Design and Synthesis of Amphiphilic Basic Peptides with Antibacterial Activity and Their Interaction with Model Membrane

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Twelve peptides having 4 to 6 basic amino acid residues in the hydrophobic amino acid sequence were designed and synthesized by the solution method to find peptides with potential activity against Gram-positive and -negative bacteria. CD study of the peptides demonstrated that they formed α-helical structures in the presence of phospholipid liposomes consequently to have amphiphilic property along the axis of helix. The peptides also induced the leakage of the dye carboxyfluorescein from phospholipid vesicles, indicating that the peptides possess the ability to perturb the membrane structure. These peptides showed antibacterial activity against Gram-positive bacteria, and some of them were also active against Gram-negative bacteria. Relationships between the structure and antibacterial activity of the synthetic peptides were discussed.

Recently, an amphiphilic α-helical structure, especially that containing basic amino acids, has been found to be a common factor in several biologically active peptides, e.g., plasma apolipoproteins,[1,2] calmodulin-binding peptides,[3,4] bee venom toxins[5,6] and antibacterial peptides.[7-10] Previously, we reported[11,12] that the basic model peptides, e.g., Ac-(Leu-Ala-Lys-Leu)$_{3,4}$-NHCH$_3$ ($\mathbf{4}_3$, $\mathbf{4}_4$), which were designed from the common feature of extension peptides of mitochondrial protein precursors, showed inhibition of import of the precursors to mitochondria, antibacterial activity against Gram-positive bacteria and perturbation of mitochondrial membrane and phospholipid bilayer. Their antibacterial activities were also correlated to the secondary structure of the peptides.[12] Thus, simple model peptides designed can be used as an effective tool to clarify the structure proposed for biological activity.

DeGrado reported[13] that a simple amphiphilic α-helical peptide, Fmoc-(Leu-Lys-Lys-Leu-Leu-Lys-Leu)$_{2\tau}$OH (F),[14] which was designed from cecropin,[7,8] a naturally occurring cationic amphiphilic antibiotic, showed the same strong antibacterial activity as cecropin against Gram-positive and -negative bacteria. Ando et al.[15] reported that an analog of gramicidin S (GS), [D-2,3-diaminopropionic acid (D-Dpr)$^{4,4'}$]GS, had an activity against Gram-positive and -negative bacteria, although GS which has a cationic amphiphilic structure with antiparallel β-sheet and β-turn is only active against Gram-positive bacteria. The reason of the difference in the activity against Gram-negative bacteria between the model peptide $\mathbf{4}_3$ or GS and peptide F or [D-Dpr$^{4,4'}$]GS should be due to the num-

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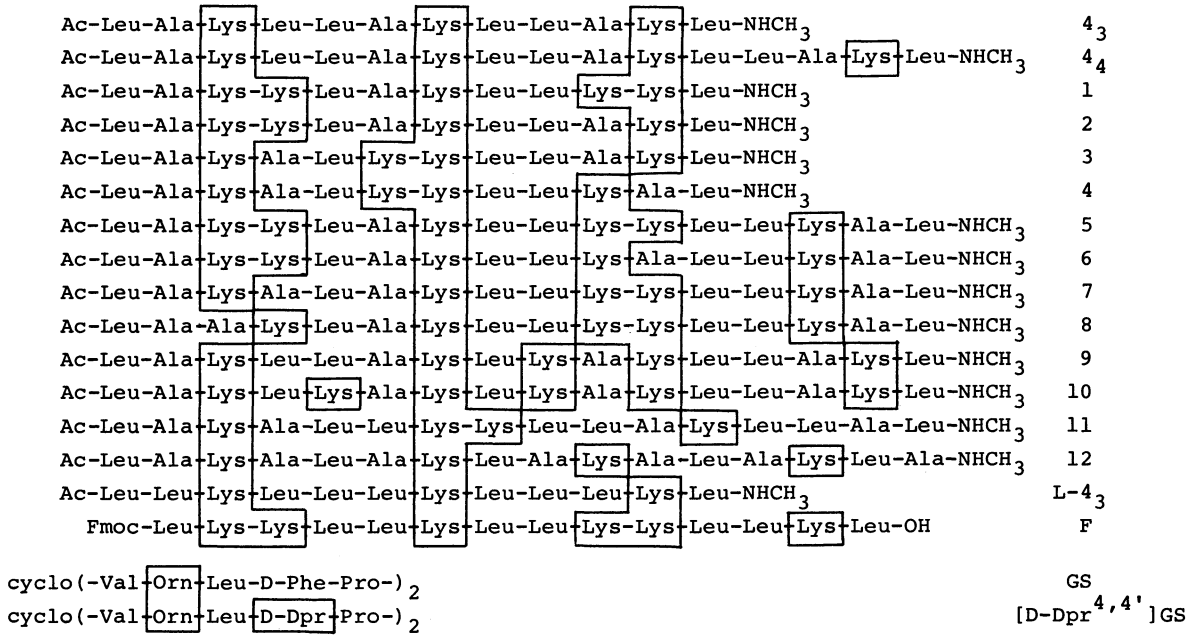

