peptides.

Cationic peptides can be classified into several groups on the basis of sequence similarities, secondary and tertiary structure, function and origin. In this review, we are attempting to provide a detailed overview of the known polycationic peptides, with emphasis on those of less than 100 amino acids and with a net charge greater than +2 (Table 1).

2. OCCURRENCE OF CATIONIC PEPTIDES IN NATURE

2.1. Peptides Involved in Mammalian Host Defence Mechanisms

The oxygen-independent microbicidal host defence mechanism of mammals involves several proteinaceous molecules that are cationic in nature. These include lysozyme, bactericidal/permeability increasing factor (BPI), cathepsin G, CAP-37, lactoferrin, defensins and the eosinophil-derived proteins: the major basic protein (MBP) and the eosinophil cationic protein (ECP) (Elsbach and Weiss, 1988). All of these proteins (except lysozyme) are granule-associated and reside in neutrophils, including polymorphonuclear leukocytes and eosinophils. Most of these polypeptides are larger than the polycationic peptides discussed here although a synthetic 24 amino acid cationic domain of CAP-37 has been shown to be the probable bactericidal domain of CAP-37 (Pereira *et al.*, 1993) and a 21 amino acid cationic domain to be the antibacterial domain of CAP-18 (Tossi *et al.*, 1994).

Defensins represent a class of small (29–35 amino acids), arginine- and cysteine-rich peptides which have been isolated from rat, rabbit, guinea pig and human leukocytes (Couto *et al.*, 1992; Eisenhauer and Lehrer, 1992). These molecules, found primarily within the cytoplasmic granules of neutrophils, may constitute between 5 and 15% of the total cellular protein. Two defensins, MCP-1 and MCP-2, are expressed in elicited rabbit alveolar macrophages (Selsted *et al.*, 1983). Cryptdins, the name given to mouse defensins, are found in the Paneth cells of the small intestine (Ouellete *et al.*, 1989), whereas the defensins HNP-5 and HNP-6 were found in the human cell counterpart (Jones and Bevins, 1992). All defensins share amino acid sequence similarities and possess a specific conserved array of six cysteine residues which form three disulphide bridges. Members of the defensin family all possess a secondary structure rich in β-pleated sheet stabilized by these intramolecular disulphide bonds, as discussed in further detail later in this review.

Defensins kill a wide variety of bacteria (being generally more lethal against Gram-positives than Gram-negatives), fungi, spirochaetes and viruses. They exert not only microbicidal activity due to permeabilization of biological membranes but they also possess chemotactic and endocrine regulatory activities (Lehrer *et al.*, 1990).

Synthesis of defensins is under tissue specific, developmental and immune regulation. The cDNA of human defensin clones shows that each defensin is synthesized as a 93–95 amino acid preprodefensin comprising a 19 amino acid signal sequence for targeting to the endoplasmic reticulum and a 40–45 amino acid anionic propiece. It has been proposed that this anionic segment acts to neutralize the cationic charge of the defensin thereby rendering the peptide inactive until cleavage occurs, releasing this segment (Michaelson *et al.*, 1992). This appears to be a general mechanism of synthesis with these biologically active cationic peptides. Piers *et al.* (1993) have supported this finding as they found that cloning a synthetic cationic peptide by fusion to a negatively charged carrier protein or inclusion of the negatively charged pro sequence used in defensin synthesis in eukaryotes, resulted in the stable production in bacteria of an inactive fusion protein, which, on cleavage from the anionic carrier protein, regained activity.

Recently, a new subset of defensins, termed β-defensins, have been characterized from bovine neutrophils (Selsted *et al.*, 1993). This family of 13 structurally homologous peptides, although possessing the six invariantly spaced cysteines forming three disulphide bridges, is distinct from other neutrophil defensins owing to their unique consensus sequences. In addition, the bovine antimicrobial peptide TAP, isolated from the trachea, contains the same triple disulphide motif as β-defensins and is synthesized as a preproprotein (Diamond *et al.*, 1991). Within the large granules of bovine neutrophils are three arginine-rich peptides, the bactenecins (Frank *et al.*, 1990; Romeo *et al.*, 1988) two of which can be subgrouped due to their high proline content. The third is a novel tryptophan-rich, 13-amino acid peptide, indolicin (Selsted *et al.*, 1992). In contrast to indolicin, which is stored in granules in its mature form, bactenecins are present as inactive proforms which are processed into their active form when the granules containing them and protease-containing azurophilic granules interact (Zanetti *et al.*, 1990).

2.2. Insect Defence Peptides

The antibacterial response of insects has been well characterized over the last 20 years. A range of inducible antimicrobial cationic peptides has been isolated, including attacins (Hultmark *et al.*, 1983), cecropins (Steiner *et al.*, 1981), coleoptericin (Bulet *et al.*, 1991), diptericins (Dimarcq *et al.*, 1988), drosocin (Bulet *et al.*, 1993), phormicins (Lambert *et al.*, 1989), sarcotoxins

Table 1 The natural cationic peptides.

Peptide	Origin	Sequence (size)	Accession number	Reference	Activity
Abaecin	Honey bee (*Apis mellifera*)	YVPLPNVPQPGRRPFPTFPGQG PFNPKIKWPQGY	P15450	Casteels *et al.* (1990)	B
Ac-AMP1	Amaranth (*Amaranthus caudatus*)	VGECVRGRCPSGMCCSQFGY	98045[a]	Broekaert *et al.* (1992)	B+ F
Ac-AMP2	,,	VGECVRGRCPSGMCCSQFGYC GKGPKYCGR	98046[a]	Broekaert *et al.* (1992)	B+ F
Adenoregulin	Two-coloured leaf frog (*Phyllomedusa bicolor*)	GLWSKIKEVGKEAAKAAAKAA GKAALGAVSEAV	P31107	Daly *et al.* (1992)	B F
AFP1	Rape (*Brassica napus*)	QKLCERPSGTWSGVCGNNNAC KNQCINLEKARHGSCNYVFPAH K	P30225	Terras *et al.* (1992)	F
AFP2	Turnip (*Brassica rapa*)	QKLCERPSGTXSGVCGNNNAC KNQCIR	P30228	Terras *et al.* (1992)	F
Andropin	Fruit fly (*Drosophila melanogaster*)	VFIDILDKVENAIHNAAQVGIGF AKPFEKLINPK	P21663	Samakovlis *et al.* (1991)	B+
Apidaecin IA	Lymph fluid of honey bee (*Apis mellifera*)	GNNRPVYIPQPRPPHPRI	P11525	Casteels *et al.* (1989)	B−
Apidaecin IB	,,	GNNRPVYIPQPRPPHPRL	P11526	Casteels *et al.* (1989)	B−
Apidaecin II	,,	GNNRPIYIPQPRPPHPRL	P11527	Casteels *et al.* (1989)	B−
AS-48	*Streptococcus faecalis* subsp. *liquefacines* S-48	7.4 kDa		Gálvez *et al.* (1989)	B+
Bactenecin	Cytoplasmic granules of bovine neutrophils	RLCRIVVIRVCR	A33799	Romeo *et al.* (1988)	B
Bac5	Cytoplasmic granules of bovine neutrophils	RFRPPIRRPPIRPPFYPPFRPPI RPPIFPPIRPPFRPPLRFP	B36589	Frank *et al.* (1990)	B−
Bac7	,,	RRIRPRPPRLPRPRPRPLPFP RPGPRPIPRPLPFPRPGPRPIP RPLPFPRPGPRPIPRP	A36589	Frank *et al.* (1990)	B−
Bactericidin B2	Tobacco hornworm larvae hemolymph (*Manduca sexta*)	WNPFKELERAGQRVRDAVISA APAVATVGQAAAIARG*	P14662	Dickinson *et al.* (1988)	B C
Bactericidin B-3	,,	WNPFKELERAGQRVRDAIISA GPAVATVGQAAAIARG*	P14663	Dickinson *et al.* (1988)	B C
Bactericidin B-4	,,	WNPFKELERAGQRVRDAIISA APAVATVGQAAAIARG*	P14664	Dickinson *et al.* (1988)	B C
Bactericidin B-5P	,,	WNPFKELERAGQRVRDAVISA AAVATVGQAAAIARGG*	P14665	Dickinson *et al.* (1988)	B C
Bacteriocin C3603	*Streptococcus mutans*	4.8 kDa		Takada *et al.* (1984)	B+
Bacteriocin IY52	*Staphylococcus aureus*	5 kDa		Nakamura *et al.* (1983)	B+
Bacteriocin plantaricin A	*Lactobacillus plantarum*	AYSLQMGATAIKQVKKLFKKW	P80214	Nissen-Meyer *et al.* (1993)	B
BNBD-1	Bovine neutrophils	DFASCHTNGGICLPNRCPGHMI QIGICFRPRVKCCRSW	127951	Selsted *et al.* (1993)	B
BNBD-2	,,	VRNHVTCRINRGFCVPIRCPGR TRQIGTCFGPRIKCCRSW	127952	Selsted *et al.* (1993)	B
BNBD-3	,,	PEGVRNHVTCRINRGFCVPIRC PGRTRQIGTCFGPRIKCCRSW	127953	Selsted *et al.* (1993)	B
BNBD-4	Bovine neutrophils	PERVRNPQSCRWNMGVCIPFL CRVGMRQIGTCFGPRVPCCRR	127954	Selsted *et al.* (1993)	B
BNBD-5	,,	PEVVRNPQSCRWNMGVCIPIS CPGNMRQIGTCFGPRVPCCR	127955	Selsted *et al.* (1993)	B
BNBD-6	,,	PEGVRNHVTCRIYGGFCVPIRC PGRTRQIGTCFGRPVKCCRRW	127956	Selsted *et al.* (1993)	B
BNBD-7	,,	PEGVRNFVTCRINRGFCVPIRC PGHRRQIGTCLGPRIKCCR	127957	Selsted *et al.* (1993)	B
BNBD-8	,,	VRNFVTCRINRGFCVPIRCPGH RRQIGTCLGPQIKCCR	127958	Selsted *et al.* (1993)	B
BNBD-9	,,	PEGVRNFVTCRINRGFCVPIRC PGHRRQIGTCLGPQIKCCR	127959	Selsted *et al.* (1993)	B
BNBD-10	,,	PEGVRSYLSCWGNRGICLLNR CPGRMRQIGTCLAPRVKCCR	127960	Selsted *et al.* (1993)	B
BNBD-11	,,	GPLSCRRNGGVCIPIRCPGPMR QIGTCFGRPVKCCRSW	127961	Selsted *et al.* (1993)	B
BNBD-12	,,	GPLSCGRNGGVCIPIRCPVPMR QIGTCFGRPVKCCRSW	127962	Selsted *et al.* (1993)	B
BNBD-13	,,	SGISGPLSCGRNGGVCIPIRCP VPMRQIGTCFGRPVKCCRSW	127963	Selsted *et al.* (1993)	B

