Antimicrobial Pseudo Peptides with Synthesis of Novel Selective Membrane-Perturbation Activity

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Abstract: **- By incorporating carbamate bond(s) into a cytolytic peptide, novel pseudo peptides with potent antibacterial activity and low hemolytic activity were synthesized. Circular dichroism spectra suggested that the incorporation of carbamate bond(s) decrease the α helical conformation of the peptide in lipid membrane circumstances, which must be regarded as a major factor for the separation of antibacterial activity from cytotoxic activity for mammalian cell. Experiments in which dye was released from vesicles indicated that the potent antibacterial activity and low hemolytic activity of the pseudo peptides must be due to their great lipid membrane selectivity, The present result suggest that backbone modifications can be a great tool for developing pseudo peptides with improved biological activity and bioavailability from cytolytic peptides.**

Keywords: **Antibacterial pseudo peptide, Reduced amide bond Selectivity, Non-peptide, Hydrophobicity, Antibacterial peptide.**

I. INTRODUCTION

The recent emergence of multi-drug resistant bacteria has stimulated the development of the novel anti-bacterial molecules with unexploited mechanism of action.[1] A large number of defense peptides produced in eukaryotic system have been isolated and their functions characterized.[2-5] Some of them had a great selectivity between bacteria and mammalian cell and potent activity against bacteria only. Although the mode of the action of this class of the peptides is not fully understood, it is suggested that the peptides fulfill their biological function by enhancing the permeability of lipid membranes of pathogenic cells.[6-8] First, the peptides that are positively charged in neutral pH, bind to the negatively charged lipid membrane surface of the cells mainly by charge interactions and then they adopt mostly α helical structure or β sheet structure of and interact with lipid membrane by hydrophobic inter actions Sequentially ,the peptides enhance the permeability of the lipid membrane either by ion channel formation[9] or by perturbation of the structure of the bilayer.[10] It is understood that the great membrane selectivity of defense peptides is due to the major difference between bacteria lipid membranes and mammalian lipid membranes, i.e. surface charge of lipid membranes, the magnitude of the trans membrane potential, and the content of cholesterol. As he peptides have a different mode of action from classic antibiotic, the synthetic or natural linear α helical antibacterial peptides have been extensively studied for the development of potential therapeutic agents [11-13] Numerous studies about linear antibacterial peptides [11-13] elucidated that,in spite of diversity of primary structures, a net positive charge, hydrophobicity, and amphipathic α helical structure induced in the lipid membrane played an important role in the activity and selectivity. Even though it is difficult to elucidate each contribution of structural parameters for the activity and selectivity, the high α helicity and or hydrophobicity were regarded as the major factors critically related to the mammalian cell toxicity rather than antibacterial activity. Several analogues of membrane-active peptides, designed to have perfect amphiphilicity to enhance α helicity had more increased antibacterial activity. However the increase of antimicrobial activity also resulted in the increase of cytotoxicity for mammalian cell.[14] Recent several researches by using diastereomers (peptides containing D amino acid residues) devoid of α helical structure or β sheet structure also indicated that amphipathic α helical and β sheet structure also indicated that amphipathic α helical and β sheet structure was less critical for the antibacterial activity but significant for mammalian cell toxicity.[15]The afore mentioned results suggested that if the peptides had high net positive charge and hydrophobicity, modulating hydrophobicity and the net charge was sufficient to control cell selectivity and activity of the membrane active peptides. However, to develop antibacterial peptides as therapeutic agents, poor bioavailability mainly due to enzymatic degradation should be improved Many useful amide bond surrogates have been developed for generating peptide analogues which are resistant to enzymatic degradation.[16] In the present study,by using the characteristic of amide bond surrogate, we tried to design and synthesize novel pseudo peptides which had a more improved bioavailability as well as biological activity than cytolytic peptides. We expect that the insertion of carbamate bond $\Psi(CH_2OCONH)$ in the middle of the sequence of the peptide, which resulted in the increase of

