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(72) Inventeurs/Inventors:
SUN, YUKUN, CN;
WU, DENGXI, CN;
ZHU, ZHIYONG, CN;
YU, GANG, CN;
SHEN, CHUNJUAN, CN;
ZHAO, SHAOLING, CN;
ZHOU, JIAXIANG, CN

(73) Propriétaire/Owner:
SHANGHAI HUAYI BIO-LAB., LTD., CN

(74) Agent: FASKEN MARTINEAU DUMOULIN LLP

(54) Titre : DERIVES DE LA MAGAININE
(54) Title: DERIVATIVES OF MAGAININ

(57) Abrégé/Abstract:
The present invention relates to a variety of Magainin derivatives and pharmaceutically acceptable salts produced thereof, with the derivatives having sequences as shown below: Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-X-Asn-Y-OH In which: X is an amino acid residue selected from the group consisting of Met, Ile and Leu, Y is the combination of two amino acid residues selected from the group consisting of Ser, Lys, Ile, Arg and Leu. The derivatives of this invention have the same or higher anti-microbial activities compared to that of the naturally occurring Magainin. They are easily produced by solid-phase synthesis, and more easily, by bioengineering techniques.

ABSTRACT

The present invention relates to a variety of Magainin derivatives and pharmaceutically acceptable salts produced thereof, with the derivatives having sequences as shown below:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-X-Asn-Y-OH

In which:

X is an amino acid residue selected from the group consisting of Met, Ile and Leu, Y is the combination of two amino acid residues selected from the group consisting of Ser, Lys, Ile, Arg and Leu. The derivatives of this invention have the same or higher anti-microbial activities compared to that of the naturally occurring Magainin. They are easily produced by solid-phase synthesis, and more easily, by bioengineering techniques.

DERIVATIVES OF MAGAININ

Field of the Invention

The present invention relates to derivatives of Magainin. Specifically, the present invention relates to derivatives of Magainin having the properties of anti-microbial activities.

Background of the Invention

There are many life forms, for example, insects, microorganisms, amphibians and human beings which may produce anti-bacterial peptide materials that protect their communities. These anti-bacterial peptides can penetrate lipids on the cell membranes and make them inactive, can also affect protozoon species, germ cells and even viruses, hence such peptides are referred to as super-antibiotics. Anti-bacterial peptides all carry various amounts of positive charge, and their anti-bacterial mechanism lies in the combination of the positive charges carried by the peptides with the negative charges carried by the phospholipids which exist in the bacteria cell wall, creating an ion path on the cell membrane, enhancing the penetrability, causing the bacteria to dissolve and die. Hence the anti-microbial activities of these peptides do not depend on the binding with any specific receptors.

The anti-bacterial peptides exhibit a broad spectrum of antimicrobial activity upon gram-positive and gram-negative bacteria, as well as aerobic and anaerobic bacteria. They are different from antibiotics in that anti-bacterial peptides do not produce drug-resistance effects, even bacteria that have resistance to many types of antibiotics could be suppressed by the anti-bacterial peptides. Further, such anti-bacterial peptides also have inhibitive effect to protozoon species and viruses. As the metabolism products of the anti-bacterial peptides are amino acids, these peptides are of low toxicity for host cells. In summary, the anti-microbial peptides are a class of compounds with wide prospects of being used for anti-microbial drugs.

Magainin is a category of naturally occurring anti-bacterial peptides derived from frog skin with anti-bacterial effects. Magainin has been extensively studied up till now, they have such features as being easy to be synthesized, low in cost and little possibility of hemolysis.

US Patent USP5589364 disclosed the method by which Magainin II (23 amino acids) can be prepared using bioengineering techniques. Magainin II is a type of the naturally occurring frog-skin anti-bacterial peptide, having amino acid sequence shown as below:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Asn-Ser-OH

In which:

Gly stands for glycine, Ile for isoleucine, Lys for lysine, Phe for phenylalanine, Leu for leucine, His for histidine, Ser for serine， Ala for alanine, Val for valine, Glu for glutamic acid, Met for methionine and Asn for asparaginate.