Table 1—continued

Bombinin	Yellow-bellied toad (*Bombina variegata*)	GIGALSAKGALKGLAKGLA ZHFAN*	P01505	Csordas and Michl (1970)	B
1	Asian toad (*Bombina orientalis*)	GIGASILSAGKSALKGLAKG LAEHFAN*	M76483	Gibson *et al.* (1991)	B
BLP-2	,,	GIGSAILSAGKSALKGLAKG LAEHFAN*	B41575	Gibson *et al.* (1991)	B
BLP-3	,,	GIGAAILSAGKSALKGLAKG LAEHF*	M76484	Gibson *et al.* (1991)	B
BLP-4	,,	GIGAAILSAGKSIIKGLANGL AEHF*	D41575	Gibson *et al.* (1991)	B
Bombolitin BI	Bumblebee venom (*Megabombus pennsylvanicus*)	IKITTMLAKLGKVLAHV*	P10521	Argiolas and Pisano (1985)	B C
Bombolitin BII	,,	SKITDILAKLGKVLAHV*	P07493	Argiolas and Pisano (1985)	B C
Bombolitin BIII	,,	IKIMDILAKLGKVLAHV*	P07494	Argiolas and Pisano (1985)	B C
Bombolitin BIV	,,	INIKDILAKLVKVLGHV*	P07495	Argiolas and Pisano (1985)	B C
Brevinin-1E	European frog (*Rana esculenta*)	FLPLLAGLAANFLPKIFCK ITRKC	S33729	Simmaco *et al.* (1993)	B C
Brevinin-2E	,,	GIMDTLKNLAKTAGKGALQS LLNKASCKLSGQC	S33730	Simmaco *et al.* (1993)	B
Cecropin	Silk moth (*Bombyx mori*)	RWKIFKKIEKVGQNIRDGIVKA GPAVAVVGQAATI	P14666	Qu *et al.* (1987)	B
Cecropin (lepidopteran A)	Silk moth (*Bombyx mori*)	RWKIFKKIEKMGRNIRDGIVKA GPAIEVIGSAKAI	P04142	Teshima *et al.* (1986)	B
Cecropin A	Silk moth (*Hyalophora cecropia*)	KWKLFKKIEKVGQNIRDGIIKA GPAVAVVGQATQIAK*	M63845	Gudmundsson *et al.* (1991)	B
Cecropin B	Silk moth (*Hyalophora cecropia*)	KWKVFKKIEKMGRNIRNGIV KAGPAIAVLGEAKAL*	X07404	Xanthopoulos *et al.* (1988)	B
Cecropin C	Fruit fly (*Drosophila melanogaster*)	GWLKKLGKRIERIGQHTRDATI QGLGIAQQAANVAATARG*	Z11167	Tryselius *et al.* (1992)	B
Cecropin D	Silk moth pupae (*Hyalophora cecropia*)	WNPFKELEKVGQRVRDAVISA GPAVATVAQATALAK*	P01510	Hultmark *et al.* (1982)	B–
Cecropin P_1	Pig small intestine (*Sus scrofa*)	SWLSKTAKKLENSAKKRISEGI AIAIQGGPR	P14661	Lee *et al.* (1989)	B–

Cecropin 1	Mediterranean fruit fly (*Ceratitis capitata*)	GWLKKIGKKIERVGQHTRDATI AVAQQAANVAATARG	JT0673	Rosetto *et al.* (1993)	
Cecropin 2	,,	GWLKKIGKKIERVGQHTRDAT IQTIGVAQQAANVAATIKG	JT0674	Rosetto *et al.* (1993)	
Charybdotoxin	Scorpion venom (*Leiurus quin-questriatus hebraeus*)	ZFTNVSCTTSKECWSVCQRLH NTSRGKCMNKKCRCYS	P13487	Schweitz *et al.* (1989)	
Coleoptericin	Beetle (*Zophobas atratus*)	8.1 kDa	A41711	Bulet *et al.* (1991)	B
Crabrolin	European hornet venom (*Vespa crabro*)	FLPLILRKIVTAL*	A01781	Argiolas and Pisano (1984)	C
Crambin	Crambe plants (*Crambe abyssinica*)	TTCCPSIVARSNFNVCRIPGTP EAICATYTGCIIIPGATCPGDYAN	P01542	Teeter *et al.* (1981)	
Cryptdin 1 (defensin)	Mouse intestine (*Mus musculus*)	LRDLVCYCRSRGCKGRERM NGTCRKGHLLYTLCCR	A43279	Selsted *et al.* (1992)	
Cryptdin 2 (defensin)	,,	LRDLVCYCRTRGCKRRERM NGTCRKGHLMYTLCCR	B43279	Selsted *et al.* (1992)	
Cryptdin 3 (defensin)	,,	LRDLVCYCRKRGCKRRERM NGTCRKGHLMYTLCCR	C43279	Selsted *et al.* (1992)	
Cryptdin 4 (defensin)	,,	GLLCYCRKGHCKRGERVRGT CGIRFLYCCPR	D43279	Selsted *et al.* (1992)	
Cryptdin 5 (defensin)	,,	LSKKLICYCRIRGCKRRERVF GTCRNLFLTFVFCC	E43279	Selsted *et al.* (1992)	
Defensin 4K	Scorpion (*Leiurus quinquestriatus*)	GFGCPLNQGACHRHCRSIRRR GGYCAGFFKQTCTCYRN	JN0613	Cociancich *et al.* (1993b)	B+
Dermaseptin	South American arboreal frog (*Phyllomedusa sauvagii*)	ALWKTMLKKLGTMALHAGKA ALGAADTISQTQ	P24302	Mor *et al.* (1991)	F
Dermaseptin 1	Sauvage's leaf frog (*Phyllomedusa sauvagei*)	ALWKTMLKKLGTMALHAGKA ALKAAADTISQGTQ	P80277	Mor *et al.* (1991)	B F
Dermaseptin 2	,,	ALWFTMLKKLGTMALHAGKA ALGAAANTISQGTQ	P80278	Mor *et al.* (1991)	F
Dermaseptin 3	,,	ALWKNMLKGIGKLAGKAALG AVKKLVGAES	P80279	Mor *et al.* (1991)	F
Dermaseptin 4	,,	ALWMTLLKKVLKAAAKALNA VLVGANA	P80280	Mor *et al.* (1991)	F

Table 1—continued

Dermaseptin 5	,,	GLWSKIKTAGKSVAKAAAKA AVKAVTNAV	P80281	Mor *et al.* (1991)	F
Dermaseptin B1	,,	AMWKDVLKKIGTVALHAGKA ALGAVADTIS	P80282	Mor and Nicolas (1994)	F
Diptericin	Nestling-suckling blowfly (*Phormia terranovae*)	9 kDa	X15851	Reichhardt *et al.* (1989)	B–
Drosocin	Fruit fly (*Drosophila melanogaster*)	GKPRPYSPRPTSHPRPIRV	S35984	Bulet *et al.* (1993)	B
Endozepine	Domestic pig (*Sus scrofa domestica*)	KQATVGDINTERPDILDKGKAK WDAWNGLKGTSKEDAMKAYI NKVEELKKKYGI	S36839	Agerberth *et al.* (1993)	B
Esculentin	European frog (*Rana esculenta*)	GIFSKLGRKKIKNLLISGLKNV GKEVGMDVVRTGIDIAGCKIK GEC	S33731	Simmaco *et al.* (1993)	B
Gastric inhibitory peptide (GIP)	Domestic pig (*Sus scrofa domestica*)	ISDYSIAMDKIRQQDFVNWLLA QKGKKSDWKHNITQ	S36840	Agerberth *et al.* (1993)	B
GNCP-1 (defensin)	Guinea pig (*Cavia cutteri*)	RRCICTTRTCRFPYRRLGTCIF QNRVYTFCC	S21169	Yamashita and Saito (1989)	B
GNCP-2 (defensin)	,,	RRCICTTRTCRFPYRRLGTCLF QNRVYTFCC	X63676	Yamashita and Saito (1989)	B
Hiastadin 1	Crab eating primate (*Macaca fascicularis*)	DSHEERHHGRHGHHKYGRKFH EKHHSHRGYRSNYLYDN	P34084	Xu *et al.* (1990)	F
Histadin 2	Human (*Homo sapiens*)	MKFFVFALILALMLSMTGADSH AKRHHGYKRKFHEKHHSHRGY RSNYLYDN	292146[a]	Sabatini and Azen (1989)	F
HNP-1 (defensin)	Azurophil granules of human neutrophils	ACYCRIPACIAGERRYGTCIYQ GRLWAFCC	P11479	Lehrer *et al.* (1991)	B F C
HNP-2 (defensin)	,,	CYCRIPACIAGERRYGTCIYQ GRLWAFCC	P11479	Lehrer *et al.* (1991)	B F C
HNP-3 (defensin)	,,	DCYCRIPACIAGERRYGTCIYQ GRLWAFCC	P11479	Lehrer *et al.* (1991)	B F C
HNP-4 (defensin)	,,	VCSCRLVFCRRTELRVGNCLI GGVSFTYCCTRVD	X65977	Wilde *et al.* (1989)	B F C
HNP-5 (defensin)	Human Paneth cells	SQARATCYCRTGRCATRESLS GVCEISGRLYRLCCR	M97925	Jones and Bevins (1992)	B F C
HNP-6 (defensin)	,,	STRAFTCHCRRSCYSTEYSYG TCTVMGINHRFCCL	M98331	Jones and Bevins (1993)	B F C
Indolicidin	Bovine neutrophils	ILPWKWPWWPWRR*	A42387	Selsted *et al.* (1992)	B
Insect defensin	Dragonfly larvae (*Aeschna cyanea*)	GFGCPLDQMQCHRHCQTITGR SGGYCSGPLKLTCTCYR	P80154	Bulet *et al.* (1992)	B+
Lactoferricin B	N-terminal region of bovine lactoferrin	FKCRRWQWRMKKLGAPSITC VRRAF	M63502	Bellamy *et al.* (1992b)	B
Lepidopteran C	Silkworm (*Bombyx mori*)	RWKLFKKIEKVGRNVRDGLIKA GPAIAVIGQAKSL	225797[a]	Teshima *et al.* (1987)	
Leukocin A-Ual 187	*Leuconostoc gelidum* UAL 187 (bacterium)	KYYGNGVHCTKSGCSVNW GEAFSAGVHRLANGGNGFW	S65611	Hastings *et al.* (1991)	B–
Magainin I	Amphibian skin (*Xenopus laevis*)	GIGKFLHSAGKFGKAFVGEIMKS	A29771	Zasloff (1987)	B F E
Magainin II	,,	GIGKFLHSAKKFGKAFVGEIMNS	A29771	Zasloff (1987)	B – Ev
Mastoparan	Wasp venom (*Vespula lewisii*)	INLKALAALAKKIL*	P01514	Bernheimer and Rudy (1986)	B+
MBP-1	Maize (*Zea mays*)	RSGRGECRRQCLRRHEGQPWE TQECMRRCR	P28794	Duvick *et al.* (1992)	F
MCP1 (defensin)	Rabbit alveolar macrophages (*Oryctolagus cuniculus*)	VVCACRRALCLPRERRAGFC RIRGRIHPLCCRR	M28883	Selsted *et al.* (1983)	B F C
MCP2 (defensin)	,,	VVCACRRALCLPLERRAGFC RIRGRIHPLCCRR	M28073	Ganz *et al.* (1989)	B+ F
Melittin	Bee venom (*Apis mellifera*)	GIGAVLKVLTTGLPALISWIK RKRQQ	P01504	Tosteson and Tosteson (1984)	B C F
Mj-Amp1	*Mirabilis jalapa*	QCIGNGGRCNENVGPPYCCSG FCLRQPGQGYGYCKNR	243904[a]	Cammue *et al.* (1992)	B+ F
Mj-AMP2	,,	CIGNGGRCNENVGPPYCCSGFC LRQPNQGYGVCRNR	78217[a]	Cammue *et al.* (1992)	B+ F
Nisin	*Lactococcus lactis* subsp. *lactis* (bacterium)	ITSISLCTPGCKTGALMGCN MKTATCHCSIHVSK	P13068	Hurst (1981)	B+
Nisin Z	,,	ITSISICTPGCKTGALMGCNM KTATCNCSIHVSK	44047[a]	Mulders *et al.* (1991)	B+