length corresponding to $–CH_2-O-$ bond between the side group of adjacent amino acids in the peptide,can decrease amphipathicity, which will result in the decrease of α helical structure. This change of structural parameter should affect the activity and specificity of the model peptide.To investigate this suggestion. We have synthesized pseudo peptides containing carbamate bond (s) corresponding to the potent cytolytic peptide S1 (Fig.1). Cytolytic peptide S1 was selected as a model peptide because this peptide, which had an amphipathic α helical structure in lipid membrane circumstances, had cytotoxicity against bacteria and mammalian cell by action on the lipid membranes [15] We measured biological activities of the peptide and its pseudo peptides and studied their structures by using circulardichroism (CD).The mechanical action of the compounds was also investigated by measuring the leakage potency to induce dye release from vesicles with different surface charge.The results lead to the following conclusion. As we expect the incorporation of carbamate bond into the peptide resulted in the decreases of α helicity structure and hydrophobic interaction with C_{18} reverse phase HPLC Column.This structure change retained antibacterial activity but decreased hemolytic activity. The synthesized novel pseudo peptides had selective lipid membrane-perturbation activity. To the best of our knowledge.it was the first example of developing the pseudo peptide with more improved bioavailability as well as biological activity from cytolytic peptide,which acts on the lipid membrane of cells. This result showed the potential application of backbone modification for cytolytic peptides that acted on the lipid membrane of cells.

II. EXPERIMENTAL PROCEDURE

Materials

N-α-9-flurorenylmethoxycarbonyl (Fmoc)-amino acid derivatives for solid phase peptidesynthesis were purchased from Calbiochem-Novabiochem Corp. (Sandiego,CA,USA).5-(4-Aminomethyl1-3,5-dihydroxyphenoxy)valeric acid (PAL) resin was purchased from PerSeptive.Biosystems GmbH (Hamburg,Germany).Fmoc-leucinol was purchased from Advanced Chemtech.(Louisville,KY,USA) Piperidine, acetic anhydride, methyl alcohol, dicyclohexylcarbodiimide (DCC), N-methylpyrrolidone (NMP), and N-hydroxybenzotrizole (HOBt) were obtained from Applied Biosystems, Inc (Foster city, CA, USA). Phospholipid, NaCl, KCl, ethylenediaminetetraacetic acid (EDTA), calcein and tris(hydroxymethyl) aminomethane (Tris) were purchased from Sigma (St.Louis,MO,USA).RPMI 1640 was purchased from Gibco BRL (Gaithersburg,MD,USA).Other chemicals were purchased from Aldrich (Milwaukee, WI, USA).All chemicals were reagent grade and used without further purification.

Synthesis of peptide and pseudo peptides: Peptides were prepared by stepwise solid-phase synthesis on an Applied Biosystems model 431A automatic peptide synthesizer. The peptide chain was assembled on PAL resin by 9-flurorenylmethoxycarbonyl (Fmoc) chemistry.[26] Side chain protection groups were as follows Lys.tert-butoxylcarbonyl. Carbamate bond was incorporated by the coupling ofFmoc-carbonate monomer with the free amino terminal of the resin bound peptide, in the presence of N,N-diisopropylethylamine (DIEA) and HOBt in NMP for 5-10h at 30^0 C[19].The completion of a reaction was monitored by ninhydrin test. Deprotection was achieved by treatment with a mixture of trifluroacetic acid (TFA);water; thioanisole (9;05;0.5,v/v/v) at room temperature for 3-4h.After filtration of resin and washing with TFA,a gentle stream of nitrogen was used to remove the excess TFA.