Fig. 1. Synthetic model peptides and gramicidin S. All amino acids are of L-configuration.

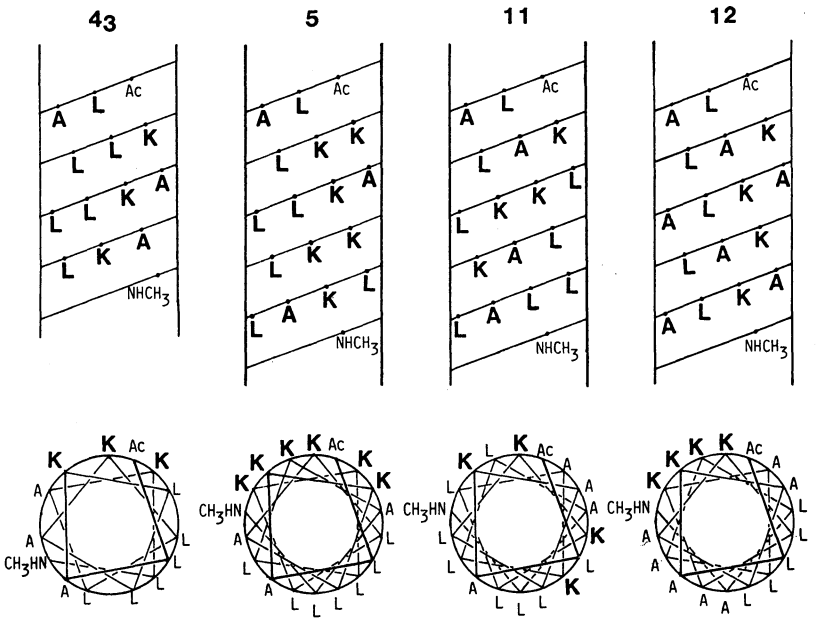

Fig. 2. α-Helical nets and wheels of peptides 4_3, **5**, **11**, and **12**. One-letter symbols of amino acids are used for concise representation: A, alanine; K, lysine; L, leucine.

ber of basic amino acids and the secondary structure, i.e., amphiphilicity. In order to study the relationships between the activity against Gram-positive or -negative bacteria and the property of amphiphilic peptides, we have designed and synthesized several basic peptides as shown in Fig. 1, and examined antibacterial activities of the peptides and their secondary structures by CD measurements. Phase-transition release experiment of carboxyfluorescein from DPPC vesicles using the peptides was also performed in order to examine whether the ability to perturb bacterial membrane relates to the antimicrobial activity.

Results

Design. The model peptides (Fig. 1) were designed on the basis of the structures of 4_3 and 4_4 showing the strongest antimicrobial activity among the previously synthesized model peptides[12] and of peptide F taking amphiphilic α-helical structure and having the activity against Gram-positive and -negative bacteria.[13] The structural differences between 4_4 and F are just the number of basic amino acids and distribution of the Lys residues in hydrophobic sequences. Therefore, to develop peptides active against Gram-negative bacteria, the number and distribution of basic amino acid Lys were altered in the peptides. In compounds **1**—**8**, one or two additional Lys residues are distributed into 4_3 or 4_4 to keep amphiphilicity postulating that these peptides take α-helices. The sequence of **5** is almost the same as that of peptide F except for the acetyl group and the Ala residues in **5** instead of the Fmoc group and the Leu residues in F, respectively. Peptides **9** and **10** were designed to disturb the amphiphilic