Table 1—continued

NP-1 (defensin)	Rabbit neutrophils (*Oryctolagus cuniculus*)	VVCACRRALCLPRERRAGFC RIRGRIHPLCCRR	P01376	Ganz *et al.* (1989)	B F C
NP-2 (defensin)	,,	VVCACRRALCLPLERRAGFCR IRGRIHPLCCRR	P01377	Ganz *et al.* (1989)	B F C
NP-3A (defensin)	,,	GICACRRRFCPNSERFSGYCR VNGARYVRCCSRR	M64599	Michaelson *et al.* (1992)	B F C
NP-3B (defensin)	Rabbit neutrophils (*Oryctolagus cuniculus*)	GRCVCRKQLLCSYRERRIGDC KIRGVRFPFCCPR	M64600	Michaelson *et al.* (1992)	B F C
NP-4 (defensin)	,,	VSCTCRRFSCGFGERASGSCT VNGVRHTLCCRR	M64601	Michaelson *et al.* (1992)	B F C
NP-5 (defensin)	,,	VFCTCRGFLCGSGERASGSCT INGVRHTLCCRR	M64602	Michaelson *et al.* (1992)	B F C
Pep 5	*Staphylococcus epidermidis*	TAGPAIRASVKQCQKTLKATR LFTVSCKGKNGCK	P19578	Kaletta *et al.* (1989)	B+
Peptide 3910	Domestic pig (*Sus scrofa domestica*)	RADTQTYQPYNKDWIKEKIYVL LRRQAQQAGK	S36841	Agerberth *et al.* (1993)	B
PGLa	Amphibian skin (*Xenopus laevis*)	GMASKAGAIAGKIAKVALKAL*	X13388	Kuchler *et al.* (1989)	B
PGQ	Amphibian stomach (*Xenopus laevis*)	GVLSNVIGYLKKLGTGALNAVLKO		Moore *et al.* (1991)	B F
Phormicin A	Nestling-suckling blowfly (*Phormia terranovae*)	ATCDLLSGTGINHSACAAHCL LRGNRGGYCNGKGVCVCRN	P10891	Lambert *et al.* (1989)	B+
Phormicin B	,,	ATCDLLSGTGINHSACAAHCLL RGNRGGYCNRKGVCVRN	P10891	Lambert *et al.* (1989)	B+
Polyphemusin I	Atlantic horseshoe crab (*Limulus polyphemus*)	RRWCFRVCYRGFCYRKCR*	P14215	Miyata *et al.* (1989)	B
Polyphemusin II	,,	RRWCFRVCYKGFCYRKCR*	P14216	Miyata *et al.* (1989)	B
Protegrin I	Porcine leukocytes (*Sus scrofa*)	RGGRLCYCRRRFCVCVGR	S34585	Kokryakov *et al.* (1993)	B F E
Protegrin II	,,	RGGRLCYCRRRFCICV	S34586	Kokryakov *et al.* (1993)	B F E
Protegrin III	,,	RGGGLCYCRRRFCVCVGR	S34587	Kokryakov *et al.* (1993)	B F E
RatNP-1 (defensin)	Rat neutrophils (*Rattus norvegicus*)	VTCYCRRTRCGFRERLSGAC GYRGRIYRLCCR	A60113	Eisenhauer *et al.* (1989)	F B
RatNP-2 (defensin)	,,	VTCYCRSTRCGFRERLSGACG YRGRIYRLCCR		Eisenhauer *et al.* (1989)	F B
RatNP-3 (defensin)	,,	CSCRTSSCRFGERLSGACRLN GRIYRLCC	B60113	Eisenhauer *et al.* (1989)	F B
RatNP-4 (defensin)	,,	ACYCRIGACVSGERLTGACGL NGRIYRLCCR	C60113	Eisenhauer *et al.* (1989)	F B
Royalisin	Royal jelly (*Apis mellifera*)	VTCDLLSFKGQVNDSACAANCL GKAGGHCEKGVCICRKTSFKD LWDKYF	P17722	Fujiwara *et al.* (1990)	B+
Rs-AFP1	Radish (*Raphanus sativus*)	QKLCERPSGTWSGVCGNNNACK NQCINLEKARHGSCNYVFPAHK	109570[a]	Terras *et al.* (1992)	B+ F
Rs-AFP2	,,	QKLCQRPSGTWSGVCGNNNACI NQCIRLEKARHGSC	109572[a]	Terras *et al.* (1992)	B+ F
Sapecin	Flesh fly (*Sacophaga peregrina*)	ATCDLLSGTGINHSACAAHCLL RGNRGGYCNGKAVCVCRN	J04053	Hanzawa *et al.* (1990)	B
Sapecin B	,,	ITCEIDRSLCLLHCRLKGYLRA YCSQQKVCRCVQ	P31529	Yamada and Natori (1993)	B+ F
Sapecin C	,,	ATCDLLSGIGVQHSACALHCVF RGNRGGYCTGKGICVCRN	P31530	Yamada and Natori (1993)	B+ F
Sarcotoxin IA	Flesh fly (*Sacrophaga peregrina*)	GWLKKIGKKIERVGQHTRDAT IQGLGIAQQAANVAATAR*	P08375	Okada and Natori (1985b)	B
Sarcotoxin IB	,,	GWLKKIGKKIERVGQHTRDAT IQVIGVAQQAANVAATAR*	P08376	Okada and Natori (1985b)	B
Sarcotoxin IC	,,	GWLRKIGKKIERVGQHTRDAT IQVLGIAQQAANVAATAR*	P08377	Okada and Natori (1985b)	B
Seminalplasmin	Bovine seminal plasma (*Bos taurus*)	SDEKASPDKHHRFSLSRYAKL ANRLANPKLLETFLSKWIGDRG NRSV	S08184	Reddy and Bhargava (1979)	B F C
Sillucin	*Rhizomucor pusillus* (fungus)	ACLPNSCVSKGCCCGBSGYWC RQCGIKYTC	P02885	Bradley and Somkuti (1979)	B+
Subtilin	*Bacillus subtilis* (bacterium)	MSKFDDFDLDVVKVSKQDSK ITPQWKSESLCTPGCVTGALQ TCFLQTLTCNCKISK	P10946	Banerjee and Hansen (1988)	
Tachyplesin I	Horseshoe crab (*Tachypleus tridentatus*)	KWCFRVCYRGICYRRCR*	P23684	Nakamura *et al.* (1988)	B F

RatNP-2 (defensin)	,,	VTCYCRSTRCGFRERLSGACG YRGRIYRLCCR		Eisenhauer *et al.* (1989)	F B
RatNP-3 (defensin)	,,	CSCRTSSCRFGERLSGACRLN GRIYRLCC	B60113	Eisenhauer *et al.* (1989)	F B
RatNP-4 (defensin)	,,	ACYCRIGACVSGERLTGACGL NGRIYRLCCR	C60113	Eisenhauer *et al.* (1989)	F B
Royalisin	Royal jelly (*Apis mellifera*)	VTCDLLSFKGQVNDSACAANCL GKAGGHCEKGVCICRKTSFKD LWDKYF	P17722	Fujiwara *et al.* (1990)	B+
Rs-AFP1	Radish (*Raphanus sativus*)	QKLCERPSGTWSGVCGNNNACK NQCINLEKARHGSCNYVFPAHK	109570[a]	Terras *et al.* (1992)	B+ F
Rs-AFP2	,,	QKLCQRPSGTWSGVCGNNNACI NQCIRLEKARHGSC	109572[a]	Terras *et al.* (1992)	B+ F
Sapecin	Flesh fly (*Sacophaga peregrina*)	ATCDLLSGTGINHSACAAHCLL RGNRGGYCNGKAVCVCRN	J04053	Hanzawa *et al.* (1990)	B
Sapecin B	,,	ITCEIDRSLCLLHCRLKGYLRA YCSQQKVCRCVQ	P31529	Yamada and Natori (1993)	B+ F
Sapecin C	,,	ATCDLLSGIGVQHSACALHCVF RGNRGGYCTGKGICVCRN	P31530	Yamada and Natori (1993)	B+ F
Sarcotoxin IA	Flesh fly (*Sacrophaga peregrina*)	GWLKKIGKKIERVGQHTRDAT IQGLGIAQQAANVAATAR*	P08375	Okada and Natori (1985b)	B
Sarcotoxin IB	,,	GWLKKIGKKIERVGQHTRDAT IQVIGVAQQAANVAATAR*	P08376	Okada and Natori (1985b)	B
Sarcotoxin IC	,,	GWLRKIGKKIERVGQHTRDAT IQVLGIAQQAANVAATAR*	P08377	Okada and Natori (1985b)	B
Seminalplasmin	Bovine seminal plasma (*Bos taurus*)	SDEKASPDKHHRFSLSRYAKL ANRLANPKLLETFLSKWIGDRG NRSV	S08184	Reddy and Bhargava (1979)	B F C
Sillucin	*Rhizomucor pusillus* (fungus)	ACLPNSCVSKGCCCGBSGYWC RQCGIKYTC	P02885	Bradley and Somkuti (1979)	B+
Subtilin	*Bacillus subtilis* (bacterium)	MSKFDDFDLDVVKVSKQDSK ITPQWKSESLCTPGCVTGALQ TCFLQTLTCNCKISK	P10946	Banerjee and Hansen (1988)	
Tachyplesin I	Horseshoe crab (*Tachypleus tridentatus*)	KWCFRVCYRGICYRRCR*	P23684	Nakamura *et al.* (1988)	B F

(Okada and Natori, 1985a), sapecins (Matsuyama and Natori, 1988a) and insect defensins (Lambert *et al.*, 1989). These antibacterial peptides have been isolated and studied from silk moths (*Hyalophora cecropia*), flesh flies (*Sarcophaga peregrina*), blow flies (*Phormia terranovae*), fruit flies (*Drosophila melanogaster*) and beetles (*Zophobus atratus*). The synthesis of molecules involved in the immune response in insects occurs primarily in the fat body.