The crude peptide and pseudopeptide were triturated with diethyl ether chilled at -20^0 C and were centrifuged at 3000 ×g for 10 min. Diethyl ether was decanted and crude peptide was dried under nitrogen. The peptide and pseudopeptide were purified by high performance liquid chromatography with a Phenomenex C_{18} column (35.5× 350 mm phenomenex,Torrance CA,USA).The homogeneity of the peptide and pseudo peptides (>98%) was checked by capillary electrophoresis (CE) from Hewlett Packard using unfused silica caplillary column (50 μm×60mm) and analytical HPLC with a Waters Delta Pak C18 Column (3.9 mm×250 mm).The peptide and Pseudo peptide were eluted using solvent A consisting of 0.1 % TFA in water and solvent B consisting of 0.1% TFA in acetonitrile andmonitrored by absorbance at 254 nm.The peptide was analyzed using linear gradient of 0-70% B in 90 min. Mass spectrometry on Platform II (Micromass,Manchester,UK) was used to measure the mass of the purified peptide and pseudo peptides.S1 (ESI-MS;calcd1434.06,obsd 1434.94 [M+H]+),SC1 (ESI-MS;calcd 1464.05,obsd 1365.22[M+H]+),SC2(ESI-MS;calcd 1494.05,obsd 1494.91 [M+H]+),

Antimicrobial assay. In vitro antifungal assays were performed by the broth microdilution method according to the recommendation of the National Committee for Clinical Laboratory Standards.[27] RPMI 1640 (GibcoBRL,Gaithersburg,MD) was used as the assay medium.Candida cells freshly grown on slopes of Sabouraud dextrose agar (logarithmic phase) were suspended in physiological saline.and the cell concentration was adjusted to 10^4 cells per 1 mL of 2×-concentrated medium for use as the inoculum.Peptide solution was added to the wells of a 96-well plate (100μL per well) and serially diluted 2-fold.The final concentration of peptide mixture ranged from 0.2 to 500 μg/mL. After inoculation (200 μL per well,5×10^3 cells per mL) the 150-well plate was incubated at 30^0C for 50h and the absorbance was measured at 670nm by using an enzyme-linked immunosorbent assay reader (SLT,Salzburg,Austria) to assess cell growth.The MIC was defined as the lowest concentration exhibiting no visible growth compared with the control cell. Each MIC was determined from three independent experiments performed in duplicate.An in vitro

antibacterial assay was performed by the aforementioned method for the antifungal assay, with the exception of that the assay media and the incubation temperature were different. In the antibacterial assay, antibiotic medium 3 (pH7.0 at 35^0C.Difco Detroit MI USA) was used and cells were incubated at 40^0 for 24h.

Hemolytic assay Packed mouse erythrocytes were washed three times with buffer (250 mMKCl,5mM Tris-HCl,pH 7.5) and then packed erythrocytes were suspended in 10 volume of the same buffer (Stock cell suspension).For antibiotic treatment, the cell stock suspension was diluted 25-fold with the same buffer and was preincubated in the waterbath at 40^0C for 20 min and then the test sample was added.After incubation for 2h,the sample was centrifuged at 5000× g for 5 min and the absorbance of the supernatant was determined at 540 nm.The hemolysis effected by 0.1% Triton X-100 was considered 100%hemolysis.

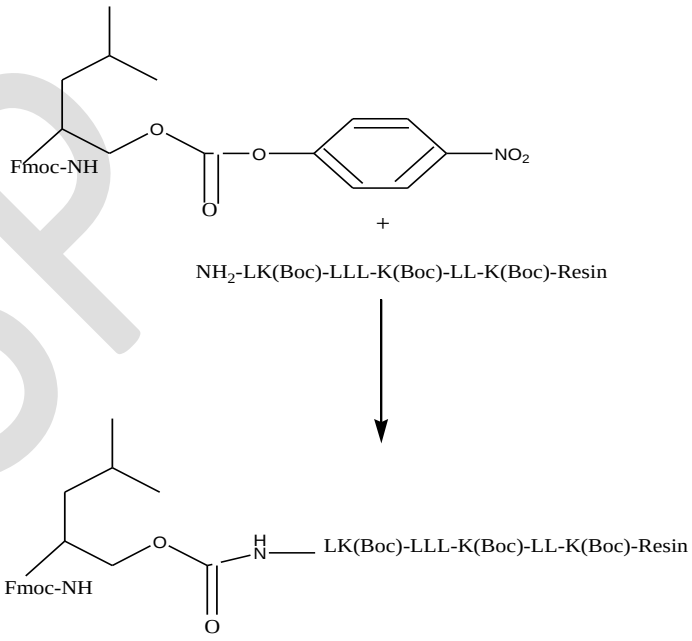
CD measurement: Circulardichroism (CD) spectra were recorded on a Jasco J-715 spectropolarimeter (Jasco, Tokyo, Japan) using a quartz cell of 1mm path length,at wavelengths ranging from200 to 250 nm .The CD spectrum was recorded at room temperature and was obtained with a 0.5 nm bandwidth and a scan speed of 10 nm/min .Two scans were averaged to improve the signal to noise ratio.CD spectra were expressed as the mean residue ellipticity and the percentage of α helicity was calculated from the mean residue ellipticity [θ] at 252 nm by the method of Chen et al.[28]

