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Commonwealth of Australia
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DECLARATION IN SUPPORT

In support of the (Convention) Application made by: THE UNITED STATES OF AMERICA, as represented by the Secretary, United States Department of Commerce

for a patent for an invention entitled: NEW SYNTHETIC BIOACTIVE COMPOUNDS & METHOD OF PRODUCING BIOACTIVE EFFECT

I ~~(We)~~^{XX} Charles A. Bevelacqua

of and care of the applicant company do solemnly and sincerely declare as follows:

a) ~~I am (We are) the applicant(s) for the patent~~

or

b) I am ~~(We are)~~ authorised by the applicant(s) for the patent to make this declaration on its behalf.

~~Delete the following if not a Convention Application~~

The basic application(s) as defined by section 141 (142) of the Act ~~was (were)~~ made

on 4 March 1987 in U.S.A.

on 23 July 1987 in U.S.A.

on 5 August 1987 in U.S.A.

by Michael Alan Zasloff

The basic application(s) referred to in this paragraph ~~is (are)~~ the first application(s) made in a Convention country in respect of the invention the subject of the application.

~~a) I am (We are) the actual inventor(s) of the invention.~~

or

b) Michael Alan Zasloff, of 14431 Woodcrest Drive, Rockville, MD 20853, United States of America

is (are) the actual inventor(s) of the invention and the facts upon which

The applicant company
is (are) entitled to make the application are as follows:

The applicant is the assignee of the invention from the said actual inventor

Springfield,
Declared at Virginia this 17 day of December 1970
THE UNITED STATES OF AMERICA

Signed



Status Director, Office of Federal Patent
Licensing

Declarant's Name Charles A. Bevelacqua

F. B. RICE & CO PATENT ATTORNEYS

This form is suitable for any type of Patent Application. No legalisation required.

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DEPARTMENT OF COMMERCE
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- (57) PGLa: GMASKAGAIAGKIAKVALKAL (NH₂)
XPF: GWASKIGQTLGKIAKVGLKELIQPK

CLAIM

1. A synthetic, substantially pure Magainin having at least in part or in whole the amino acid sequence selected from the group consisting of:

(NH₂)GIGKFLHSAGKFGKAFVGEIMKS(OH),
(NH₂)GIGKFLHSAKKFGKAFVGEIMNS(OH),
(NH₂)GIGKFLHSAKKFGKAFVGEIMN(OH),
(NH₂)IGKFLHSAKKFGKAFVGEIMNS(OH),
(NH₂)GKFLHSAKKFGKAFVGEIMNS(OH),
(NH₂)KFLHSAKKFGKAFVGEIMNS(OH),
(NH₂)FLHSAKKFGKAFVGEIMNS(OH),
(NH₂)LHSAKKFGKAFVGEIMNS(OH),
(NH₂)GIGKFLHSAKKFGKAFVGEIMNS(NH₂), and
(NH₂)GIGKYLHSAKKFGKAFVGEIMNS(NH₂).

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13. A method of treating or controlling microbial infection comprising administering to a host, tissue or material susceptible to ^{or} afflicted with microbial infection an antimicrobial ^{effective} amount of a polypeptide selected from the group consisting of XPF, PGLa, Magainin, and a combination thereof.

PCT

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(54) Title: NEW SYNTHETIC BIOACTIVE COMPOUNDS AND METHOD OF PRODUCING BIOACTIVE EFFECT

(57) Abstract

A new class of broad spectrum bioactive polypeptides termed "Magainin" have been described. These peptides have a molecular weight of about 2500 or less, are highly water soluble, amphiphilic and non-hemolytic. A new method of producing antimicrobial effect by contacting a subject susceptible to microbial invasion or contamination with XPF, PGLa, a Magainin has also been.