Two forms of attacins have been isolated from *Hyalophora cecropia*. Both have molecular masses of approximately 20 kDa and have 80% amino acid similarity, but differ in their isoelectric point, one being acidic and the other basic (Hultmark *et al.*, 1983). A protein with homology to the basic attacin, sarcotoxin IIA, has been isolated from *Sarcophaga peregrina* and shown to possess the same antibacterial activity towards Gram-negative bacteria (Ando and Natori, 1988). It has been postulated that attacin works to increase the permeability of the membrane thereby making it more susceptible to the action of lysozyme and cecropins (Engström *et al.*, 1984). *Phormia terranova* possesses antibacterial activity that can be divided into three groupings, diptericins, insect defensins (phormicins) and cecropins, based on the activity and abundance of these molecules. Lysozyme has not been detected in this species and cecropins are not a major component. The diptericins act on Gram-negative bacteria and insect defensins primarily on Gram-positives, with both of these molecules being produced from cells of mesodermal origin (Dimarcq *et al.*, 1990). So far, no homologues to diptericins have been identified in vertebrates, in contrast to the defensins. For production of the mature dipteric in peptide, two functions are required: cleavage from a pro molecule and amidation of the C-terminus (Reichhart *et al.*, 1989). Although both of these molecules are present in unchallenged *Diptera*, the level of transcription is greatly induced upon challenge or trauma (Dimarcq *et al.*, 1990).

Recently, a new and novel 19-residue, proline-rich peptide has been isolated and characterized from *Drosophila*. This peptide, known as drosocin, is unique in that it appears to require an O-glycosylation modification before it is active (Bulet *et al.*, 1993).

Defensin-like molecules, also known as sapecin, phormicin and sarcotoxin I, have been isolated from several different insect species (Dimarcq *et al.*, 1990; Lambert *et al.*, 1989; Matsuyama and Natori, 1988a). Although termed defensins and possessing six cysteine residues, these molecules have different tertiary structures when compared with the mammalian defensin family, because the intramolecular disulphide bridges are in a distinct arrangement resulting in a different solution structure (Hoffman and Hetru, 1992). Initially it was thought that the insect and mammalian defensins evolved from a common ancestor, based on sequence similarity seen between a rabbit defensin and the insect defensin molecule isolated from *Phormia* (Lambert *et al.*, 1989). However, on elucidation of the structural data, this does not

seem to be the case. Instead it appears that insect defensins have greater similarity to royalisin from bees, charybdotoxin from scorpion venom and the newly isolated scorpion defensin (Bontems *et al.*, 1991; Cociancich *et al.*, 1993b; Fujiwara *et al.*, 1990). These molecules not only share amino acid similarity with respect to the cysteine array, but also possess a similar tertiary structure (Bontems *et al.*, 1991; Hoffman and Hetru, 1992).

As with the mammalian system, insect defensins are synthesized as preproproteins requiring cleavage to produce the mature peptide (Dimarcq *et al.*, 1990). The genetic structure of the defensin gene in *Drosophila* is known and appears to contain an upstream transcriptional control region which is related to that involved in inducing mammalian interleukin 6 (Isshiki *et al.*, 1991). Recently, a similar system has been identified for cecropin genes. A kappa B-motif has been identified which in mammals binds transcription factor NF-kappa B thereby regulating the immune and acute-phase responses (Engström *et al.*, 1993).

One of the main groups of antibacterial components in the insect humoral response is the cecropins. These molecules constitute a class separate from those described above and differ from other bacteriolytic peptides produced by insects by not lysing mammalian cells. Cecropins contain 31–39 residues, do not possess cysteine residues and therefore do not form any disulphide bridges. They can be classified into different groups based on their amino acid sequence, which usually differs by only a few residues (Hultmark *et al.*, 1982). As with other molecules, cecropins are known by a variety of names depending on their origin (Dunn *et al.*, 1985; Okada and Natori, 1985a; Teshima *et al.*, 1986). Similar to diptericin and other antibacterial peptides, the C-terminus contains an amidated residue but non-amidated variants of cecropins have been found in *Hyalophora* (Hultmark *et al.*, 1982), *Sarcophaga* (Matsuyama and Natori, 1988b) and *Drosophila* (Samakovlis *et al.*, 1990). Nevertheless, there are indications that C-terminal amidation may be required for activity against Gram-positive bacteria (Callaway *et al.*, 1993). The cecropin family, members of which have a totally different tertiary structure to the defensin family (see following section), have similarities to another basic amphipathic molecule, melittin, the toxic component of bee stings. Cloning of cDNA corresponding to cecropin genes has revealed the presence of a signal peptide and a pro region which is cleaved off (von Hofsten *et al.*, 1985).

Cecropin-like peptides have also been isolated from pig intestine (Lee *et al.*, 1989). This 31-amino acid peptide differs from insect cecropins by not containing an amidated C-terminus, and by its solution structure (Sipos *et al.*, 1992), discussed in the following section.

2.3. Bee-derived Peptides

The honeybee *Apis mellifera* possesses some novel antimicrobial peptides, namely abaecin (Casteels *et al.*, 1990), apidaecins (Casteels *et al.*, 1989),

hymenoptaecin (Casteels *et al.*, 1993), magainins (Zasloff, 1987), royalisin (Fujiwara *et al.*, 1990) and a bee defensin (Casteels *et al.*, 1993). Apidaecins (IA, IB and II) identified by Casteels and co-workers (1989) from the immune haemolymph of honeybees, are a family of proline-rich peptides which are primarily active against Gram-negative and plant-associated bacteria. They are specifically induced and, owing to their high proline content, are stable at high temperatures and low pH. Similar to other peptides, apidaecins are synthesized as an inactive form with additional residues (8–10) at the N-terminus, requiring the action of a dipeptidyl aminopeptidase for maturation. Recently, the genetic structure of the apidaecin precursor has been characterized in detail (Casteels-Josson *et al.*, 1993). It appears from these studies that the apidaecin precursor contains multiple copies of the mature peptide, with a common prepro structure. Casteels-Josson *et al.* (1993) have postulated that the processing of the preproprotein into its biologically active form is via a mechanism reminiscent of that described for the yeast pheromone system (Singh *et al.*, 1983).

Abaecin, a 34-amino acid peptide containing 30% proline, with sequence similarity to the apidaecins, has been classified into a new group based on its different spectrum of anti-bacterial activity and its delayed activity in contrast with the immediate action of apidaecin (Casteels, 1990). Hymenoptaecin, a recently identified peptide, is composed of 93 amino acids with a glycine content of 19%. It exhibits no amino acid homology to other previously characterized molecules in bees, as it does not possess a high proline content and, unlike defensins, is devoid of cysteines (Casteels *et al.*, 1993).

The representative of the insect defensin family in bees is the 51-residue peptide royalisin isolated from royal jelly (Fujiwara *et al.*, 1990). This peptide appears to contain the characteristic array of cysteine residues forming three disulphide bridges. The N-terminal half of the molecule is hydrophobic, whereas the C-terminal half is hydrophilic. Bees also contain an α-helical peptide, mellittin, where the polarity of the hydrophobic versus the charged domains is reversed compared with cecropins (Suchanek and Kreil, 1977). Melittin is the major component (50%) of bee venom. It is a 26-residue peptide which has a wide spectrum of biological effects, including antibacterial activity (Piers and Hancock, 1994), membrane permeabilization leading to cell lysis, and interference with various enzymatic activities (Habermann, 1972). Melittin has been very well characterized with respect to its tertiary structure and its mode of action (discussed in detail later). Analogues of melittin and hybrid molecules with cecropins have shed some light on the functional residues within these molecules (Sipos *et al.*, 1991, 1992).

Yet another class of antimicrobial peptides has been isolated from the venom of the bumblebee, *Megabombus pennsylvanicus*. These five structurally related peptides called bombolitins possess a high percentage of hydrophobic amino acids (Argiolas and Pisano, 1985). Bombolitins share

functional similarities to other venom peptides including melittin from bees, mastoparan from wasps, and crabrolin from hornets (Argiolas and Pisano, 1985).

2.4. Amphibian Peptides

It has been discovered that the skin of frogs contains a wide array of biologically active peptides in glands located in the skin and also in the gastric mucosa (Erspamer and Melchiorri, 1980; Moore *et al.*, 1991). One peptide was originally identified in the species *Bombina* and was subsequently named bombinin (Csordas and Michl, 1970). Homologues of this 26-amino acid cationic peptide, named bombinin-like peptides (BLPs), have been found in different species of *Bombina*. The significant difference between these peptides and the original bombinin is that BLPs possess no haemolytic activity (Gibson *et al.*, 1991). The genes encoding BLPs have been cloned and analysed, revealing that peptides within this family are expressed as a precursor protein (Gibson *et al.*, 1991; Simmaco *et al.*, 1991). These peptides have a predicted alpha-helical structure reminiscent of the cecropin family.

Magainins, another family of amphibian cationic peptides, produced in the African clawed frog (*Xenopus laevis*), are also amphipathic and alpha-helical in structure (Chen *et al.*, 1988). They have been well characterized structurally, and chemical synthesis of synthetic magainin analogues has helped understand the structures required for the biological activity (Chen *et al.*, 1988; Cuervo *et al.*, 1988). The cDNA for magainin has been cloned, as have those corresponding to PGLa, PGQ and xenopsin, three related amphibian antibiotic peptides (Hoffman *et al.*, 1983; Moore *et al.*, 1991; Sures and Crippa, 1984; Terry *et al.*, 1988; Zasloff, 1987). As with the peptides described above, magainins are also synthesized as precursor molecules containing a signal sequence which shows considerable homology throughout the magainin family. They all possess a common processing motif and are produced with an acidic N-terminal pro region.

In 1991, Mor and colleagues isolated a novel antimicrobial peptide from the skin of the South American arboreal frog (*Phyllomedusa sauvagii*) and named it dermaseptin because of its antiseptic activity. This peptide has no sequence homology to the other amphibian cationic peptides; however, it is thought to permeabilize membranes in a similar fashion, owing to its amphipathic nature (Mor *et al.*, 1991). It is unique in its spectrum of antimicrobial activity as it inhibits the growth of pathogenic moulds (Mor *et al.*, 1991).

Simmaco and co-workers (1993) isolated three antimicrobial peptides from the skin of the European green frog (*Rana esculenta*). Two of these peptides, brevinin 1-E and 2-E, share homology and all three possess a single

C-terminal disulphide bond. These peptides differ in their antibacterial spectrum (Simmaco *et al.*, 1993).

2.5. Plant Peptides

Plants have been shown to combat infections by the production of specific cationic peptides, thionins. For example, barley produces a leaf-specific thionin, BTH6 (Bohlmann *et al.*, 1988). A wide variety of plants produce proteins that belong to the superfamily of highly basic, cysteine-rich peptides, which include thionins and mammalian and insect defensins. These include peptides from the seeds of *Amaranthus caudatus*, Ac-AMP (Broekaert *et al.*, 1992) and *Mirabilis jalapa*, Mj-AMP (Cammue *et al.*, 1992). As with defensins, these plant peptides are toxic to fungi and are more active against Gram-positive bacteria than Gram-negative bacteria.