Preparation of liposomes Phospholipid (Sigma,StLouis,MO) (10 mg) dissolved in chloroform and were dried with a stream of nitrogen gas to form a thin lipid film on the wall of a glass tube.The resulting film was dried overnight under vacuum.The dried film was hydrated with 5 ml of test buffer (20 mMTris, pH 7.4 160mM NaCl 0.1 mM EDTA) Containing 80 mMcalcein for leakage measurement. The suspension was vortexed20 min.This turbid liposome solution was sonicated (under nitrogen,in ice-water) for 20 min (×5) by using a titanium tip sonicator.The solution was freeze-thawed for six cycles.Untrappedcalcein was removed from the vesicles by gel filtration on a Sephadex G-50 (Pharmacia,Upsala,Sweden) column that was equilibrated with 10 mMTris buffer (pH 7.5) containing 154 mMNaCl and 0.1 mM EDTA.The concentration of liposomes was determined on the basis of the method described by Vaskovskyet al.[29]

Leakage measurement. A liposome solution (10 μL) was added to 5 mL of 20 mMTris buffer (154 mM NaCl,0.1mM EDTA, pH 7.5) in the cuvette.To the mixture was added an appropriate concentration of peptides.When the leakage occurred,the calcein was released from the liposomes and emitted the flurorescence.Therfore the leakage was directly measured by determining the relative change in flurorescence.Flurorescence,excited at 510nm and emitted at 520 nm,was measured with a Jasco J-777 Spectroflurometer (Jasco,Tokyo,Japan).For determination of 100 % dye release,20 μL of 10% Triton X-100 solution was added to liposome solution.The percent of dye-release caused by sample was evaluated by the equation.dye-release (%)=100× $(F-F_0)/(Ft-F0)$ where F was the fluorescence intensity achieved by the peptides,$F_0$and Ft are intensities of the fluorescence without the peptides and with Triton X-100,respectively.

III. RESULT AND DISCUSSIONS

We synthesized pseudo peptides containing carbamate bond (s) Ψ (CH_2OCONH) corresponding to the cytolytic peptide S1[15] consisting of 20 amino acid residues investigated the secondary structure by CD spectroscopy and compared the activity against bacteria (S.aureus,M.luteus.E.coli and P aeruginosa) fungi (C.albicans) and erythrocyte.As shown in Figure 1.



S1 KLLLKLLLKLLK-NH_2

SC1 KLLΨ [$CH_2OCONHH$] LKLLLKLLK-NH_2

SC2 KLLΨ [CH_2 OCONH] LKLLLΨ [CH_2OCONH]KLLK-NH2

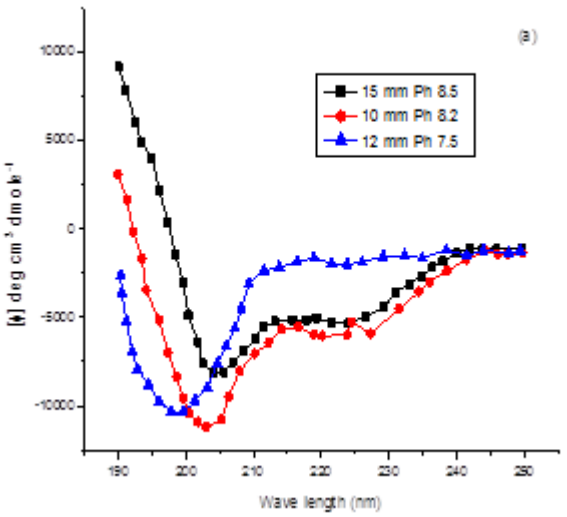
.Fig.1. General Method for the synthesis of carbamate bond in SPPS and primary structure of S1 and its pseudo peptides.