This document contains the amendments made under Section 49 and is correct for printing

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1 NEW SYNTHETIC BIOACTIVE COMPOUNDS
2 AND METHOD OF PRODUCING BIOACTIVE EFFECT

3 BACKGROUND OF THE INVENTION

4 The present invention relates generally to
5 bioactive compounds and a new method of producing
6 bioactive effect. More particularly, the present
7 invention is related to a new class of synthetic
8 polypeptides, designated herein "Magainins", which have a
9 broad range of bioactive properties. The new method of
10 producing bioactive effect comprises, contacting a
11 subject susceptible to microbial invasion or
12 contamination with antimicrobial amount of Magainins, XPF
13 or PGLa polypeptides.

14 Prior Art

15 Although "Magainin" family of compounds having the
16 properties as described herein have never heretofore been
17 discovered or known, XPF and PGLa polypeptides have been
18 described in the literature and are characterized by the
19 following amino acid sequence:

20 PGLa: GMASKAGAIAGKIAKVALKAL (NH₂)
21 XPF: GWASKIGQTLGKIAKVGLKELIQPK

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1 A review of XPF and PGLa can be found in Hoffman,
2 et al. EMBO J., 2:711-714 (1983); Andreu, et al., J.
3 Biochem, 149:531-535 (1985); Gibson, et al., J. Biol.
4 Chem., 261:5341-5349 (1986); and Giovannini, et al.,
5 Biochem. J., 243:113-120 (1987). It should be noted that
6 no function or useful property of these peptides has been
7 identified in the literature.

8 SUMMARY OF THE INVENTION

9 It is, therefore, an object of the present
10 invention to provide a novel family of synthetic
11 compounds, termed "Magainins."

12 It is a further object of the present invention to
13 provide a novel class of antimicrobial compounds capable
14 of inhibiting the growth or proliferation of such
15 organisms as gram-positive and gram-negative bacteria,
16 fungi, virus, protozoan species and the like.

17 It is another object of the present invention to
18 provide a new use of a known compound.

19 It is a still further object of the present
20 invention to provide a method of controlling, preventing
21 or eradicating microbial growth or proliferation by
22 contacting the microbes with antimicrobial amount of XPF
23 or PGLa polypeptides.

24 It is yet another object of the present invention
25 to provide a method of producing bioactive effect
26 employing XPF and PGLa in bioactive amounts.

27 Various other objects and advantages will become
28 apparent from the detailed description of the invention.

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DETAILED DESCRIPTION OF THE INVENTION

The above and various other objects and advantages of the present invention are achieved by Magainins which are defined as a class of substantially pure, homogeneous polypeptides composed exclusively of about 25 amino acids, having a molecular weight of about 2500 or less, being water soluble at a concentration of greater than 5mg per ml at neutral pH or in an aqueous solution of physiologic ionic strength, being amphiphilic, surface-seeking and having a broad spectrum of properties at physiologic ionic strength and pH. The Magainins are distinguishable from all other peptides reported in the literature at least with respect to their amino acid sequence. By computer search no analogous peptide with significant homology was found in the NIH protein and nucleic acid Data Banks (GenBank, 1986 Edition). Furthermore, the Magainins cannot be generated through simple substitutions of peptides of other classes with similar properties, such as cecropin, xenopsin, bombesin, mellitin and the like. Hence, Magainins represent a unique family of chemical substances, clearly defined at least in part by their unique amino acid sequence.

Table I shows the amino acid sequence (single letter code) of certain Magainin polypeptides of the present invention.

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TABLE I

Primary Sequence of Magainin PolypeptidesMagainin I: (NH₂)GIGKFLHSAGKFGKAFVGEIMKS(OH)Magainin II: (NH₂)GIGKFLHSAKKFGKAFVGEIMNS(OH)Magainin III: (NH₂)GIGKFLHSAKKFGKAFVGEIMN(OH)

Certain other objects of the present invention are achieved by a method of treating or controlling microbial growth, comprising administering to a host susceptible to microbial infection, or contacting the microbes directly or systemically, with antimicrobial amount of a polypeptide selected from the group consisting of XPF, PGLa and a combination thereof to prevent or eradicate microbial growth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

The term "bioactive" as used herein means having biological effect such as illustrated herein infra.