US patent No. 6183992 disclosed a method to produce MSI-78 (22 amino acids), a derivative of magainin, having the amino acid sequence shown as below:

Gly-Ile-Gly-Lys-Phe-Leu-Lys-Lys-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Lys-Ile-Leu-Lys-Lys-NH_2

It has been reported in ADIS NEW DRUG PROFILE by Harriet M. Lamb, etc., that the Magainin derivative MSI-78 shows obvious curative effect in treating trauma infection and crura ulceration caused by the diabetes mellitus.

Object of the Invention

The object of the present invention is to provide some derivates of Magainin, which broadens the category of Magainin derivatives. Such derivatives can be prepared by synthetic chemical method, and more easily, by the recombinant techniques, which makes it possible to reduce the cost of production. The anti-microbial effects of such derivatives are the same or higher than that of the naturally occurring Magainin.

Summary of the Invention

The present invention involved in some derivatives of Magainin having amino acid sequence as shown below and pharmaceutically acceptable salts produced thereof:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-X-Asn-Y-OH

In which:

X is an amino acid residue selected from the group consisting of Met， Ile and Leu, Y is the combination of two amino acid residues selected from the group consisting of Ser, Lys, Ile, Arg and Leu.

The Magainin derivates of the present invention have the amino acid sequences shown in <210>1 of the Sequence Listing.

The Magainin derivatives of the present invention are amphoteric compounds, and may be sufficiently acidic or sufficiently basic to react with any of a number of inorganic bases, and inorganic and organic acids, to form a salt. Acids commonly employed to form acid addition salts are acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenyl-sulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid,

and the like. Examples of such salts include the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate, glycolate, tartrate, methanesutfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate, and the like. Preferred acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and, especially, hydrochloric acid.

Alkalis may also be employed to react with the Magainin derivatives to form salts. Representative examples of such alkalis include ammonium, alkali metals, alkali metal hydroxides, carbonates and bicarbonates. Typically, such an alkali may be sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, and the like.

The amino acids of Magainin derivatives of the present invention may be the L-typed or D-typed isomers.

One aspect of the present invention provides a method to produce the Magainin derivatives.

The said method is to produce the Magainin derivatives by recombinant techniques, comprising: synthesizing gene fragments by the amino acid sequence of a Magainin derivative, ligating the gene fragments, constructing a recombinant plasmid, cloning and obtaining the product after the steps of fermentation, separation, purification and lyophilization.

Another aspect of the present invention provides a method to produce the Magainin derivates.

The said method is to produce the Magainin derivates by solid phase synthesis, which comprises of using HMP resin as a solid phase carrier, protecting alpha-amine of a residue with 9-fluorenyl methoxycarbonyl (Fmoc), synthesizing the peptide on a peptide synthesizer, and obtaining products after the steps of separation, purification and lyophilization.

In still another aspect, the present invention provides the uses of such Magainin derivatives and pharmaceutically acceptable salts thereof in the preparation of drugs/pharmaceuticals having anti-microbial effects.

This invention provides a variety of Magainin derivatives, broadening the category of Magainin derivatives. Such Magainin derivatives may be prepared by chemical synthesis, and more easily, by recombinant techniques, which makes it possible to decrease the production cost. Such derivatives have the same or higher anti-microbial effects compared to the naturally occurring Magainin.

It has been shown by the anti-microbial effect experiment, the time-kill study, the hemolysis and the acute toxicity tests that the anti-microbial effect on *Escherichia coli* of the Magainin derivatives of this invention is equivalent to that of the naturally occurring Magainin, and such Magainin derivatives also have inhibitory effects on *Staphylococcus aureus.* The anti-microbial experiment was conducted by testing the effects of the Magainin derivatives of the present invention to 50 μl of the bacterial solution (10^6 bacteria/ml), more than 90% of the bacteria were killed within three hours with the dosage of 0.5 μg, while with the dosage of 1 μg, almost all the bacteria were killed within three hours. The hemolysis studies of the derivatives of the present invention showed that the ratio of the effective concentrations for hemolysis of 50% of the bacteria (HC_{50}) to inhibition of 50% of the bacteria (IC_{50}) was about 50:1, while the ratio of the naturally occurring Magainin was about 24:1, which demonstrates the safety of the products of this invention is superior to that of the naturally occurring Magainin. Further, the acute toxicity studies showed the Magainin derivatives of the present invention are of low toxicity.