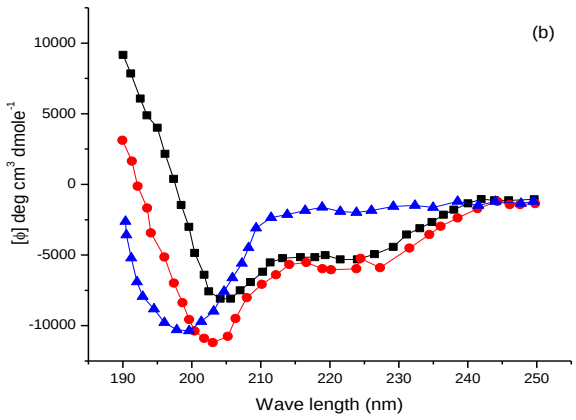
Considering the fact that one turn of α helix contain 4.5 amino acid residues carbamate bond(s) Ψ(CH_2O-CONH) were introduced after six amino residues and /or seven amino acid residues in the terminal of the peptides for decreasing amphiphilicity of the target peptide.The pseudo peptides were synthesized by a solid phase approach on the PAL resin using aFmoc-based strategy. When carbamate bond was introduced,Fmoc-carbonate monomer (10 equiv each) instead

of Fmoc amino acid was used in solid peptide synthesis.in the presence of **DIEA and HOBt** (fig.1).[16] This coupling reaction was repeated until no color change was observed in ninhydrin test.Cleavage from the resin and HPLC purification provided the desired pseudo peptide in greater than 20% yield, which comparable yield of the synthesis of the peptide. The purity of the synthetic peptide and pseudo peptides was above 98% as measured by analytical **HPLC and CE** respectively and the mass of all compounds were characterized by ESI mass spectrometry.

As shown in Table1 the introduction of carbamate bond did not affect the net positive charge of the peptide. However incorporation of carbamate bond decreased the retention time of the peptide on the C18 reverse HPLC column. It was reported that retention time which reflected hydrophobic interactions between peptide and C18 stationary phase,was parallel to the hydrophobicity of the peptides or to the α helicity of the peptides with the same amino acid composition.[17]The hydrophobic domain generated by amphipathic α helical structure of the peptide in hydrophobic environment affects hydrophobic interactions between the peptides and reverse-phase HPLC. The hydro-phobicity of the pseudo peptides should be greater than that of the peptide because carbamate bond(s) itself is more hydrophobic than amide bond.The retention time of the pseudopeptide with carbamate bond,however was shorter than that of the peptide,which suggested that the incorporation of carbamate bond (s) must decrease α helicity of the peptide.