The term "antibiotic" as used herein means that the polypeptides of the present invention produce effects

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1 adverse to the normal biological functions of the cell,
2 tissue or organism including death or destruction and
3 prevention of the growth or proliferation of the
4 biological system.

5 The term "antimicrobial" as used herein means that
6 the polypeptides of the present invention inhibit,
7 prevent or destroy the growth or proliferation of
8 microbes such as bacteria, fungi, virus and the like.

9 The term "substantially pure" as used herein means
10 as pure as it is possible to obtain by using the methods
11 known to one of ordinary skill in the art.

12 Of course, having described the amino acid
13 sequence of the Magainins, these polypeptides can be
14 routinely synthesized in substantially pure form by
15 standard techniques well known in the art, such as by
16 commercially available peptide synthesizers and the
17 like. Such standard techniques of polypeptide synthesis
18 can be found described in such publications as
19 Merrifield, J. Chem. Soc., 85:2149-2154 (1963);
20 Hunkapillar, et al., Nature, 310:105-111 (1984).

21 It is noted, however, that the amino acid
22 sequences of Magainins listed in Table I show only
23 essential portions required for antibiotic activity. In
24 otherwords, the polypeptides are not limited to the
25 sequences shown in Table I, but must have, at least in
26 part or in whole the amino acid sequence shown in Tables
27 I and II. Of course, various analogs and derivatives of
28 Magainins can be easily predicted, generated and/or
29 prepared by such standard and common methods as NMR
30 (nuclear magnetic resonance), computer modeling and the
31 like and all such analogs or derivatives which are
32 equivalent in structure and function to the Magainins as
33 defined herein are encompassed within the scope of the
34 disclosure contained herein. Some derivatives of

1 Magainin II and their antimicrobial activities, for
2 instance, are shown in Table II. It is clear from the
3 results presented in Table II that the Magainins could be
4 manipulated in various ways, for example, adding a
5 carboxyl-terminal amide, progressively made shorter at
6 the amino terminus, substituted with different amino acid
7 residues at different positions of the molecule and the
8 like so long as they retain or augment antimicrobial
9 potency and/or other biological properties.

10 Table III shows the antimicrobial spectrum of the
11 synthetic Magainin peptides of the present invention.

12 TABLE II
13 Antimicrobial Activity of Truncated Synthetic
14 Derivatives of Magainin II and Other Peptides

15	Derivative (Single Letter Code)	Zone of Inhibition on
16		Lawn of <u>E. coli</u> Y1088
17		Generated by 50ug of
18		the Peptide (mm)
19		
20	Magainin II(a) (NH ₂)IGKFLHSAKKFGKAFVGEIMNS(OH)	10
21	Magainin II(b) (NH ₂)GKFLHSAKKFGKAFVGEIMNS(OH)	10
22	Magainin II(c) (NH ₂)KFLHSAKKFGKAFVGEIMNS(OH)	10
23	Magainin II(d) (NH ₂)FLHSAKKFGKAFVGEIMNS(OH)	2
24	Magainin II(e) (NH ₂)LHSAKKFGKAFVGEIMNS(OH)	1
25	Magainin II(f) (NH ₂)HSAKKFGKAFVGEIMNS(OH)	0
26	Magainin II(g) (NH ₂)GIGKFLHSAKKFGKAFVGEIMNS(NH ₂)	15
27	Magainin II(h) (NH ₂)GIGKYLHSAKKFGKAFVGEIMNS(NH ₂)	7
28	PGLa	10
29	XPF (NH ₂)	15

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TABLE III

Antimicrobial Spectrum of Synthetic Magainin Peptides

Organism(ATCC)	Magainin I	Magainin II	Magainin III
(Minimal Inhibitory Concentration (µg/ml))			