2.6. Peptides from Other Species

There are various other antimicrobial peptides that do not possess homology to any other family of peptides. Bovine seminalplasmin, a 47-amino acid protein, has been studied to identify the residues responsible for its antibacterial activity. A synthetic peptide corresponding to a 13-amino acid hydrophobic region has been found to possess the same activity as the intact protein (Sitaram and Nagaraj, 1990).

Bacteria also naturally produce antimicrobial peptides. The Gram-positive bacterium *Staphylococcus epidermidis* produces a tricyclic antibiotic pep5, containing the unusual amino acids dehydrobutyrine, lanthionine and 3-methyllanthionine (Kaletta *et al.*, 1989; Weil *et al.*, 1990). This molecule is classified into a group of peptide antibiotics termed lantibiotics that are synthesized using multienzyme complexes rather than ribosomes and mRNA templates. Also included in this group is nisin, a bacteriocin from *Lactococcus lactis* (Hurst, 1981). Antibacterial peptides have also been isolated from fungi. *Rhizomucor pusillus*, a thermophilic fungus, produces a defensin-like peptide, sillucin, that is active against Gram-positive bacteria (Bradley and Somkuti, 1979).

Horseshoe crabs (*Limulus polyphemus*, *Carcinoscorpius rotundicauda* and *Tachypleus gigas*), in response to bacterial infection, produce two classes of antimicrobial peptides, tachyplesins and polyphemusins, which are contained in cytoplasmic granules (Miyata *et al.*, 1989; Muta *et al.*, 1990; Ohta *et al.*, 1992). Tachyplesins contain 17 amino acids with a C-terminal arginine amide; polyphemusins consist of 18 residues. Both groups contain cysteines that participate in two disulphide linkages. These peptides are abundant within the haemolymph and act on Gram-negative and Gram-positive bacteria and fungi (Nakamura *et al.*, 1988).

Recently, a class of peptides produced in porcine leukocytes has been identified and shown to possess not only the characteristics of tachyplesin but also of the mammalian defensins (Kokryakov *et al.*, 1993). Protegrins, as they are called, consist of 16–18 residues and contain two intramolecular disulphide linkages.

3. STRUCTURE–FUNCTION RELATIONSHIPS

3.1. Structure of Polycationic Peptides

There are basically two major structural classes of natural polycationic peptides: those that form an α-helical structure in membranes, but are often disordered in aqueous solution, and those that form an antiparallel β-sheet containing β-hairpin turns (Figs 1, 2). The former helical structure often comprises a helix–turn–helix arrangement with a 9–16 amino acid amphiphilic α-helix near the N-terminus, a 2–4 residue turn and an 11–14 amino acid hydrophobic helix near the C-terminus (Fig. 1A). Examples of peptides that have been shown by two-dimensional nuclear magnetic resonance (NMR) to possess such a configuration include cecropins A and B (Holak *et al.*, 1988), melittin (Bazzo *et al.*, 1988), the magainins (Marion *et al.*, 1988) and a synthetic cecropin–melittin hybrid (Sipos *et al.*, 1991). Another variation on the theme is provided by cecropin P1 which comes from the pig intestine. This seems to have an uninterrupted amphiphilic helix of 24 residues bounded

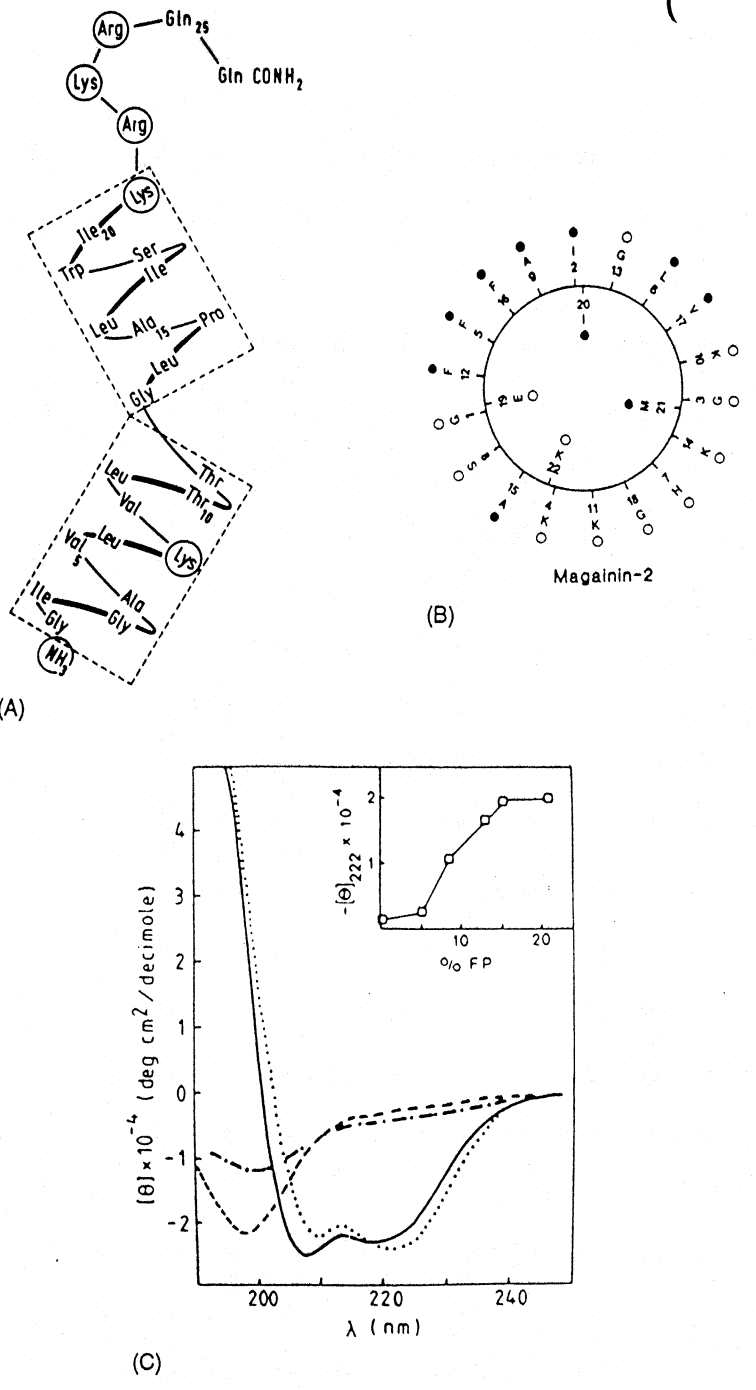

Figure 1 Structure of the α helical peptides. (A) Monomeric membrane-associated melittin showing the helix–hinge–helix structure. Charged residues are circled. (Reproduced by copyright permission from Vogel and Jähnig (1986). © Biophysical Society.) (B) Helical wheel diagram (axial projection) for magainin-2 demonstrating the amphipathic nature of the α helix. Solid circles represent hydrophobic residues and open circles represent hydrophilic residues including the five clustered lysine (K) residues. Single letter code is used for amino acids. (Reproduced by copyright permission from Kini and Evans (1989) © *International Journal of Peptide and Protein Research.*) (C) Transition in cecropin structure dependent on the suspension medium. Circular dichroism spectra are shown for cecropin A in buffer (- - -; showing largely random coil structure); cecropin A in 20% 1,1,1,3,3,3-hexafluoro-2-propanol (FP) (–; showing approximately 81% α-helix); cecropin B in a liposone containing solution (–·–·; showing approximately 20% helix). A simulated spectrum of cecropin A containing 81% α-helix, 7% β-sheet and 12% random coil is shown (. . . . .). The inset shows the increase in α-helix content (ellipticity θ at 222 nm) as a function of the % FP (reflecting the hydrophobicity of the environment). (Reproduced by permission of Steiner (1982) © Federation of European Biomedical Societies.)

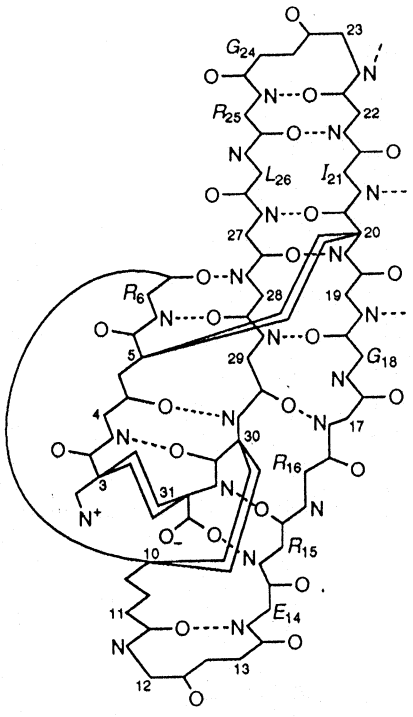

Figure 2 Model of the mammalian defensin structure. The triple stranded anti-parallel β-sheet structure of an HNP-3 monomer. The disulphide bonds are represented as "lightning bolts". Charged residues are indicated as R = arginine and E = glutamate. (Reproduced by copyright permission of Hill *et al.* (1991) © American Association for the Advancement of Science.)

by 2–4 residues at the N- and C-termini (Sipos *et al.*, 1992). Several other peptides can be fitted in part to a helical wheel diagram (Kini and Evans, 1989) which shows a tendency to form an α-helix with one hydrophobic face and one positively charged face (Fig. 1B). In the case of cecropins A and B and magainins, it has been determined that the peptides are random in aqueous solution and 80% α-helical in organic solvents (Fig. 1C) (Steiner, 1982; Marion *et al.*, 1988; Bechinger *et al.*, 1992).

The second class of structures, typified by the defensins, shows a disulphide-linked β-sheet structure (Zhang *et al.*, 1992). This structure (Fig. 2) contains an antiparallel β-sheet in addition to a short region of triple-stranded β-sheet, with the β-strands interconnected by β-turn regions. Defensins have been crystallized (HNP3) and studied by two-dimensional NMR techniques (HNP1, NP2, NP5) with quite similar results (Pardi *et al.*, 1988; Hill *et al.*, 1991; Zhang *et al.*, 1992), despite differences in amino acid composition including numbers of basic amino acids. From a three-

Table 2 Influence of selected amino acid changes on the minimal inhibitory concentrations (MIC) of synthetic cecropin A analogues. Data selected from Andreu *et al.* (1985).