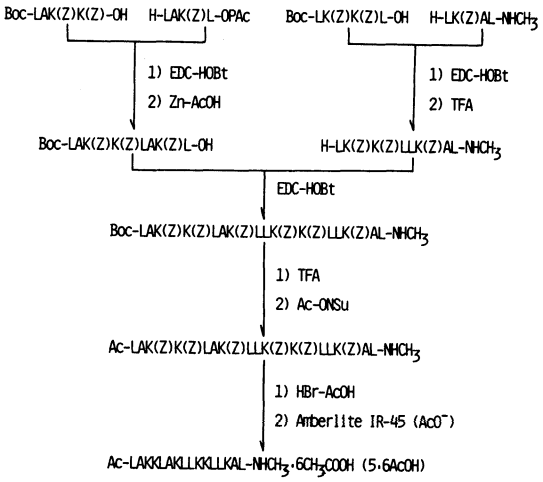

Fig. 3. Synthetic route for Ac-hexadecapeptide-$NHCH_3$ (**5**).

property by the substitution of Lys for the Leu residues in hydrophobic cluster. Orientation of the Lys residues in peptide **11** with α-helical structure is topologically similar to that of four basic amino acid residues in [D-Dpr$^{4,4'}$]GS. α-Helical nets and wheels of representative peptides are shown in Fig. 2. In peptide **12**, the Leu and Lys residues lie straight along the axis of the α-helix. Moreover, peptide L-4_3 containing Leu residues instead of Ala residues in 4_3 and peptide F were also synthesized.

Synthesis. The synthetic route for **5** is shown in Fig. 3. The tetrapeptides were prepared by stepwise elongation of the Boc-amino acid active esters.[16] The Boc-dipeptide-OPac was prepared by the mixed anhydride method.[17] After the cleavage of phenacyl ester

or Boc group, the tetrapeptides were coupled by the EDC-HOBt method[18] to give Boc-octapeptide-OPac or -$NHCH_3$. The phenacyl ester and Boc group were removed with Zn powder in acetic acid and with TFA, respectively. The resulting acid and amine components were coupled by the EDC-HOBt method to give Boc-hexadecapeptide-$NHCH_3$. The Boc group was cleaved with TFA followed by acetylation with Ac-ONSu to give Ac-peptide-$NHCH_3$. The Z groups were removed with 25% HBr in AcOH and peptide hydrobromide obtained was passed through a column of Amberlite IR-45 (acetate form) to give **5** as a tetraacetate. The purity was confirmed by TLC, paper electrophoresis, amino acid analysis and elemental analysis. Other peptides were prepared similarly. Yields and physical constants of protected peptides and final products are shown in Tables 1 and 2.