Eschericia coli D31	1-20	1-10	1-10
Acinetobacter caloaceticus(19606)	7-35	1-7	7-35
Shigella sonnei (25931)	7-35	1-7	7-35
Enterobacter sonnei (23355)	7-35	1-7	7-35
Eschericia coli (25922)	7-35	1-7	7-35
Streptococcus pyogenes (19615)	7-35	1-7	7-35
Shigella flexneri (12022)	35-70	7-35	7-35
Citrobacter freundii (8090)	>100	7-35	7-35
Enterobacter aerogenes (13048)	>100	7-35	>100
Klebsiella pneumonia (13883)	>100	7-35	>100
Staphyloccus epidermidis (1228)	>100	7-35	35-70

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TABLE III (Continued)

Antimicrobial Spectrum of Synthetic Magainin Peptides

Organism(ATCC)	Magainin I	Magainin II	Magainin III
(Minimal Inhibitory Concentration (μ g/ml))			

Streptococcus faecalis (19433)	>100	35-70	>100
Pseudomonas aeruginosa (27853)	>100	35-70	35-70
Salmonella typhimurium (14028)	>100	35-70	35-70
Staphylococcus aureus (25923)	>100	35-70	>100
Candida albicans (14053)	>100	35-70	>100
Proteus vulgaris (13315)	>100	>100	>100
Serratia marcescens (8100)	>100	>100	>100

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1 Table III also lists the range of minimal
2 inhibitory concentrations for various organisms assayed
3 in trypticase soy broth. These determinations were made
4 following standard techniques well known in the art and
5 described in such publications as, DIFCO Manual, 10th
6 edition, 1984, pages 78-80.

7 Table IV lists the approximate concentration of
8 Magainins which induces physical lysis of several
9 representative protozoan species. These determinations
10 were made by visual assessment of the treated organisms
11 by light microscopy. Of course, the optimal effective
12 antimicrobial concentration of a particular Magainin can
13 be easily determined in a routine manner without undue
14 experimentation by techniques well known to one of
15 ordinary skill in the art.

16 It is pointed that the addition of a
17 carboxyl-terminal amide to Magainin II results in about a
18 5-fold increase in potency of the peptide in several
19 systems when compared to Magainin II. This increase in
20 activity can be seen against bacteria, fungi, protozoa,
21 and as shown in Table V, against tumor cells. This
22 simple modification illustrates one of several ways of
23 modifying the polypeptides of the present invention.

24 It is clear from the results presented herein
25 supra that the Magainin polypeptides of the present
26 invention have a broad range of potent biological
27 activity including antimicrobial potency. Hence, it is
28 apparent that the Magainins of the present invention
29 allow a method of treating or controlling microbial
30 infection caused by those organisms which are sensitive
31 to Magainin. Such treatment comprises administering to a
32 host or tissue susceptible to or afflicted with microbial
33 infection of an antimicrobial amount of Magainin.

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1 Clearly, due to their antibiotic properties, the
2 Magainins of the present invention can also be used as
3 preservatives or sterilants of materials susceptible to
4 microbial contamination.

TABLE IV

Antiprotozoan Activity of Synthetic Magainin Peptides

Organism	Magainin I	Magainin II	Magainin III
(Concentration required for osmotic lysis ($\mu\text{g/ml}$))			

Paramecium caudatum	5	5	5
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Amoeba proteus	5	5	5
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Euglena gracilis	5	5	5
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TABLE V

Comparison of Potencies of Magainin II
and Magainin II(g)

Organism	MIC(μ g/ml)	
	Magainin II	Magainin II(g)
E, coli D-31	10	2
Pseudomonas aeruginosa	70	15
Saccaromyces cervisiae	50	10
Acanthomeba cstellani	15	3

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1 The substitution of phenylalanine at residue 5 by
2 tyrosine results in a slight decrease in MIC of the
3 derivative compared to Magainin II; however, a
4 compensatory advantage of the tyrosine residue allows
5 coupling of radioactive moieties such as ¹²⁵I and the
6 like with the polypeptide molecule so that such
7 radioactive derivatives can be employed in standard
8 radio-immunoassay procedures to determine Magainin
9 levels.