	MIC (μM)			
Cecropin A analogue	*E. coli*	*P. aeruginosa*	*B. megaterium*	*M. luteus*
Native	0.4	2.6	0.6	1.4
Lys^1 Trp^2 deletion	2.6	90	13	>110
$Trp^2 \rightarrow Phe^2$	0.3	3.5	0.8	7.4
$Trp^2 \rightarrow Glu^2$	3.2	170	39	>170
$Leu^4 \rightarrow Pro^4$	0.4	8.1	11	87
$Lys^6 \rightarrow Leu^6$	0.6	120	0.8	7.3
$Lys^6 \rightarrow Glu^6$	0.6	34	2.2	4.7
$Ile^8 \rightarrow Pro^8$	0.5	15	31	80

dimensional perspective, defensins have a predominant hydrophobic face with the charged residues spread out along one face of the structure (Zhang *et al.*, 1992). NMR evidence suggests that they dimerize. The insect defensin, sapecin, has, like animal defensins, three disulphide bonds, but differs substantially in sequence and by the presence of lysines and histidines as positively charged amino acids in the former (Hanzawa *et al.*, 1990). Moreover, the solution structure of sapecin is quite different, containing a flexible loop, an 8-amino acid residue α-helix and two extended regions (Hanzawa *et al.*, 1990). This difference in structure has been attributed to the different arrangement of cysteine disulphides in the animal and insect defensins. However, scorpion toxins with an arrangement of disulphides similar to the animal defensins have a hybrid structure containing both a short region of triple-stranded β-sheet as well as a 9-amino acid α-helix (Bontems *et al.*, 1991). It is important to note that sapecin has, like the animal defensins, a hydrophobic surface and a region rich in basic residues.

3.2. Structure–Function Studies

Several studies have examined the influence of deletion or modification of specific amino acids on the activity of specific families of peptides. One example is the cecropins (Table 2). This and other studies have revealed the following general principles. (1) There is considerable specificity in the way that changes in amino acid sequence influence activity (Andreu *et al.*, 1985; Blondelle and Houghten, 1991). For example, introduction of a turn-promoting proline at positions 4 or 8 in the first α-helical segment of cecropins has a substantial effect on activity against *M. luteus*, lesser effects on *B. megaterium* and *P. aeruginosa*, and no effect against *E. coli* (Table 2).

(2) For the α-helical peptides, changes that increase the tendency to form an α-helix in aqueous solution tend to increase activity (Andreu *et al.*, 1985; Steiner *et al.*, 1988; Frohlich and Wells, 1991; Blondelle and Houghten, 1991). (3) There is no absolute relationship between the numbers of positive charges and activity, although the position of specific positive charges is important (Blondelle and Houghten, 1991). (4) Enantiomers (i.e. all D-amino acids *vs*. all L-amino acids) have equal activity (Bessalle *et al.*, 1990; Wade *et al.*, 1990) showing that chirality is not important. (5) There is no absolute relationship between ability to lyse liposomes or ability to bind to bacterial cells and minimal inhibitory concentration (MIC) against bacteria, although trends are observable (i.e. decreased lysis or binding tends to correlate with decreased MIC) (Steiner *et al.*, 1988). (6) Finally, for the disulphide-bonded peptides, reduction of the cysteine disulphides destroys activity (Kagan *et al.*, 1990).

Three studies have indicated general methods of enhancing activity. The reduction in size of cecropin–melittin hybrids from 26 amino acids to 14 amino acids did not influence activity so long as these compounds maintained an α-helical structure (Andreu *et al.*, 1992). In a study of magainins, it was demonstrated that the addition of 10 or more basic amino acids to the N- or C-termini, but not 4 basic or 10 non-polar amino acids, resulted in a 10-fold enhancement of antibacterial activity (Bessalle *et al.*, 1992). In contrast, addition of two positive charges to the carboxy terminus (hydrophobic domain) of a cecropin–melittin hybrid protein actually decreased the MIC for some bacteria while enhancing the interactions with endotoxin and with bacterial outer membranes (Piers *et al.*, 1994). As above, treatments that enhanced α-helicity also increased activity. In a third study, it was demonstrated that human defensin HNP4 had increased hydrophobicity compared with other human defensins and a 100-fold greater potency against *E. coli* (Wilde *et al.*, 1989).

4. INTERACTIONS WITH LIPIDS AND MEMBRANES

4.1. Lipid Interactions

Where studied, antibacterial peptides undergo a conformational change on interaction with liposomes and/or apolar solvents (Knoppel *et al.*, 1979; Andreu *et al.*, 1985; Lee *et al.*, 1986; Marion *et al.*, 1988; Williams *et al.*, 1990; Agawa *et al.*, 1991; Bechinger *et al.*, 1992; Jackson *et al.*, 1992). The process is initiated by binding of the positively charged peptides to lipids (Batenburg *et al.*, 1987; Matsuzaki *et al.*, 1991; Sekharam *et al.*, 1991). Binding to negatively charged lipids is extremely rapid (Sekharam *et al.* 1991). The extent of binding corresponds to the zeta potential of the lipids involved and is inhibited by salt, leading one to conclude that it is electrostatic in nature (Matsuzaki *et al.*, 1991; Sekharam *et al.*, 1991). In contrast, binding to zwitterionic lipids is slower and, in the case of melittin, demonstrates negative cooperativity (presumably because the interaction of the cationic peptides with the surface of such lipids increases the surface positive charge, causing charge repulsion of other peptide molecules; Sekharam *et al.*, 1991).

Subsequently, the cationic peptides insert into the lipid bilayer (in many cases under the influence of an appropriate membrane potential (Cruciani *et al.*, 1991; de Kroon *et al.*, 1991)) and undergo a conformational change. In the case of the "α-helical" peptides such as melittin (Vogel and Jähnig, 1986), magainins (Bechinger *et al.*, 1992; Williams *et al.*, 1990) and cecropins (Andreu *et al.*, 1985), the transition is from unstructured or β-sheet conformation to α-helix. At the same time, the lipids themselves undergo changes in phase and/or motion (Smith *et al.*, 1992). In some cases these peptides are thought to end up spanning the bilayer (Steiner, 1982; Vogel and Jähnig, 1986; Sipos *et al.*, 1992; Andreu *et al.*, 1992) although, as discussed below, they appear to form channels owing to assembly into multimeric complexes. Other peptides are considered to be too short to span the bilayer and, in these cases, aggregation or multimerization may be critical to permit the spanning of membranes and channel formation (Williams *et al.*, 1990; Agawa *et al.*, 1991; Andreu *et al.*, 1992).

In many cases (Lee *et al.*, 1986; Steiner *et al.*, 1988; Katsu *et al.*, 1990; Frohlich and Wells, 1991; Matsuzaki *et al.*, 1991; Grant *et al.*, 1992) it has been demonstrated that interaction with biomembranes leads to leakiness (i.e. permeabilization) of these membranes and, with more extreme treatments, lysis. A common assay for measuring liposome leakiness is leakage of carboxy fluorescein. There is a general correlation between ability to disrupt model liposomes and activity against the most sensitive target bacteria (Steiner *et al.*, 1988; Agawa *et al.*, 1991). In more complex eukaryotic cell membranes, Bashford *et al.* (1986) argued for a common mechanism of membrane damage by cationic proteins and peptides, as well as complement, viruses, toxins and detergents. They found the following common features of these agents when applied to Lettre cells: sensitivity to changes in ionic strength and divalent cations, positive cooperativity, synergy between diverse agents and a nearly identical sequence of permeability changes. These authors concluded that the action of these agents (including melittin and polylysine) was a detergent-like disruption of permeability, although this presumably reflects the lytic action of these agents as distinct from the formation of defined channels, as discussed in the next section. Kini and Evans (1989) also suggested that peptides that function as cytolysins have common features. Another factor that has been suggested to enhance lytic capability is the spontaneous aggregation of magainin 2 and a melittin analogue into large oligomers (Urrutia *et al.*, 1989; John and Jähnig, 1992).

4.2. Planar Lipid Bilayer Studies

Several cationic peptide channels have been examined in planar lipid bilayers. In this system a lipid bilayer is constituted across a hole in a teflon divider separating two aqueous compartments, each of which contains an electrode. Addition of specific cationic peptides to one of the aqueous compartments (e.g. the cis side), and application of a negative voltage to the trans side (such that the positive ions would tend to move from the cis to the trans compartments), leads to an observed increase in conductance as the cationic peptides enter the membrane and form channels (Hanke *et al.*, 1983; Christensen *et al.*, 1988; Kordel *et al.*, 1988; Kagan *et al.*, 1990; Cociancich *et al.*, 1993a). In several cases studied, the reversal of the sign of the voltage not only prevents or substantially decreases the rate of formation of channels, but actually results in an exponential decrease in the conductance of membranes into which peptides had already been inserted, with a half-time of around 30 s (Christensen *et al.*, 1988). Thus, channel formation may actually be "driven" by electrophoresis of the cationic peptide towards the membrane and reversed by electrophoresis towards an aqueous compartment. This is consistent with the situation in bacterial cytoplasmic membranes, in which the $\Delta\psi$ (electrical potential gradient) is oriented interior-negative (see below). Alternatively, Kordel *et al.* (1988), on the basis of chemical modification experiments, suggested that the requirement for a trans-negative voltage for Pep5 channel formation reflected the orienting action of the transmembrane voltage.

Formation of channels is generally voltage-dependent, as observed with both the β-structured defensins (Kagan *et al.*, 1990) and the helix–turn–helix structured melittin (Tosteson *et al.*, 1987), cecropin (Christensen *et al.*, 1988) and magainin (Duclohier *et al.*, 1989) peptides. In one case, that of Pep5, there is actually a threshold (turn-on) potential of approximately -100 mV that must be applied before channel formation is observed (Kordel *et al.*, 1988). However, this is unusual. Far more usual is the observation that, as the voltage increases, there is an exponential, rather than linear, increase in current. This could be due to voltage-induced gating (i.e. opening of channels), or the effects of voltage on the rate of channel formation or the rate of aggregation of channels in the membrane. The voltage dependence of the formation of α-helical peptide channels may be related to the existence of a flexible (turn) segment between the N-terminal amphipathic and the C-terminal hydrophobic regions, because synthetic variants of the cecropins, which lacked the turn segment, were not voltage-dependent (Christensen *et al.*, 1988).

The interaction of the peptides with membranes is related to the charge and folding of the cationic peptides. Thus, succinylated Pep5 forms channels with a greater voltage dependence (Kordel *et al.*, 1988), whereas reduced and carboxymethylated defensins do not form channels (Kagan *et al.*, 1990).

In addition, positively charged phospholipids and cholesterol decrease cecropin channel formation by 5- and 60-fold, respectively (Christensen *et al.*, 1988). Since eukaryotic membranes are rich in cholesterol (which affects both the fluidity and dipole potential of bilayers), this may explain in part the selectivity of several cationic peptides for bacterial cells, which lack cholesterol and have very low levels of positively charged phospholipids.