Antibacterial Activity. Antibacterial assays were

Table 1. Yields and Physical Constants of Protected-Peptides

Peptides	Formula (MW)[a]	Yield %	Mp θ_m/°C	$[\alpha]_D^{20}$[b]	TLC R_f[c]
Boc-AL-OPac (13)	$C_{22}H_{32}O_6N_2$ (420.5)	89	powder	—	0.81
Boc-AK(Z)-OPac (14)	$C_{30}H_{39}O_8N_3$ (569.7)	89	105-110	-19.8	0.68
Boc-K(Z)A-OPac (15)	$C_{30}H_{39}O_8N_3$ (569.7)	82	53-54	-30.1	0.67
Boc-K(Z)K(Z)-OPac (16)	$C_{41}H_{52}O_{10}N_4 \cdot 1/2H_2O$ (769.9)	91	63-64	-36.4	0.70
Boc-AL-$NHCH_3$ (17)	$C_{15}H_{29}O_4N_3$ (315.4)	90	162-165	-33.8	0.64
Boc-LA-$NHCH_3$ (18)	$C_{15}H_{29}O_4N_3$ (315.4)	82	170-171	-18.1	0.66
Boc-AAK(Z)-OPac (19)	$C_{33}H_{44}O_9N_4$ (640.7)	86	119-124	-18.4	0.73
Boc-AK(Z)A-OPac (20)	$C_{33}H_{44}O_9N_4$ (640.7)	90	134-136	-27.5	0.64
Boc-LAK(Z)-OPac (21)	$C_{36}H_{50}O_9N_4$ (682.8)	95	89-92	-21.5	0.76
Boc-K(Z)AL-OPac (22)	$C_{36}H_{50}O_9N_4$ (682.8)	92	83-86	-27.4	0.69
Boc-LK(Z)L-OPac (23)	$C_{39}H_{56}O_9N_4$ (724.9)	91	148-150	-31.8	0.80
Boc-AK(Z)K(Z)-OPac (24)	$C_{44}H_{57}O_{11}N_5$ (832.0)	95	103-105	-17.2	0.75
Boc-LK(Z)K(Z)-OPac (25)	$C_{47}H_{63}O_{11}N_5$ (874.0)	94	116-121	-20.6	0.76
Boc-K(Z)K(Z)L-OPac (26)	$C_{47}H_{63}O_{11}N_5$ (874.0)	87	120-121	-21.9	0.74
Boc-LAL-$NHCH_3$ (27)	$C_{21}H_{40}O_5N_4$ (428.6)	85	220-222	-32.0	0.63
Boc-K(Z)AL-$NHCH_3$ (28)	$C_{29}H_{47}O_7N_5$ (577.7)	97	189-193	-20.2	0.63
Boc-K(Z)LA-$NHCH_3$ (29)	$C_{29}H_{47}O_7N_5$ (577.7)	84	181-183	-21.8	0.62
Boc-LK(Z)L-$NHCH_3$ (30)	$C_{32}H_{53}O_7N_5 \cdot 1/2H_2O$ (628.8)	80	183-187	-27.2	0.68
Boc-K(Z)K(Z)L-$NHCH_3$ (31)	$C_{40}H_{60}O_9N_6$ (769.0)	96	147-150	-16.8	0.65
Boc-LAAK(Z)-OPac (32)	$C_{39}H_{55}O_{10}N_5$ (753.9)	94	117-122	-22.4	0.71
Boc-LAK(Z)A-OPac (33)	$C_{39}H_{55}O_{10}N_5 \cdot 1/2H_2O$ (762.9)	94	168-170	-30.2	0.68
Boc-LLAK(Z)-OPac (34)	$C_{42}H_{61}O_{10}N_5$ (796.0)	95	157-161	-29.5	0.63
Boc-LK(Z)AL-OPac (35)	$C_{42}H_{61}O_{10}N_5 \cdot 1/2H_2O$ (805.0)	97	117-119	-34.2	0.74
Boc-LLK(Z)L-OPac (36)	$C_{45}H_{67}O_{10}N_5$ (838.1)	84	170-173	-36.1	0.75
Boc-LAK(Z)K(Z)-OPac (37)	$C_{50}H_{68}O_{12}N_6 \cdot 1/2H_2O$ (954.1)	86	115-119	-18.7	0.75
Boc-K(Z)AK(Z)L-OPac (38)	$C_{50}H_{68}O_{12}N_6 \cdot 1/2H_2O$ (954.1)	94	151-154	-34.6	0.70
Boc-LLK(Z)K(Z)-OPac (39)	$C_{53}H_{74}O_{12}N_6 \cdot 1/2H_2O$ (996.2)	80	127-129	-24.9	0.69
Boc-LK(Z)K(Z)L-OPac (40)	$C_{53}H_{74}O_{12}N_6 \cdot 1/2H_2O$ (996.2)	93	134-136	-27.2	0.77
Boc-LAAK(Z)-OH (41)	$C_{31}H_{49}O_9N_5$ (635.8)	81	71-78	-14.2	0.59
Boc-LAK(Z)A-OH (42)	$C_{31}H_{49}O_9N_5$ (635.8)	90	131-133	-19.5	0.52
Boc-LLAK(Z)-OH (43)	$C_{34}H_{55}O_9N_5$ (677.8)	87	136-139	-24.2	0.56
Boc-LK(Z)AL-OH (44)	$C_{34}H_{55}O_9N_5$ (677.8)	88	powder	—	0.66
Boc-LLK(Z)L-OH (45)	$C_{37}H_{61}O_9N_5 \cdot 1/2H_2O$ (728.9)	87	108-110	-31.2	0.66
Boc-LAK(Z)K(Z)-OH (46)	$C_{42}H_{62}O_{11}N_6 \cdot 1/2H_2O$ (836.0)	81	124-128	-13.6	0.69
Boc-K(Z)AK(Z)L-OH (47)	$C_{42}H_{62}O_{11}N_6$ (827.0)	93	81-82	-16.0	0.65
Boc-LK(Z)K(Z)L-OH (48)	$C_{45}H_{68}O_{11}N_6$ (869.1)	96	74-76	-22.8	0.67
Boc-LLAL-$NHCH_3$ (49)	$C_{27}H_{51}O_6N_5$ (541.7)	90	257-260	-43.2	0.65
Boc-AK(Z)LA-$NHCH_3$ (50)	$C_{32}H_{52}O_8N_6 \cdot 1/3H_2O$ (654.8)	95	179-181	-23.5	0.66