10 Anti-Protozoan Activity:

11 Magainin II(g) has also been found to be active
12 against the Malarial parasite, Plasmodium gallinaceum.
13 Observations of activity were conducted by direct light
14 microscopy. A sample of female and male zygotic forms of
15 the chicken malarial parasite were suspended in phosphate
16 buffered saline. Magainin II(g) was added to each at 100
17 ug/ml. Within seconds both forms underwent evident
18 physical lysis. A control consisting of the addition of
19 Magainin II(f) had no visible effect. The susceptibility
20 of this strain of malaria suggests that the Magainins may
21 be utilized as an anti-malarial agent in human Plasmodium
22 infections.

23 It may be noted that although at optimum
24 anti-microbial concentrations, Magainins are not
25 cytotoxic, however, at higher concentrations Magainins
26 exhibit cytotoxic activity. This activity is considered
27 a significant attribute of the Magainin peptides in that
28 it permits their use in the killing of animal cells. The
29 activity of these peptides has been tested against a
30 panel of a variety of cells listed in Table VI. As seen,
31 Magainin II(g) immediately permeates a broad spectrum of
32 cell type. The most resistant appears to be the

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1 epthelial cell from the oral mucosa. The most sensitive
2 is a transformed monkey kidney cell. These results
3 indicate the utility of Magainins as therapeutic
4 cytotoxic agents. This includes anti-tumor activity, in
5 vivo cytotoxicity or growth inhibition of malignant cells,
6 such as in leu.emias and selective reduction in specific
7 cell populations (e.g., removal of lymphocytes from
8 blood).

9 Cells were suspended in standard minimal Eagles
10 medium containing 10% fetal calf serum. VERO cells and
11 KB cells were obtained from the American Type Culture
12 Collection. Human diploid fibroblasts were propagated
13 from a primary dermal biopsy. Human lymphocytes were
14 collected from a buffy coat preparation by centrifugation
15 in ficoll-hypaque medium. Human buccal epithelium was
16 obtained by scraping the oral mucosa with a glass
17 cover-slip and resuspending the cellular scraping in
18 MEM. Cytotoxicity was determined by loss of Trypan Blue
19 exclusion, following addition of dye to the medium.
20 Cytotoxicity was assessed 30 minutes after addition of
21 the peptide. Concentration noted represents the
22 concentration of peptide resulting in 50% toxicity as
23 determined visually by light microscopic examination of
24 the treated cells.

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TABLE VI

Cytotoxic Activity of Magainin(g)

<u>Cell</u>	<u>Concentration (µg/ml)</u>
Vero Cells	25
Peripheral Human Lymphocytes	75
Human KB Cells	125
Human Diploid Fibroblasts	250
Human Buccal Epithelial Cells	500

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Spermicidal Activity of Magainin Peptides

The activity of Magainin II(g) was determined against human sperm since the peptides were shown to exhibit cytotoxic activity against a variety of human cells. Magainin II(g) was added in various concentrations to a suspension of human sperm in phosphate buffered saline. Motility was assayed by visual inspection under a light microscope. Observations were conducted at room temperature (about 22°-25°C). The approximate number of motile sperm observed in 20 independent fields was noted as a function of concentration. Magainin II(g) inhibits sperm motility by 50% at 35 ug/ml and by 100% at 75 ug/ml. These results indicate that Magainin II(g) and other derivatives are useful as spermicide and hence, as contraceptive agent. The association of anti-microbial activity of the Magainin peptides along with its spermicidal activity indicates that this family of peptides represents ideal spermicidal agents.