Many structure-activity studies about linear antimicrobial peptides indicated that the secondary structure in membrane-mimetic environments rather than that in phosphate buffer was correlated well with the activity.The CD spectra of the compounds were measured in phosphate buffer with or without sodium dodecylsulfate (SDS) or trifluoroethanol (TFE) respectively.**TFE** structure-inducing solvent is commonly used for membrane-mimic environment[18] SDS micelles have been frequently used as a membrane-mimic system because SDS consisting of an aliphatic tail and a negatively charged head group forms micelles that mimic the negatively charged lipid membrane environment.[19,20] As shown in Figure 2a, CD spectra measured in phosphate buffer indicated that the peptide (S1) and the pseudo peptide (SC1) with one carbamate bond had considerable α helical conformations whereas pseudo peptide (SC2) with two carbamate bonds had random conformations.However, as shown in Figure 2b.CD spectra with negative ellipticity at 250 nm and 270 nm revealed that S1 formed a well-defined α helical structure in the presence of sodium dodecylsulfate (SDS) micelles where as SC1 Still formed α helical structure but the content of α helicity (25%) was much lower than that of S (60%). AS we expected,SC2 did not have α helical conformation and adoped random conformations as a major structure in the presence of SDS micelles.The fractional helicity of the compound measured in the presence of SDS micelles is summarized in Table1.As shown in Figure 2c,CD spectra measured in the presence of 70% **TFE (v/v),**indicated that the α helicity of each molecules was more increased than that obtained in the presence of **SDS** micelles however the structure difference trend of each molecules was similar to that obtained in the presence of SDS micelles CD Spectra indicated that the incorporation of carbamate bond seemed to have a medium dependent perturbation effect on the α helical structure and incorporation of two carbamate bonds into the peptide totally disturbed amphipathic α helical structure.As we expected the decrease of α helicity was mainly due to the fact that the incorporation of carbamate bonds decreased amphipathicity resulting in the induction of low α helical conformation in lipid membrane.In addition there is some possibility that carbamate bond itself should disturb the hydrogen bonding for maintaining α helical structure and even this effect additionally this effect additionally help to disturb α helical structure.





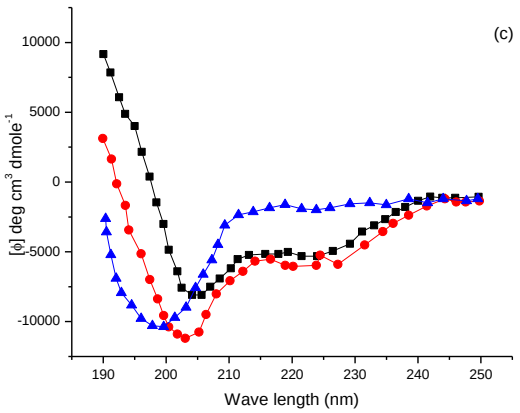


Fig. 2.CD spectra of S1 and its pseudo peptides:

(a) in 15 mM sodium phosphate buffer (pH,8.5),(S1;SC1;SC2)

(b) in 10 mM sodium phosphate buffer (pH,8.2)containing 30 mM SDS,S1,SC2.

(c) in 10 mM sodium phosphate buffer (pH,7.5) containing 60% TFE (v/v),S1,SC1;SC2.

AS shown in Table 1,the activities of S1and its pseudo peptides were measured against microorganisms including bacteria and fungi. The model peptide S1 and its pseudo peptides completely inhibited the growth of bacteria and fungi in the assay concentration range. However the introduction of carbamate bond (s) had a little different effect on the activity against bacteria and fungi,respectively. The single carbamate bond replacement retained antibacterial activity but seemed to decrease antifungal activity; the minimum inhibition concentration (MIC) of S1 for C.albicans was 7.4µg/mL whereas the MIC of SC1 for C.albicans was 11.5µg/mL.

Table1: Antimicrobial activities of S1 and its Pseudo peptides

Minimum Inhibitor Concentration (MIC) (µg/ml)

Name	**Net charge**	α helicity (%)	**Retention Time (min)**	**Staphylococcusaureus (ATCC6538)**	**micrococcuslutetus (ATCC9341)**	**Esherichia coli (ATCC2592)**	**Pseudomonas aerug in osa (ATCC9027)**	**Candida albicans (ATCC9027)**
S1	12	28	84.06	10.5	3.5	12.1	5.6	2.8
S2	12	05	78.26	5.6	4.1	8.4	10.8	9.7
S3	12	10	71.51	9.8	5.2	10.2	6.2	18.2

As shown in table 1, CD spectra was measured in the presence of SDS micelles The α helicity of the peptide was determined from the mean residue ellipticity [θ] at 300nm according to the relation $[\theta]_{300}$=-30300 [α]-2350 (where [α] is the amount of helix).

[b]The MIC values were calculated from three independent experiments performed in duplicate,which provided in a standard deviation below 40.