Table 1. (Continued)

Peptides	Formula (MW)[a]	Yield %	Mp θ_m/°C	$[\alpha]_D^{20}$[b]	TLC R_f[c]
Boc-LK(Z)AL-$NHCH_3$ (51)	$C_{35}H_{58}O_8N_6$ (690.9)	91	186–189	−26.6	0.67
Boc-LLK(Z)L-$NHCH_3$ (52)	$C_{38}H_{64}O_8N_6 \cdot H_2O$ (751.0)	87	190–193	−30.1	0.68
Boc-LK(Z)K(Z)L-$NHCH_3$ (53)	$C_{46}H_{71}O_{10}N_7$ (882.1)	85	207–210	−20.2	0.64
Boc-LAK(Z)LA-$NHCH_3$ (54)	$C_{38}H_{63}O_9N_7$ (762.0)	85	216–219	−24.7	0.65
Boc-LAK(Z)ALAK(Z)-OPac (55)	$C_{62}H_{89}O_{15}N_9$ (1200.4)	95	245–248(d)	−28.5[d]	0.68
Boc-LK(Z)K(Z)LLK(Z)L-OPac (56)	$C_{79}H_{114}O_{17}N_{10} \cdot 3H_2O$ (1529.9)	96	215–224(d)	−23.6	0.67
Boc-LAK(Z)ALAK(Z)-OH (57)	$C_{54}H_{83}O_{14}N_9 \cdot 3/2H_2O$ (1109.3)	95	265–270(d)	−20.9[d]	0.47
Boc-LK(Z)K(Z)LLK(Z)L-OH (58)	$C_{71}H_{108}O_{16}N_{10} \cdot 2H_2O$ (1393.7)	96	240–245(d)	−25.6	0.58
Boc-LAAK(Z)LAK(Z)L-OPac (59)	$C_{68}H_{100}O_{16}N_{10} \cdot H_2O$ (1331.6)	95	181–184(d)	−27.6	0.67
Boc-LAK(Z)ALAK(Z)L-OPac (60)	$C_{68}H_{100}O_{16}N_{10} \cdot 1/2H_2O$ (1322.6)	91	265–267(d)	−30.1	0.62
Boc-LAK(Z)LLAK(Z)L-OPac (61)	$C_{71}H_{106}O_{16}N_{10} \cdot H_2O$ (1373.7)	93	211–216(d)	−28.8	0.64
Boc-LAK(Z)ALLK(Z)K(Z)-OPac (62)	$C_{79}H_{113}O_{18}N_{11} \cdot 1/2H_2O$ (1513.8)	92	225–230(d)	−24.6	0.66
Boc-LAK(Z)ALK(Z)K(Z)L-OPac (63)	$C_{79}H_{113}O_{18}N_{11} \cdot H_2O$ (1522.9)	91	225–228(d)	−25.4	0.68
Boc-LAK(Z)LK(Z)AK(Z)L-OPac (64)	$C_{79}H_{113}O_{18}N_{11} \cdot H_2O$ (1522.9)	96	208–210(d)	−26.0	0.67
Boc-LAK(Z)K(Z)LAK(Z)L-OPac (65)	$C_{79}H_{113}O_{18}N_{11} \cdot H_2O$ (1522.9)	96	245–247(d)	−25.1	0.66
Boc-LAAK(Z)LAK(Z)L-OH (66)	$C_{60}H_{94}O_{15}N_{10} \cdot 3/2H_2O$ (1222.5)	95	230–234(d)	−28.8[d]	0.59
Boc-LAK(Z)ALAK(Z)L-OH (67)	$C_{60}H_{94}O_{15}N_{10} \cdot H_2O$ (1213.5)	95	265–267(d)	−26.8	0.57
Boc-LAK(Z)LLAK(Z)L-OH (68)	$C_{63}H_{100}O_{15}N_{10} \cdot H_2O$ (1255.6)	93	295–300(d)	−29.6[d]	0.56