Description of the Figures

The present invention will be further illustrated with reference to the following figures and examples.

Figure 1 is the diagram showing the time-kill study of the Magainin derivative obtained from example 1 on *Escherichia coli.*

Description of the Invention

The following examples are illustrative and are not intended to limit the scope of the present invention by any means.

Example 1

Preparation of the Magainin derivative of the present invention by bioengineering techniques wherein X is Leu and Y is Ser-Arg

(1) Synthesizing the following gene fragments by the amino acid sequence of the above Magainin derivative:

(a) 5'-AAT TCC ATG GGT ATC GGT AAA TTT CTG CAC AGC GCG AAA AAA

(b) 5'-TTT GGT AAA GCG TTT GTG GGT GAA ATC CTG AAC AGC CGT TAG A

(c) 5'-AG CTT CTA ACG GCT GTT CAG GAT TTC ACC CAC AAA CGC TTT

(d) 5'- ACC AAA TTT TTT CGC GCT GTG CAG AAA TTT ACC GAT ACC CAT GG

(2) Litigation of DNA fragments:

Fragment (a), (b), (c) and (d) with their optical density at 260nm(A_{260nm}) equaling to 0.1 were taken, and fragment (a) and (d), as well as fragment (b) and (c) were drawn into two tubes separately. The polynucleotide kinase buffer, polynucleotide kinase and ATPs were added to the two tubes respectively. The reaction mixture was incubated at 37℃ for 60 minutes, then incubated in a water bath of 95℃ for 10 minutes, and then was naturally cooled down to room temperature. The T4 ligase buffer and the ATP solution were added, followed by the addition of the T4 ligase, the mixture was incubated overnight at 15℃ for completion of the fragment ligation.

(3). Cloning

Plasmids containing the P_L promotor (or Lac, or Tac) were digested with restriction endonucleases EcoRI and HindIII, extracted with hydroxybenzene: chloroform solvent, washed for three times with chloroform, precipitated with isopropanol solvent , and collected by centrifugation.

The digested plasmids were ligated with the Magainin derived fragments, and the plasmids containing the Magainin derived gene fragments were obtained. Such plasmids were transformed into E. coli JM103 or JM 109 host cells. Bacterial colonies were screened after cultivation by agar plate.

(4) Examination

The plasmid containing the gene fragment of the Magainin derivative was extracted from mono-cloned bacteria, and was double digested with EcoRI and HindIII. The electrophoresis was conducted on a 1% agar gel, followed by dying with ethidum bromide. The cloned gene fragment was examined by comparison with the marker, and further tested by DNA sequence analysis.

(5). Fermentation:

The bacterial strain was incubated in a shaking bottle with the capability of 1 liter (10 bottles in total), each containing 300 ml of LB liquid media consisted of 10g of peptone, 5g of yeast extract, 5g/L of sodium chloride. 0.2 mM of Isopropyl beta-D-Thiogalactopyranoside (IPTG) was added at 37℃ for the induction of the protein to be expressed. The bacterial cells

were incubated overnight and harvested by centrifugation. When using the plasmid with the temperature-controlled promotor P_L, the bacterial cells were cultured at 30℃ for eight hours. Then the temperature of the media was increased to 42℃, and the bacterial cells were maintained for four hours to make the gene expressed.

(6) The bacterial cell walls were broken up under the effect of lysozyme at 37℃ for an hour. The precipitate was treated with 6M of guanidine hydrochloride. After centrifugation, dialysis and further centrifugation steps, the inclusion bodies of protein were obtained. The inclusion bodies were washed three times, with the wash solution containing 1% sodium chloride, 0.1 % Triton X-100 and Tris-HCL buffer (20 mM, pH8). The fusion protein was identified through polyacrylamide gel electrophoresis (PAGE) containing 12% sodium dodecanesulphonate (SDS).