Examination of the influence of peptide concentration on the conductance of membranes reconstituted with defensins or melittin reveals a linear relationship when conductance induced by the peptide is plotted as a function of peptide concentration, with a slope of 1.5 to 4 pS/mg ml^{-1} (Tosteson *et al.*, 1987; Kagan *et al.*, 1990). This suggests that the functional units are oligomers rather than monomers (in this latter case, a slope of 1 would be predicted). Where examined, these channels have a weak preference for chloride over sodium (i.e. 2:1) (e.g. Kagan *et al.*, 1990; Kordel *et al.*, 1988). This means that the positive charges of the individual subunits of the oligomeric channel must be distant from one another, because closely spaced charges would tend to make the channel anion-specific. This could be caused by the formation of large channels, as suggested by certain authors (Kordel *et al.*, 1988; Christensen *et al.*, 1988), with the charges spaced at distances of up to 1 nm (i.e. similar to the spacings observed for the weakly selective bacterial porins; Cowan *et al.*, 1992).

When the increases in current are examined more closely, they can be resolved into smaller increments, i.e. single channels. Single channel conductances for a given cationic peptide generally vary between 10 and 2000 pS (Hanke *et al.*, 1983; Kordel *et al.*, 1988; Duclohier *et al.*, 1989; Kagan *et al.*, 1990), the latter being similar to the value for porins (Benz *et al.*, 1985) (cf. the cecropins, Christensen *et al.*, 1988; Wade *et al.*, 1990). This multistate channel behaviour has been observed for the channel-forming peptide alamethicin (Boheim, 1974), for which it has been proposed that after monomers of alamethicin are induced by voltage to span the membrane, the monomers then associate or dissociate with various rate constants, resulting in aggregates of different sizes or lifetimes. These aggregates are then proposed to align like the staves of a barrel, with a central channel that represents the conducting pore. The size of this pore and the resultant conductance would depend on the number of monomers (staves) making up the conducting unit. The lifetime of individual channels is in the order of several milliseconds to seconds (e.g. Kordel *et al.*, 1988).

A credible model to explain the above events for cecropins is presented in Fig. 3. This suggests alignment of the positive charges of cecropins with the negatively charged lipid head groups, followed by insertion of the hydrophobic segment into the membrane, and a major (voltage-induced) conformational rearrangement which results in channel formation. This is rather analogous to the proposal for alamethicin (Boheim, 1974).

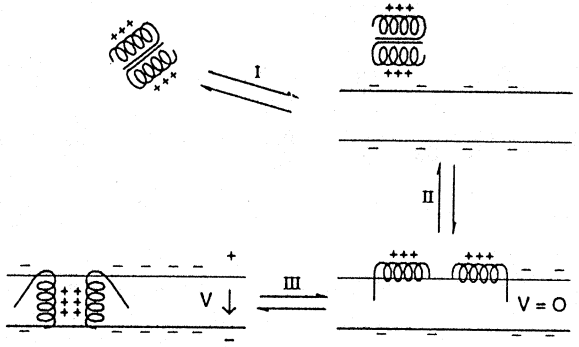

Figure 3 Tentative model for the interaction of cecropins with a lipid bilayer membrane. Aggregates adsorb to the bilayer–water interface by electrostatic forces. (I). Only a dimer is sketched for the sake of simplicity, but larger aggregates are likely to occur. The next step (II) would be insertion of the hydrophobic segment into the membrane core. Upon application of voltage (positive on the side of the peptide addition), a major conformational rearrangement takes place (III), which results in channel formation. This rearrangement could be insertion of the positively charged amphipathic helix into the membrane or opening of preformed, closed channels. (Reproduced by copyright permission from Christensen *et al.* (1988) © The National Academy of Sciences of the United States of America.)

4.3. Channel Structure in Lipids

As discussed above, several of the cationic peptides undergo a conformational change to an α-helical configuration when placed in solvents with reduced water activity (i.e. ones that create a hydrophobic membrane-like environment), such as hexafluoropropanol. An ability to form discrete conductance units (channels) as opposed to an erratic increase in membrane conductance was found only for those α-helical model peptides that were long enough to span the membrane (i.e. >20 residues, Agawa *et al.*, 1991). In the case of melittin, there is a glycine at residue 12 that causes a kink in the α-helix, giving the resultant channel the appearance of a tetramer of bent α-helices with charged and hydrophilic residues pointing into the channel of the tetramer, and hydrophobic residues facing the non-polar membrane core (Fig. 4), as modelled from Raman spectroscopy and fluorescence transfer data (Vogel and Jähnig, 1986). The cecropins, which are thematically similar to melittin, have been modelled by Durell *et al.* (1992) at an atomic scale. They propose two types of channels comprising a star-shaped arrangement of six dimers with a 0.56 nm internal channel, and a circular arrangement of six dimers with a 1.1–1.5 nm channel, a model consistent with the two discrete conductance increments (0.4 and 1.9 nS) reported by Christensen *et al.* (1988).

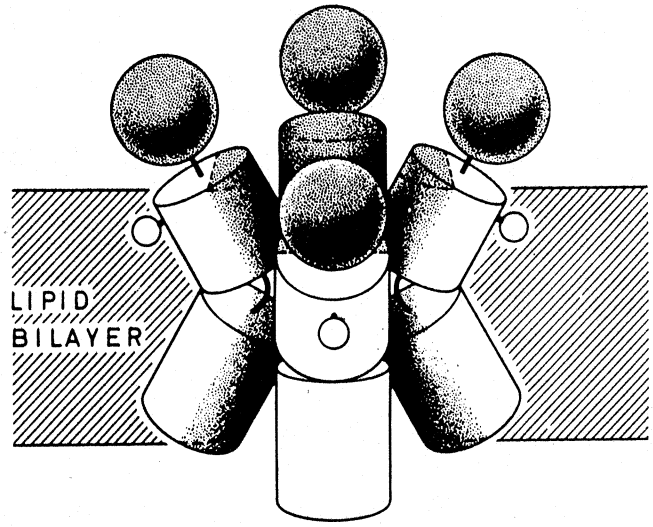

Figure 4 Model of a membrane-associated tetramer of melittin containing a central channel. The orientation of each melittin monomer is identical to that depicted in Fig. 1C. The shaded areas represent hydrophilic amino acid residues and the unshaded areas hydrophobic residues. (Reproduced by copyright permission from Vogel and Jähnig (1986) © Biophysical Society.)

Williams *et al.* (1990) have used Raman spectroscopy to follow the interaction of magainin 2a and PGLa with negatively charged liposomes. The basic interaction scheme proposed was quite similar to that based on planar bilayer studies.

4.4. Interactions with Bacterial Outer Membranes

The outer membranes of Gram-negative bacteria constitute a semi-permeable barrier to penetration of substances from the external medium (Nikaido and Vaara, 1985; Hancock, 1987, 1991; Benz, 1988; Vaara, 1992). The existence of channel-forming proteins, called porins, gives outer membranes their characteristic size-dependent exclusion limit. Thus, with one prominent exception, hydrophilic substances below a certain size can permeate the weakly ion-selective, chemically non-selective water-filled channels of these porins, whereas substances exceeding this size will not. For *E. coli*, for example, it has been suggested that substances equal to, or larger than, tetrasaccharides (e.g. stachyose) or pentapeptides (e.g. pentalysine) will diffuse very slowly or not at all across the outer membrane (Payne and Gilvarg, 1968; Nikaido and Vaara, 1985). In addition, many Gram-negative

bacteria limit the rate of uptake of hydrophobic substances by virtue of the tight packing and divalent cation stabilization of their surface glycolipid, lipopolysaccharide (LPS) molecules (Hancock, 1984; Nikaido and Vaara, 1985). An exception to these generalizations is provided by the cationic peptides described here, which are too large and bulky to pass through the porins of, for example, *E. coli*, whose crystal structure is now known (Cowan *et al.*, 1992). Instead, these molecules have been proposed to cross the outer membrane via a non-porin pathway termed the self-promoted uptake pathway (Hancock, 1984, 1991; Sawyer *et al.*, 1988).

Early studies on polymyxin B, a cationic cyclic peptide with a fatty acyl tail, suggested that it interacted with Gram-negative outer membranes, causing structural perturbations and increased outer membrane permeability (Schindler and Teuber, 1975). To explain these data and results with a mutant of *P. aeruginosa* that was cross-resistant to EDTA, aminoglycosides and polymyxins, it was proposed that these compounds promoted their own uptake across the outer membrane (Nicas and Hancock, 1980; Hancock *et al.*, 1981; Young *et al.*, 1992). This hypothesis was later extended to embrace the cationic peptides (Hancock, 1984; Sawyer *et al.*, 1988), melittin and two other α-helical peptides (Piers and Hancock, 1994; Piers *et al.*, 1994).

Self-promoted uptake is initiated by the interaction of the cationic antibiotic with anionic, divalent cation-binding sites on LPS. Direct interaction of defensins (Sawyer *et al.*, 1988), magainins (Rana *et al.*, 1991) and melittin (David *et al.*, 1992) with LPS has been demonstrated. Using dansyl polymyxin as a probe, it was shown that the interaction of defensins (Sawyer *et al.*, 1988), melittin and two cecropin–melittin-derived hybrids (Piers and Hancock, 1994; Piers *et al.*, 1994) occurs at divalent cation-binding sites on LPS. However, the above peptides, for example, have binding affinities that are three orders of magnitude higher than the normally resident divalent cations, making binding to this LPS site an efficient process. Interestingly, the kinetics of probe displacement by defensins from purified LPS exactly mirrored the kinetics of displacement from intact cells, suggesting that LPS binding could explain binding to Gram-negative bacteria. It was demonstrated that addition in CEMA of two positive charges to the carboxyl terminus of a cecropin melittin hybrid CEME, enhanced the interaction of CEMA with LPS and with the outer membrane (Piers *et al.*, 1994). By Fourier transform-infrared spectroscopy studies of *S. typhimurium* mutant LPS molecules of different chain lengths, it was concluded that magainin interaction with LPS (and with the cells from which these LPS molecules are derived) depends on the magnitude of LPS charge rather than chain length *per se* (Rana *et al.*, 1991). ^{31}P-NMR studies were consistent with others that suggest that the LPS binding sites comprise negatively charged phosphate residues (Rana *et al.*, 1991; Peterson *et al.*, 1985, 1987; Schindler and Osborn, 1979), of which there are 5–15 per molecule of LPS.

Interaction of cationic substances, including magainins, with purified LPS causes the LPS to undergo a conformational change including an alteration in the mobility of both the hydrophilic portion and the fatty acyl chains (Peterson *et al.*, 1985, 1987). This disorganization can have one of two observable consequences. In electron micrographs of cells treated with sarcotoxin (Okada and Natori, 1984) or defensins (Sawyer *et al.*, 1988; Lehrer *et al.*, 1989), it was manifested as structural perturbations that have been visualized in one case as blebbing of the outer membrane (Sawyer *et al.*, 1988). Similar blebbing has been observed in cells treated with the cationic antibiotic polymyxin and some, but not all, other polycations (Gilleland and Murray, 1976; Vaara and Vaara, 1983; Vaara, 1992). In some cases, LPS is actually released from cells by treatment with polycations (Vaara and Vaara, 1983), although we consider this to be a more extreme manifestation of the blebbing phenomena and one that generally occurs at higher polycation concentrations. It has been proposed that these structural perturbations reflect the formation of transient cracks (Martin and Beveridge, 1986). A more easily assayed phenomenon is breakdown of the outer membrane permeability barrier, which has been assessed as increased permeation of hydrophobic fluorescent probes such as 1-N-phenylnapthylamine, which are normally excluded (Hancock and Wong, 1984; Sawyer *et al.*, 1988; Piers *et al.*, 1994), lysozyme (Piers and Hancock, 1994) and chromogenic β-lactams such as pyridium-2-azo-p-dimethylanaline cephalothin (PADAC) and nitrocefin, which normally have limited access to periplasmic β-lactamase (Sawyer *et al.*, 1988; Lehrer *et al.*, 1989; Skerlavaj *et al.*, 1990). The ability of cationic peptides to promote their own uptake has not as yet been formally demonstrated, although this seems to be a plausible hypothesis.