Boc-LAK(Z)ALLK(Z)K(Z)-OH (69)	$C_{71}H_{107}O_{17}N_{11} \cdot 2H_2O$ (1422.7)	94	227–233(d)	−26.4	0.54
Boc-LAK(Z)ALK(Z)K(Z)L-OH (70)	$C_{71}H_{107}O_{17}N_{11} \cdot 3/2H_2O$ (1413.7)	96	229–234(d)	−22.9	0.52
Boc-LAK(Z)LK(Z)AK(Z)L-OH (71)	$C_{71}H_{107}O_{17}N_{11} \cdot 3/2H_2O$ (1413.7)	94	240–245(d)	−24.4[d]	0.51
Boc-LAK(Z)K(Z)LAK(Z)L-OH (72)	$C_{71}H_{107}O_{17}N_{11} \cdot 3/2H_2O$ (1413.7)	96	264–269(d)	−21.9	0.54
Boc-LLAK(Z)LLAL-$NHCH_3$ (73)	$C_{56}H_{96}O_{12}N_{10} \cdot 1/2H_2O$ (1110.5)	94	257–261(d)	−41.5[d]	0.63
Boc-LK(Z)ALLK(Z)AL-$NHCH_3$ (74)	$C_{64}H_{103}O_{14}N_{11} \cdot 1/2H_2O$ (1259.6)	95	255–258(d)	−21.2	0.58
Boc-LLK(Z)LLLK(Z)L-$NHCH_3$ (75)	$C_{70}H_{115}O_{14}N_{11} \cdot H_2O$ (1352.8)	96	284–287(d)	−41.2[d]	0.64
Boc-K(Z)AK(Z)LLAK(Z)L-$NHCH_3$ (76)	$C_{72}H_{110}O_{16}N_{12} \cdot 1/2H_2O$ (1408.8)	94	267–270(d)	−25.6[d]	0.64
Boc-LK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (77)	$C_{75}H_{116}O_{16}N_{12} \cdot H_2O$ (1459.8)	89	255–260(d)	−17.2	0.55
Boc-LAK(Z)ALAK(Z)LA-$NHCH_3$ (78)	$C_{64}H_{102}O_{15}N_{12} \cdot 1/2H_2O$ (1288.6)	89	276–280(d)	−27.2[d]	0.56
Boc-LAK(Z)K(Z)LAK(Z)LLK(Z)K(Z)L-$NHCH_3$ (79)	$C_{112}H_{168}O_{24}N_{18} \cdot 3/2H_2O$ (2177.7)	96	305–310(d)	−22.0[d]	0.58
Boc-LAK(Z)K(Z)LAK(Z)LLAK(Z)L-$NHCH_3$ (80)	$C_{101}H_{155}O_{22}N_{17} \cdot 3/2H_2O$ (1986.5)	96	296–299(d)	−22.0[d]	0.61
Boc-LAK(Z)ALK(Z)K(Z)LLAK(Z)L-$NHCH_3$ (81)	$C_{101}H_{155}O_{22}N_{17} \cdot 2H_2O$ (1995.5)	95	290–295(d)	−22.6[d]	0.62
Boc-LAK(Z)ALK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (82)	$C_{101}H_{155}O_{22}N_{17} \cdot 2H_2O$ (1995.5)	95	292–297(d)	−25.8[d]	0.62
Boc-LAK(Z)K(Z)LAK(Z)LLK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (83)	$C_{141}H_{213}O_{30}N_{23} \cdot 2H_2O$ (2746.4)	89	295–300(d)	−31.1[d]	0.70