(7) The inclusion bodies were dissolved in 8M of carbamide solution. Under the existence of 50mM of hydrochloric acid, cyanogen bromide was added for the lysis of inclusion bodies. The solution was stirred with the protection of nitrogen and shunning of the light. After completion of the lysis reaction, crude product of the Magainin derivative was obtained through Sephadex G-25 with fast protein liquid chromatography (FPLC, AKTA™ manufactured by Amersham Pharmacia Biotech), and final product of the Magainin derivative was acquired through purification with high performance liquid chromatography (HPLC, C_{18} column) and gradient elution with CH_3CN/0.1% TFA buffer. The HPLC analysis result of the obtained product is consistent with those products prepared by chemical synthesis.

Example 2

Preparation of the Magainin derivative of the present invention by bioengineering techniques wherein X is Leu and Y is Ser-Lys.

A. Synthesizing the following gene fragments by the amino acid sequence of the above Magainin derivative:

(a)5' AAT TCC ATG GGT ATC GGT AAA TTT CTG CAC AGC GCG AAA AAA

(b) 5' TTT GGT AAA GCG TTT GTG GGT GAA ATC CTG AAC AGC AAG TAG A

(c) 5' AG CTT CTA CTT GCT GTT CAG GAT TTC ACC CAC AAA CGC TTT

(d)5' ACC AAA TTT TTT CGC GCT GTG CAG AAA TTT ACC GAT ACC CAT GG

The steps (2)~(7) of this example are the same as that of example 1, and the HPLC

analysis result of the obtained Magainin derivative is consistent with those results prepared by chemical synthesis.

Example 3

Preparation of the Magainin derivative of the present invention by solid-phase synthesis wherein X is Leu and Y is Lys-Arg.

(1) Amino acid monomers:

Fmoc-L-Ala-OH	Fmoc-L-Lys(Boc)-OH
Fmoc-L-Asn(Trt)-OH	Fmoc-L-Met-OH
Fmoc-L-Asp(OtBu)-OH	Fmoc-L-Phe-OH
Fmoc-L-Gln(Trt)-OH	Fmoc-L-Pro-OH
Fmoc-L-Glu(OtBu)-OH	Fmoc-L-Ser(tBu)-OH
Fmoc-L-Gly-OH	Fmoc-L-Thr(tBu)-OH
Fmoc-L-His(Trt)-OH	Fmoc-L-Trp-OH
Fmoc-L-Ile-OH	Fmoc-L-Tyr(tBu)-OH
Fmoc-L-Leu-OH	Fmoc-L-Val-OH

In which:

Fmoc stands for 9-fluorenyl methoxycarbonyl

BOC for tert-butyloxycarbonyl

Trt for trityl

OtBu for tertiary butyl ester, and

TBu for tert-butyl.

(2) Apparatus and Reagents:

Apparatus: Model 433A peptide synthesizer (Applied Biosystem, US)

Reagents:

N-methyl ketopyrrolidine, methylene chloride, hexahydropyridine, methanol, dimethylaminopyridine/ DMF N, N-diisopropylethylamine/NMP, 100 mmole HBTU / 0.5 M HOBT in DMF, N, N-Dicyclohexylcarbodiimide/NMP

In which:

DMF stands for N, N-Dimethylformamide

NMP for N-methylpyrrolidone

HOBT for 1-Hydroxybenzotriazole, and

HBTU for 2-(1H-benzotriazole-yl-1,1,3,3-tetramethyl-Uronium hexafluorophosphate)

(3) Procedures:

a. Synthesis

Take the synthesis scale of 0.25 mmol for example, the synthesis process was described as follows. 0.25g of HMP resin was weighed and placed in a reactor vessel of the synthesizer. 1 mmol of various residues, each coupled with protecting groups, were weighed and arrayed in the synthesizer by the amino acid sequence of the insulinotropic peptide derivate from the carboxy terminal to the amino terminal. At room temperature of 25℃, reactions for removing Fmoc protection, activating a residue and attaching the activated residue to HMP resin were automatically performed under the control of a computer program. Such reactions were circulated until the whole peptide was synthesized. After completion of the synthesis, the residue-attached resin, with each residue coupled with side chain protecting groups, was air dried on a peptide synthesizer and then weighed.

b. Removal of protecting groups and detachment of resin:

The residue-attached resin, with each residue of the insulinotropic peptide derivative coupled with protecting groups, was placed in a plugged erlenmeyer flask, and followed by addition of cleavage reagents as shown below.