Introduction of two carbamate bonds into the peptide retained the activity against bacteria but decreased the activity against fungi by 6 times;the MIC of S1 for C.albicans was 7.4 µg/mLwhere as the MIC of SC2 was 25 ug/mL.This activity difference was clearly beyond our normal experimental error.We also tested the cytotoxicity of the peptide and its pseudo peptides against mouse erythrocytes tofind out the cytotoxicity for mammalian cell.As shown in Figure 3, the peptide S1 caused 100% lysis of erythrocytes at a concentration greater than 100 µg/mL and SC1 did 100 % lysis at a concentration greater than than 100 µg/mL.However SC2 did not cause 100% lysis of erythrocytes even at a concentration greater than 50 ug/mL and SC1 did 100% lysis at a concentration greater than 100 µg/ml. However, SC2 did not cause 100% lysis of erythrocytes even at a concentration up to 250 µg/mL.Well-known cytolytic peptide, melittin[21]caused 100% lysis at concentration greater than 512µg/mL in the same assay condition.The biological activity indicated that the introduction of carbamate bond into the cytolytic peptide separated bacteria cell toxicity from mammalian cell toxicity.

The membrane-permeable activities of S1 and its pseudo peptides were investigated bymeasuring dye release from vesicles with different surface charge density. We prepared the neutral charged large unilamellar vesicle (LUV)consisting of egg phosphatidylcholine (PC) and highly negative charged LUV consisting of egg phosphatidylglycerol (PG) to mimic the surface charge of lipid membrane of mammalian cell and bacterial cell,respectively. After the addition of S1 or its pseudo peptides,

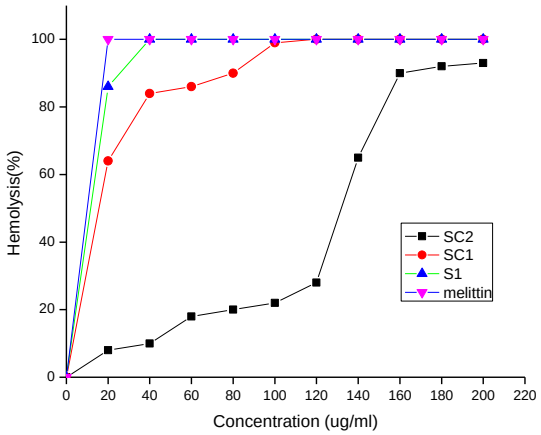


Fig.3. Hemolytic activity of S1 and its pseudo peptides.Erythrocytes were incubated in the Tris buffer (200 mM KCl,10mMTrisHCl, pH 7.4 with various concentration of sample for 1 h at 40^0c.

the release of fluorescence dye,calcein encapsulated in vesicle was determined by measuring the decrease of self-quenching on spectroflurorometer (Ex:520nm,Em:620nm). As shown in Figure 4a,all compounds employed in this study showed the similar membrane-perturbation activity on egg PG vesicles in the same assay concentration range, Which was consistent with the antibacterial activity measured in this study. As shown in Figure 4b,4 μg/ml of the peptide (S1)induced 100% leakage of calcein from PC LUV whereas even 10 μg/ml of the pseudopeptide with one carbamate bond (SC1) did not induce 100% of leakage in the PC LUV. The decreased leakage activity for PC LUV was more clearly observed in the pseudo peptide (SC2);SC2 just induced 30% of leakage of the dye at a concentration up to 10 μg/ml.This leakage potency of the peptide and its pseudo peptides for the vesicles with neutral charge density was consistent with hemolytic activity.

The leakage potency of the molecules was correlated with their biological activity, suggesting that the biological action of the molecules must be carried out through the lipid membrane as a primary target. And the separation of bacterial cell toxicity from mammalian cell toxicity must be due to the great membrane selectivity of the pseudo peptide.