Boc-LAK(Z)K(Z)LAK(Z)LLK(Z)ALLK(Z)AL-$NHCH_3$ (84)	$C_{130}H_{200}O_{28}N_{22} \cdot 2H_2O$ (2555.2)	95	305–310(d)	−17.6[d]	0.68
Boc-LAK(Z)ALAK(Z)LLK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (85)	$C_{130}H_{200}O_{28}N_{22} \cdot 2H_2O$ (2555.2)	95	290–295(d)	−21.4[d]	0.62
Boc-LAAK(Z)LAK(Z)LLK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (86)	$C_{130}H_{200}O_{28}N_{22} \cdot 2H_2O$ (2555.2)	96	295–300(d)	−18.8[d]	0.67
Boc-LAK(Z)LLAK(Z)LK(Z)AK(Z)LLAK(Z)L-$NHCH_3$ (87)	$C_{130}H_{200}O_{28}N_{22} \cdot 5/2H_2O$ (2564.2)	95	275–280(d)	−15.2[d]	0.68
Boc-LAK(Z)LK(Z)AK(Z)LK(Z)AK(Z)LLAK(Z)L-$NHCH_3$ (88)	$C_{138}H_{207}O_{30}N_{23} \cdot 2H_2O$ (2704.3)	92	285–290(d)	−12.8[d]	0.67
Boc-LAK(Z)ALLK(Z)K(Z)LLAK(Z)LLAL-$NHCH_3$ (89)	$C_{122}H_{193}O_{26}N_{21} \cdot 2H_2O$ (2406.0)	95	265–270(d)	−30.0[d]	0.68
Boc-LAK(Z)ALAK(Z)LAK(Z)ALAK(Z)LA-$NHCH_3$ (90)	$C_{113}H_{175}O_{26}N_{21} \cdot 4H_2O$ (2315.8)	96	229–231(d)	−21.4[d]	0.62
Boc-(LLK(Z)L)$_3$-$NHCH_3$ (91)	$C_{102}H_{166}O_{20}N_{16} \cdot H_2O$ (1954.6)	96	305–310(d)	−33.3[d]	0.70
Boc-(LK(Z)K(Z)LLK(Z)L)$_2$-OPac (92)	$C_{145}H_{212}O_{30}N_{20} \cdot 2H_2O$ (2751.4)	92	295–300(d)	−21.6[d]	0.68
Ac-LAK(Z)K(Z)LAK(Z)LLK(Z)K(Z)L-$NHCH_3$ (93)	$C_{109}H_{162}O_{23}N_{18} \cdot 2H_2O$ (2128.6)	95	293–297(d)	−20.4[d]	0.55
Ac-LAK(Z)K(Z)LAK(Z)LLAK(Z)L-$NHCH_3$ (94)	$C_{98}H_{149}O_{21}N_{17} \cdot H_2O$ (1919.4)	96	280–284(d)	−17.2[d]	0.53
Ac-LAK(Z)ALK(Z)K(Z)LLAK(Z)L-$NHCH_3$ (95)	$C_{98}H_{149}O_{21}N_{17} \cdot H_2O$ (1919.4)	86	251–253(d)	−28.9[d]	0.58
Ac-LAK(Z)ALK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (96)	$C_{98}H_{149}O_{21}N_{17} \cdot H_2O$ (1919.4)	93	285–290(d)	−26.3[d]	0.56
Ac-LAK(Z)K(Z)LAK(Z)LLK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (97)	$C_{138}H_{207}O_{29}N_{23} \cdot 2H_2O$ (2688.3)	86	287–292(d)	−12.0[d]	0.60