Reagent	Dosage
Water	0.50 ml
Methyl phenate	0.50 ml
Phenol	0.75 g
Mercaptoethanol	0.20 ml
trifluoroacetic acid	10.0 ml

The electromagnetic stirring reaction was carried out at constant temperature of 30 ℃ for 6 hours. After filtration step, the aqueous filtrate was collected. The resin was washed with small amount of trifluoroacetic acid. Then the collected aqueous filtrate and the washing solution were mixed together, and ether was added for precipitation. The mixture was filtrated, and the resulted precipitate was washed with small amount of ether. After evaporation in a dehumidifier, the crude product was obtained.

c. Purification by HPLC and lyophilization

Separation and purification of the crude product was achieved by using preparative HPLC. Final product was obtained after the steps of freezing and lyophilization. Through joint analysis of chromatogram and mass spectrogram, the molecular weight of the derivative was found to be

consistent with the theoretical value.

Example 4

Pharmacodynamic Studies

The anti-microbial experiment of the Magainin derivative obtained from Example 1 was conducted according to the following procedures, with the comparison of that of the naturally occurring Magainin.

The tested strains of Escherichia coli JM103 and staphylococcus aureus were cultured separately, and diluted to 1×10^6 bacteria/ ml. 20 mM of the sterilized Tris-HCl buffer (pH6.5) was added. Then the Magainin derivative and the naturally occurring Magainin II with various concentrations were added respectively, and incubated at 37℃ for different time limit. 50 μl of the cultures was taken and spread on an agar plate, and incubated at 37℃ overnight. The remaining bacteria colonies were accounted for, and the percent of the killed bacteria was calculated.

(1) Table showing the anti-microbial effects:

Comparison of the anti-microbial effects between the Magainin derivative obtained from Example 1 and the naturally occurring Magainin II

Sample	Concentration μg/ml	Bacteria colonies remained			Bacteria killed %	
		0hr	3hr	4hr	3hr	4hr
Magainin derivative obtained from Example 1	0	407 228				
		317				
	10		109 73	160 195	71.3	44.2
			91	177		
	20		9 5	2 5	97.8	99.1
			7	3		
Naturally occurring Magainin II	10		99 57	52 44	75.4	84.9
			78	48		
	20		12 4	6 4	97.5	99.2
			8	5		

Table showing the anti-microbial effect of the Magainin derivative obtained from Example 1 on Staphylococos anreus

Concentration μg/ml	Bacteria colonies remained			Bacteria killed within two hours %
	0hr	1hr	2hr	
0	267			
	225			
50	158	18	2	99
	185	18	2	99
	172	18	2	99

(2) Figure showing the result of time-kill study: The result of time-kill study for the Magainin derivative obtained from Example 1 was referred to in Figure 1.

(3) Table showing the results of hemolysis test:

Group		Dosage (μg/ml)	Hemolysis %
Test Group	Naturally-occurring Magainin	100	0
		500	0
		1000	1.9
	Magainin derivative obtained from Example 1	100	0
		500	0
		1000	2
Control Group	Tritonx-100	0.1%	100

(4) Acute toxicity test: Acute toxicity test was conducted on two mice of Kunming species. The mice were injected abdominally with the Magainin derivative obtained from Example, and the livability of the two mice were observed after receiving injection for one hour.