Anti-Viral Activity of Magainin Peptides

The anti-viral activity of Magainin was tested against the presumptive AIDS virus, HTLV III in vitro and has been found in three repeated studies to exhibit anti-viral activity. HTLV III was obtained from culture media from the producing cell line A.3101. The media was diluted with MEM containing 10% fetal calf serum (FCS) to a viral concentration of about 10^4 pfu/ml. Magainin II(g) was added to the viral inoculum (1 ml) to a concentration of 200 ug/ml and incubated at 37°C for 30 min. The viral inoculum was then added to a well containing 10^3 tester cells (cell line, SUPT1) and 1 ml of MEM with 10% FCS. Viral activity was assessed by

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1 formation of syncytia and cell death in the test cell
2 culture as compared with cell cultures unexposed to
3 Magainin peptides. After an incubation period of 2 weeks
4 Magainin-exposed cultures appeared identical to cultures
5 not exposed to virus, while untreated cultures were fully
6 destroyed. Assay of reverse transcriptase activity in
7 the culture fluids of untreated and treated wells
8 revealed that Magainin treated samples exhibited no
9 greater reverse transcriptase activity than uninfected
10 cells, corroborating the visual assessment of cellular
11 protection.

12 These studies demonstrate that the Magainin
13 peptides have the potential to reduce the infectivity of
14 the AIDS virus, HTLV III. The use of the Magainin
15 peptides as a sterilant against this virus or as a
16 chemotherapeutic agent for treating AIDS is thus
17 indicated.

18 The activity of the Magainin peptides against HTLV
19 III in vitro suggests that these peptides will exhibit
20 anti-viral activity against other viral agents. The
21 property of the Magainin peptides to disrupt certain
22 membrane structures leads to the obvious application of
23 these peptides against other membrane enclosed viruses,
24 of which HTLV III is only a representative organism.
25 Other candidates include herpes viruses, hepatitis B
26 virus, retroviruses such as feline leukemia virus and
27 HTLV I, and influenza virus.

28 Of course, pharmaceutical compositions comprising
29 Magainin polypeptides of the present invention as an
30 active ingredient in an amount sufficient to produce
31 antibiotic effect in susceptible host or tissue and a
32 pharmaceutically acceptable, non-toxic sterile carrier
33 can be easily prepared based on the data provided herein.

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1 Such carriers could be fillers, non-toxic buffers,
2 physiological saline solution and the like. The
3 preparation can be used topically or systemically and may
4 be in any suitable form such as liquid, solid or
5 semi-solid, which include injectable solutions, tablets,
6 ointments, lotions, pastes, capsules and the like. Of
7 course, the Magainins may also be administered in
8 combination with other adjuvants, protease inhibitors, or
9 compatible drugs where such combination is seen desirable
10 or advantageous in controlling the infection caused by
11 harmful microorganisms including protozoa, viruses and
12 the like.

13 Table VII shows the minimal inhibitory
14 concentrations of XPF, PGLa and Magainin(g) against
15 different bacteria and fungi. As can be appreciated, the
16 relative potencies of each of the peptides differs
17 somewhat with respect to a particular organism, but the
18 activity is quite similar.

19 Table VIII lists the minimal effective
20 concentrations of XPF, PGLa and Magainin(g) necessary to
21 physically disrupt several different species of
22 protozoa. In each case, the peptide induces osmotic
23 swelling indicating a common mechanism.

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TABLE VII

Spectrum of Antimicrobial
Activity of Synthetic Peptides

ORGANISM	MINIMAL INHIBITORY CONCENTRATION (µg/ml)		
	Magainin II(g)	PGLa	XPF
E. coli (25922)	10-50	10-50	10-50
P. aeruginosa (27883)	50-100	200-500	50-100
S. aureus (250230)	500	50-100	100-200
S. pyogenes (19615)	10-50	10	10-50
S. cervisiae ()	50-100	100-200	100-200
C. albicans (14053)	200-500	100-200	200-500

10⁵ Organisms were inoculated from late-log phase cultures into 0.5 ml of TSB broth containing synthetic peptide at concentrations of 10, 50, 100, 200 and 500 µg/ml. Cultures were incubated at either 37°C (bacteria) or 30°C (fungi) for 24 hours. Concentrations of peptide at which no visible growth of inoculum occurred is listed.