Aswe mentioned before. The net positive charge hydrophobicity and amphipathic α helical structure of the peptide played an important role in the membrane perturbation activity and selectivity. In this study,we incorporated carbamate bond (s) into the peptide to disturb the amphiphilicity,which resulted in thedecrease α helicity,Amphiphilicity was frequently used as a parameter to modulated α helicity because the amphiphilicity of the peptide played an important role inducing α helical structure and β sheet structure in lipid membrane.[22] However under the circumstance without the detail structure information of the pseudo peptides in lipid membrane we cannot remove the possibility that carbamate bond itself disturbed the hydrogen bonding for α helical structure resulting in the decrease of α helicity.

The synthesized pseudo peptides had a greater selectivity between bacteria and mammalian cell than the peptide without the decrease of antibacterial activity.It was suggested that a great selectivity of some antibacterial peptide is due to the difference between bacteria lipid

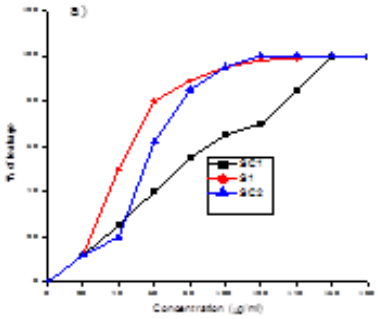


Fig. 4.Leakage of the dye from liposomes by S1 and pseudo peptides.

(a)The phospholipid of liposomes (10 μM) consisted of phosphatidyl –glycerol.Each Compound was incubated with liposomes for 0.8 h at 450 C; S1; o, SC1; SC2.

(b) The phospholipid of liposomes (15μM) consisted of phosphatidycholine.Each compound was incubated with liposomes for 0.8 h at 450C S1; O, SC1; SC2.

Membranes and mammalian lipid membranes. The surface charge was regarded as a most distinct difference the outer membranes of erythrocytes consisting of phosphatidycholine and sphingomyelin were almost electrical neutral whereas the bacterial lipid membranes consisting of mainly phosphatidylglycerol were negative charged. Our result also indicated that the surface charge difference in the lipid membrane of target cells and the loss of α helical structure of the pseudo peptides accompanying with decrease of hydrophobic interaction to the surface of lipid membranes are main factors for lipid membrane selectivity.

In a previous study, we have for the first time incorporated amide bond surrogate (carbamate bond and reduce amide bond)into the N-terminal of the membrane activity decapeptide[23] and developed potent antimicrobial pseudopeptide with increased stability in the presence of serum.[24]However, in the previous study as the membrane-activity decapeptide itself did not have hemolytic activity and their structure were not greatly changed by an incorporation of amide bond surrogate,we could not observe the real effect amide bond surrogate on the hemolytic activity.In the present study, we designed and synthesized novel pseudo peptides with selective membrane-perturbation activity by using the characteristics of amide bond surrogates. We did not compare

in vivo stability between the peptide and its pseudo peptides described here. However on the basis of our previous result and the fact that introduction of amide surrogate(s) of peptides,located away from a protesase labile bond, also increased the resistance against enzymatic degradation,[25] we expect that the bioavailability of the pseudopeptide should be improved, In the present study the introducation of carbamate bond(s) into the peptide for improving bio-availability simultaneously improved the lipid membrane selectivity resulting in the retention of the antibacterial activity and decrease of mammalian cell toxicity.The present result also suggests that backbone modifications easily applied in solid phase synthesis can be a great tool for developing non-peptide or pseudopeptide drug with improved activity as well as improved bioavailability from membrane-active peptides.

IV. CONCLUSION

By using the characteristic of amide bond surrogate novel pseudo peptides which have more improved bio-availability as well as improved biological activity were synthesized from a cytolyticpeptide that acted on the lipid membrane.The incorporation of the carbamate bonds decreased α helicity structure and hydrophobic interactions to lipid membrane,which must be a major factor for the separation of antibacterial activity from hemolytic activity of the cytolytic peptide.The present hemolytic activity of the cytolytic peptide. The present study suggested that backbone modifications,easily applied in solid phase synthesis, can be a great tool for developing antibacterial from membrane-active peptides.

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