Dosage of abdominal injection	Livability
100μg	100%
200μg	100%
1000μg	100%

SEQUENCE LISTING

<110> Shanghai Huayi Bio Lab

<120> Derivates of Magainin

<130> USP 6183992

<150> CN 01112855.0
<151> 2001-05-10

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<170> PatentIn version 3.1

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<213> Magainin analogue

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<223> Lys or Ile or Arg or Leu

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<221> VARIANT
<222> (24)..(24)
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<400> 1

Gly Ile Gly Lys Phe Leu His Ser Ala Lys Lys Phe Gly Lys Ala Phe
1 5 10 15

Val Gly Glu Ile Met Asn Ser Ser
20

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A Magainin derivative peptide having the amino acid sequence of the general formula:

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Lys-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-X-Asn-Y-Arg-OH in which:

X at position 21 is an amino acid selected from the group consisting of Met, Ile and Leu;

Y at position 23 is an amino acid selected from the group consisting of Ser, Lys, Ile, Leu and Arg, and

wherein X at position 21 is not Met when Y at position 23 is Ser.

2. The peptide of claim 1, wherein: X at position 21 is not Met when Y at position 23 is Arg; X at position 21 is not ILe when Y at position 23 is Arg; and X at position 21 is not Leu when Y at position 23 is Arg.

3. The peptide of claim 2, wherein: X at position 21 is ILe and Y at position 23 is Ser; or X at position 21 is Leu and Y at position 23 is Ser.

4. The peptide of claim 3, wherein X at position 21 is Leu and Y at position 23 is Ser.

5. A pharmaceutical composition comprising the Magainin derivative peptide of claim 1 and a pharmaceutically acceptable carrier and/or pharmaceutically compatible binding agents.

6. A pharmaceutical composition, comprising the Magainin derivative peptide of claim 2 and a pharmaceutically acceptable carrier and/or pharmaceutically compatible binding agents.

7. A pharmaceutical composition, comprising the Magainin derivative peptide of claim 3 and a pharmaceutically acceptable carrier and/or pharmaceutically compatible binding agents.

8. A pharmaceutical composition, comprising the Magainin derivative peptide of claim 4 and a pharmaceutically acceptable carrier and/or pharmaceutically compatible binding agents.

9. A method to produce the Magainin derivative peptide described in any one of claims 1 to 4 by recombinant techniques, comprising:

(a) synthesizing gene fragments encoding the amino acid sequence of the Magainin derivative peptide;

(b) ligating the gene fragments;

(c) constructing a recombinant plasmid;

(d) transforming the recombinant plasmid in to a host cell;

(e) culturing the host cell to express the Magainin derivative peptide;

(f) extracting the Magainin derivative peptide from the host cell; and

(g) purifying the Magainin derivative peptide by lyophilisation.

10. A method to produce the Magainin derivative peptide described in any one of claims 1 to 4 by solid phase synthesis, comprising:

(a) using HMP resin as a solid phase carrier, and protecting alpha-amine of an amino acid with 9-fluoroenyl methoxycarbonyl (Fmoc);

(b) synthesizing the Magainin derivative peptide using a peptide synthesizer; and

(c) isolating, purifying and lyophilizing the synthesized Magainin derivative peptide.

11. A use of the Magainin derivative peptide and pharmaceutically acceptable salts thereof as described in any one of claims 1 to 4 in the preparation of drugs having anti-microbial effects.

12. The peptide of claim 1, wherein said peptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15.

13. The peptide of claim 12, wherein said peptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 15.

14. The peptide of claim 13, wherein said peptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 6 and SEQ ID NO: 11.

15. The peptide of claim 14, wherein said peptide has an amino acid sequence as shown in SEQ ID NO: 11.

16. A pharmaceutical composition comprising the Magainin derivative peptide of claim 12 and a pharmaceutically acceptable carrier and/or pharmaceutically compatible binding agents.

17. A pharmaceutical composition, comprising the Magainin derivative peptide of claim 13 and a pharmaceutically acceptable carrier and/or pharmaceutically compatible binding agents.

18. A pharmaceutical composition, comprising the Magainin derivative peptide of claim 14 and a pharmaceutically acceptable carrier and/or pharmaceutically compatible binding agents.

19. A pharmaceutical composition, comprising the Magainin derivative peptide of claim 15 and a pharmaceutically acceptable carrier and/or pharmaceutically compatible binding agents.