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TABLE VIII

Sensitivity of Protozoa to Synthetic Peptides

ORGANISM	MINIMAL DISRUPTIVE CONCENTRATION (μ g/ml)		
	Magainin II	PGLa	XPF
Paramecium caudatum	10	5	10
Tetrahymena pyriformis	20	20	20
Acanthameoba castellani	2	2	2

Protozoa (about 10^2) were suspended into 200 μ l of 1% trypticase soy broth on a glass depression slide. Actively growing cultures of P. caudatum and T. pyriformis were obtained from Nasco (Wisc) and A. castellani from B Bowers (NHLBI). Peptides were added to various concentrations and the organism visually assessed by light microscopy within 15 minutes of exposure. The concentrations listed represent the minimal peptide concentration at which physical disruption of all protozoa occurred.

It is clear from the results presented herein that XPF and PGLa are potent antimicrobial agents.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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1 WHAT IS CLAIMED IS:

2 1. A synthetic, substantially pure Magainin
3 having at least in part or in whole the amino acid
4 sequence selected from the group consisting of:

5 (NH₂)GIGKFLHSAGKFGKAFVGEIMKS(OH),
6 (NH₂)GIGKFLHSAKKFGKAFVGEIMNS(OH),
7 (NH₂)GIGKFLHSAKKFGKAFVGEIMN(OH),
8 (NH₂)IGKFLHSAKKFGKAFVGEIMNS(OH),
9 (NH₂)GKFLHSAKKFGKAFVGEIMNS(OH),
10 (NH₂)KFLHSAKKFGKAFVGEIMNS(OH),
11 (NH₂)FLHSAKKFGKAFVGEIMNS(OH),
12 (NH₂)LHSAKKFGKAFVGEIMNS(OH),
13 (NH₂)GIGKFLHSAKKFGKAFVGEIMNS(NH₂), and
14 (NH₂)GIGKYLHSAKKFGKAFVGEIMNS(NH₂).

15 2. The Magainin of Claim 1 being
16 (NH₂)GIGKFLHSAGKFGKAFVGEIMKS(OH).

17 3. The Magainin of Claim 1 being
18 (NH₂)GIGKFLHSAKKFGKAFVGEIMNS(OH).

19 4. The Magainin of Claim 1 being
20 (NH₂)GIGKFLHSAKKFGKAFVGEIMN(OH).

21 5. The Magainin of Claim 1 being
22 (NH₂)IGKFLHSAKKFGKAFVGEIMNS(OH).

- 21 -

1 6. The Magainin of Claim 1 being
2 (NH₂)GKFLHSAKKFGKAFVGEIMNS(OH).

3 7. The Magainin of Claim 1 being
4 (NH₂)KFLHSAKKFGKAFVGEIMNS(OH).

5 8. The Magainin of Claim 1 being
6 (NH₂)FLHSAKKFGKAFVGEIMNS(OH).

7 9. The Magainin of Claim 1 being
8 (NH₂)LHSAKKFGKAFVGEIMNS(OH).

9 10. The Magainin of Claim 1 being
10 (NH₂)GIGKFLHSAKKFGKAFVGEIMNS(NH₂).

11 11. The Magainin of Claim 1 being
12 (NH₂)GIGKYLHSAKKFGKAFVGEIMNS(NH₂).

13 12. A pharmaceutical composition comprising as an
14 active ingredient a Magainin of Claim 1 ~~in an amount~~
15 ~~sufficient to produce bioactive or antimicrobial effect~~
16 and a pharmaceutically acceptable carrier.