It must be stated that ability to permeabilize the outer membrane to probe molecules is not equivalent to self-promotion of uptake. As discussed in detail by Vaara (1992), molecules such as the deacylated derivative of polymyxin B, polymyxin B nonapeptide (PMBN), are effective at permeabilizing outer membranes at concentrations orders of magnitude below the MIC. This probably reflects the inability of PMBN to transfer to and/or from channels in the cytoplasmic membrane, since its parent compound polymyxin B has a similar outer membrane permeabilizing concentration (0.3–1 μg/ml) but a far lower MIC (1 *vs.* $\geq$300 μg/ml). Consistent with this proposal, polymyxin B but not PMBN causes voltage-dependent channels in asymmetric planar bilayers (Schroeder *et al.*, 1992). Thus, it is not surprising that human defensins (which carry three to four net positive charges) permeabilize the outer membrane of *E. coli* at concentrations close to the minimal growth inhibitory concentration (Sawyer *et al.*, 1988; Vaara *et al.*, 1988). Similar results were observed for a cecropin–melittin hybrid peptide CEME (Piers and Hancock, 1994). Such results are consistent with the concept that self-promoted passage across the outer membrane is the rate-limiting step for many of these peptides. In the case of rabbit defensins, at low pH values which inhibited the killing of cells (Lehrer *et al.*, 1983), permeabilization

actually increased (Sawyer *et al.*, 1988). These data suggest a potential relevance *in vivo* for the phenomenon of permeabilization, because the release of defensins into phagocytic vacuoles containing bacteria, i.e. phagosomes, is accompanied by rapid vacuole acidification (Cech and Lehrer, 1984). Thus, permeabilization at low pH may be required to permit penetration of other potentially bactericidal substances that would otherwise be excluded by the outer membrane. Permeabilization may also have clinical relevance. Darveau *et al.* (1991) demonstrated that magainins were therapeutically ineffective against systemic *E. coli* infections, but worked synergistically with sub-inhibitory doses of the β-lactam antibiotic, cefepime.

Some cationic peptides are relatively ineffective against Gram-negative bacteria. Three of these, nisin, mastoparan and melittin, have been shown to have enhanced efficacy in wild-type cells after treatment of outer membranes with EDTA or, in two cases against mutant cells, with truncated lipopolysaccharides (Katsu *et al.*, 1985; Rana *et al.*, 1991; Stevens *et al.*, 1992). These treatments would be expected to enhance the accessibility of LPS-binding sites; indeed, melittin has been shown to bind with reasonably high affinity to lipid A ($K_d = 2.5 \times 10^{-6}$ M; David *et al.*, 1992) and LPS (Piers *et al.*, 1994). Thus, the reason for the limited or poor activity of these and similar compounds against Gram-negative bacteria is that they have limited ability to access divalent cation-binding sites on cell surface LPS and thus cannot initiate cooperative binding/permeabilization. A similar explanation was used to explain the generalized polycation resistance of *P. cepacia* (Moore and Hancock, 1986).

The intrinsic resistance of some strains of *S. typhimurium* to the peptides melittin and protamine has been found to be determined by an ATP-binding cassette (ABC) transporter SapABCDF encoded by the *sapABCDF* gene (Parra-Lopez *et al.*, 1993). The proteins SapBCDF are thought to be associated with the inner membrane while SapA is believed to be a periplasmic binding protein. Resistance requires the presence of all five subunits and is believed to be involved in transport of the toxic peptide to the cytoplasm.

4.5. Bacterial Cytoplasmic Membranes

The cytoplasmic membranes of Gram-negative bacteria have a major role in maintaining cytoplasmic integrity, vectorial transport of substrates into and out of the cytoplasm, exclusion of many non-substrate molecules, synthesis and export of molecules found external to the cytoplasmic membrane, generation and maintenance of cellular energization including synthesis of ATP and macromolecules, maintenance of a transmembrane proton gradient, and energization of transport (Cronan *et al.*, 1987). As described above,

cationic peptides form weakly anion-selective channels in planar lipid bilayers. Thus, it is no surprise that these peptides have a dramatic effect on bacterial cytoplasmic membrane integrity and that their antibacterial action probably, in part, reflects this, despite the rather complex phenotypic changes that arise from altered cytoplasmic membrane integrity.

Bacteria maintain across their cytoplasmic membranes a protonmotive force of approximately −170 mV (Bakker and Mangerich, 1981). According to Mitchell's chemiosmotic hypothesis, this protonmotive force comprises two individual forces that reflect the properties of protons, namely the electrical potential gradient, $\Delta\psi$, and ΔpH. These gradients are oriented so that the cytoplasm is negatively charged and alkaline (pH 7.8) relative to the external face of the cytoplasmic membrane. Treatment of cells with cations such as magainins (Juretic *et al.*, 1989; Westerhoff *et al.*, 1989), sarcotoxin (Okada and Natori, 1985b), insect defensin (Cociancich *et al.*, 1993a), nisin or pep5 (Kordel and Sahl, 1986) leads to dissolution of the $\Delta\psi$ as revealed by increased uptake of the lipid soluble cation triphenyl phosphonium. This apparently occurs at concentrations approaching the minimal effective concentration. The decrease in the protonmotive force is also manifest as an increased respiration rate as the cells attempt to compensate with an increase in respiration-driven proton pumping (Juretic *et al.*, 1989). This increased respiration rate occurs as a sigmoidal function of peptide concentration suggesting that magainins act in a cooperative fashion on cytoplasmic membranes. Another manifestation of this breakdown in cell integrity is K^+ leakage that has been shown to occur on treatment of Gram-positive bacteria with mastoparan or melittin (Katsu *et al.*, 1990). Mastoparan is relatively ineffective against Gram-negative bacteria. However, destruction of outer membrane integrity with EDTA causes a similar effect on susceptibility to killing and K^+ release in Gram-positive and Gram-negative bacteria (Katsu *et al.*, 1990). These data strongly support the hypothesis that loss of cytoplasmic membrane integrity is responsible for cell death, and that the resistance of Gram-negative bacteria to mastoparan is mediated by the outer membrane.

One phenomenon associated with cationic peptides is their generally weaker antibacterial activity at low pH (5.5) compared with that at mid-range pH values (7.5) (Lehrer *et al.*, 1983; Kordel *et al.*, 1988). This is probably not due to effects on the outer membrane, because Sawyer *et al.* (1988) demonstrated that the ability of rabbit defensins to permeabilize the outer membrane actually increases at low pH. One plausible hypothesis might relate to the relatively lower $\Delta\psi$ that exists at low pH, possibly due to a pH-sensitive K^+ pump which maintains the overall magnitude of the protonmotive force by causing a compensatory decrease in $\Delta\psi$ in response to an increase in ΔpH (Yamasaki *et al.*, 1980; Bakker and Mangerich, 1981). The $\Delta\psi$ in this case would be required for uptake of cationic peptides, in

keeping with the data discussed above. Similarly, the role of anaerobiosis and/or energy inhibitors in protecting bacterial cells (Walton and Gladstone, 1976) might reflect decreases in $\Delta\psi$. In the case of the model compound 48/80, it has been shown that membrane permeability changes occur only above the phase transition for *E. coli* lipids, a result consistent with reduced uptake of this polycation into gel-phase lipid bilayers (Katsu *et al.*, 1985).

Loss of cytoplasmic membrane integrity has also been followed by examining uptake of the normally excluded substrate ortho-nitrophenylgalactoside permitting cleavage by the cytoplasmic enzyme β-galactosidase in *E. coli*. However, this phenomenon, which occurs in cells treated with defensins (Lehrer *et al.*, 1989) and seminalplasmin (Sitaram *et al.*, 1992), has been demonstrated only at high concentrations of peptide and, in the case of defensins, after a considerable lag time. Thus it may not reflect a primary action of these cationic peptides. Similarly, the influence of cationic peptides on DNA, RNA and protein synthesis (Lehrer *et al.*, 1989) may reflect a decrease in cellular ATP levels or other secondary manifestations of ion leakage.

Seminalplasmin may lead to cellular lysis through activation of intrinsic autolysin activity (Chitnis *et al.*, 1990). Similar activation of autolytic activity has been observed after treatment with the polycationic antibiotics polymyxin B and the aminoglycosides (Nicas and Hancock, 1980) and with other cationic peptides (Piers *et al.*, 1994).

4.6. Effects on Mammalian Cells

Some of the antibacterial compounds have very weak activity against mammalian cells. Others are quite toxic. For example, indolicidin and, to a lesser extent, bactenecin are strongly toxic to rat and human T lymphocytes (Schluesener *et al.*, 1993). The determinative factor appears to be the ability to bind to (Steiner *et al.*, 1988) and/or enter into (Christensen *et al.*, 1988) the membranes of mammalian cells. In the latter case, the partitioning of peptides into membranes has been shown to depend on lipid charge, lipid composition (Christensen *et al.*, 1988; Sekharam *et al.*, 1991) and/or transmembrane potential (de Kroon *et al.*, 1991). Nevertheless, it is possible to design synthetic compounds with excellent antibacterial activity and weak haemolytic activity (Steiner *et al.*, 1988; Boman *et al.*, 1989). Of assistance may be a detailed comparison of the structural features of peptides that favour cytolytic activity (Kini and Evans, 1989). Another significant therapeutic consideration is the demonstrated ability of polycations to enhance phagocytosis of bacteria (Peterson *et al.*, 1984; Sawyer *et al.*, 1988), whereas the ability of those polycations to bind to lipid A/LPS makes them candidates as anti-endotoxins (since endotoxin = lipid A).

5. OUTLOOK

Cationic peptides represent the first novel antibiotic structures in 20 years. They have several features that confer some advantages over existing antibiotics. They are broad in their antimicrobial spectrum, including action against known antibiotic-resistant clinical isolates, do not induce resistant mutants at measurable frequencies, have bonus activities that include permeabilizer, anti-endotoxin and antifungal activities and can be manufactured recombinantly. We believe that the next 5–10 years will see the first marketing of these antibiotics, and that studies of structure–activity relationships will result in steady improvements of activity. These studies will expand beyond the β-structured and α-helical classes to other classes (loops, tryptophan or proline rich, etc.). Furthermore, intensive future studies should help to define what we believe will prove to be an important and currently undervaluated role in human non-specific defences against infection.

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