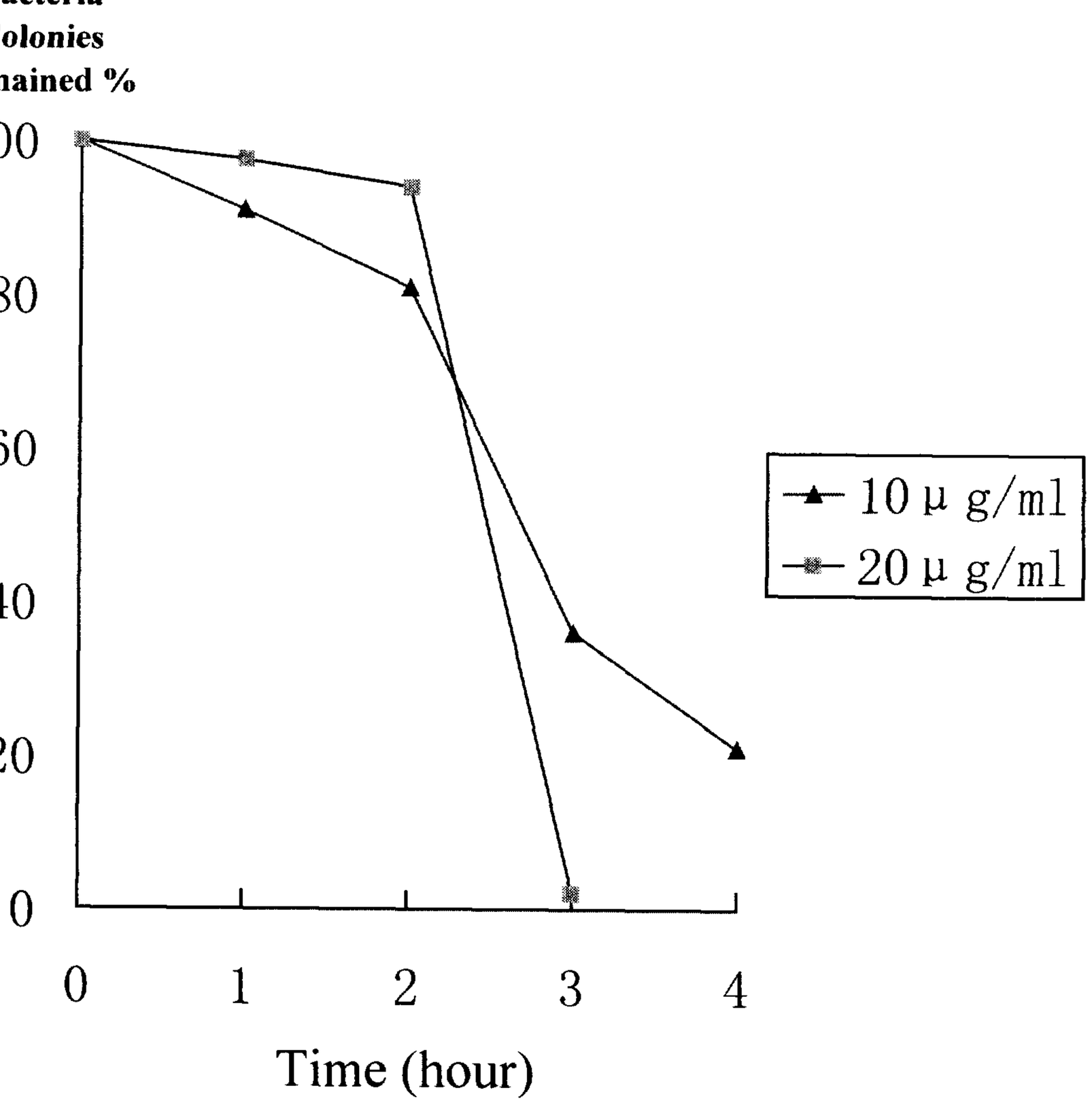
Bacteria
Colonies
Remained %
100
80
60
40
20
0
0
1
2
3
4
Time (hour)
10 μ g/ml
20 μ g/ml

Figure 1