17 13. A method of treating or controlling microbial
18 infection comprising administering to a host, tissue or
19 material susceptible to or afflicted with microbial
20 infection an antimicrobial ^{effective} amount of a polypeptide
21 selected from the group consisting of XPF, PGLa,
22 Magainin, and a combination thereof.



- 22 -

1 14. The method of Claim 13, wherein said
2 polypeptide is XPF.

3 15. The method of Claim 13, wherein said
4 polypeptide is PGLa.

5 16. The method of Claim 13, wherein said
6 polypeptide is a Magainin.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US88/00520

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
INT Cl. --4th Ed.-- C07K 7/10; A61k 37/02		
U.S. Cl. 530/326; 514/13,21		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S.	530/326,842; 514/13,21	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	The Journal of Biological Chemistry, Vol. 261, No. 12, issued 25 April 1986, B. Gibson, "Novel Peptide Fragments originating from PGL ^a and the Caerulein and Xenopsin Precursors from <i>Xenopus laevis</i> , pp. 5341-5349.	13,14,15
A	Biochem. 149, issued 1985, Andreu, "Solid-phase synthesis of PYLa and isolation of its natural counterpart, PGLa [PYLa-(4-24)] from skin secretion of <i>Xenopus laevis</i> ", pp. 531-535.	1-16
A	Ann. Rev. Biochem., 53, issued 1984, D. Eisenberg, "Three-dimensional structure of membrane and surface proteins", pp. 545-623.	1-16
* Special categories of cited documents: ¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
05 MAY 1988	21 JUN 1988	
International Searching Authority	Signature of Authorized Officer	
ISA/US	TERESA D. WESSENDORF	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

- | | | |
|---|--|------|
| A | Proc. Natl. Acad. Sci. USA, Vol. 81,
issued January 1984, I. Sures, "Xenopsin:
The neurotensin-like octapeptide from
Xenopus skin at the carboxyl terminus
of its precursor", pp. 380-384. | 1-16 |
| A | The Journal of Biological Chemistry
Vol. 257, No. 5, issued 10 March 1982,
C. Kempf, "Voltage-dependent
trans-bilayer orientation of Melittin",
pp. 2469-2476. | 1-16 |

VI. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter ¹¹ not required to be searched by this Authority, namely:
2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹², specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application, for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

III DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages **	Relevant to Claim No. 1 *
A	Proc. Natl. Acad. Sci. USA, Vol. 80, issued February 1983, E. Kaiser, "Secondary structures of proteins and peptides in amphiphilic environments (A Review)," pp. 1137-1143.	1-16
A	Nature, Vol. 292, issued 16 July 1981, H. Steiner, "Sequence and specificity of two antibacterial proteins involved in insect immunity", pp. 246-248.	1-16
A	Biochemistry, 21, issued 1982, R. Merrifield, "Synthesis of the Antibacterial peptide Cecropin A (1-33)," pp. 5020-5031.	1-16
A	Toxicon, Vol. 7, issued 1969, A. Csordas "Primary structure of two Oligopeptides of the Toxin of Bambusa Variegata L.", pp. 103-108.	1-16
A	Peptides, Vol. 6, Suppl. 3, issued 1985, K. Richter, "Biosynthesis of Peptides in the skin of <i>Xenopus laevis</i> Isolation of novel peptides predicted from the sequence of cloned cDNAs," pp. 17-21.	1-16
P,X	Biochem. J., 243, issued 1987, M. G. Giovannini "Biosynthesis and degradation of peptides derived from <i>Xenopus laevis</i> prohormones", pp. 113-120.	1-16
A	The EMBO Journal, Vol. 2, No. 5, issued 1983, W. Hoffmann, "A novel peptide designated PYLa and its precursor as predicted from cloned mRNA of <i>Xenopus</i> <i>laevis</i> skin," pp. 711-714.	1,13,15