Table 1. (Continued)

Peptides	Formula (MW)[a]	Yield %	Mp θ_m/°C	$[\alpha]_D^{20}$[b]	TLC R_f[c]
Ac-LAK(Z)K(Z)LAK(Z)LLK(Z)ALLK(Z)AL-$NHCH_3$ (98)	$C_{127}H_{194}O_{27}N_{22}\cdot 2H_2O$ (2497.1)	88	240–245(d)	−16.0[d]	0.68
Ac-LAK(Z)ALAK(Z)LLK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (99)	$C_{127}H_{194}O_{27}N_{22}\cdot H_2O$ (2479.1)	93	240–250(d)	−21.5[d]	0.57
Ac-LAAK(Z)LAK(Z)LLK(Z)K(Z)LLK(Z)AL-$NHCH_3$ (100)	$C_{127}H_{194}O_{27}N_{22}\cdot 3H_2O$ (2515.1)	87	265–270(d)	−16.8[d]	0.64
Ac-LAK(Z)LLAK(Z)LK(Z)AK(Z)LLAK(Z)L-$NHCH_3$ (101)	$C_{127}H_{194}O_{27}N_{22}\cdot 3H_2O$ (2515.1)	95	275–280(d)	−12.0[d]	0.66
Ac-LAK(Z)LK(Z)AK(Z)LK(Z)AK(Z)LLAK(Z)L-$NHCH_3$ (102)	$C_{135}H_{201}O_{29}N_{23}\cdot 2H_2O$ (2646.3)	91	285–290(d)	−11.6[d]	0.65
Ac-LAK(Z)ALLK(Z)K(Z)LLAK(Z)LLAL-$NHCH_3$ (103)	$C_{119}H_{187}O_{25}N_{21}\cdot 4H_2O$ (2384.0)	92	280–285(d)	−22.6[d]	0.59
Ac-LAK(Z)ALAK(Z)LAK(Z)ALAK(Z)LA-$NHCH_3$ (104)	$C_{110}H_{169}O_{25}N_{21}\cdot 4H_2O$ (2257.7)	93	250–255(d)	−17.4[d]	0.51
Ac-$(LLK(Z)L)_3$-$NHCH_3$ (105)	$C_{99}H_{160}O_{19}N_{16}\cdot 3/2H_2O$ (1905.4)	96	305–310(d)	−30.3[d]	0.72
Fmoc-$(LK(Z)K(Z)LLK(Z)L)_2$-OPac (106)	$C_{155}H_{214}O_{30}N_{20}\cdot H_2O$ (2855.5)	81	270–280(d)	−23.2[d]	0.78
Fmoc-$(LK(Z)K(Z)LLK(Z)L)_2$-OH (107)	$C_{147}H_{208}O_{29}N_{20}\cdot 4H_2O$ (2791.5)	88	290–295(d)	−17.6[d]	0.57

a) The results of elemental analysis agreed with calculated values within ±0.3%. b) *c* 1.0, DMF. c) $CHCl_3$–MeOH (5:1, v/v). d) *c* 0.5, DMSO.

Table 2. Yields and Physical Constants of the Model Peptides[a]

Peptide	Formula (MW)[b]	Yield/%	TLC/R_f[c]	Pep(R_{Lys})[d]
Ac-LAKKLAKLLKKL-$NHCH_3\cdot$5AcOH (1·5AcOH)	$C_{69}H_{132}O_{13}N_{18}\cdot 5C_2H_4O_2\cdot 7H_2O$ (1848.3)	88	0.30	0.90
Ac-LAKKLAKLLAKL-$NHCH_3\cdot$4AcOH (2·4AcOH)	$C_{66}H_{125}O_{13}N_{17}\cdot 4C_2H_4O_2\cdot 6H_2O$ (1713.1)	82	0.39	0.80
Ac-LAKALKKLLAKL-$NHCH_3\cdot$4AcOH (3·4AcOH)	$C_{66}H_{125}O_{13}N_{17}\cdot 4C_2H_4O_2\cdot 5H_2O$ (1695.0)	89	0.43	0.79
Ac-LAKALKKLLKAL-$NHCH_3\cdot$4AcOH (4·4AcOH)	$C_{66}H_{125}O_{13}N_{17}\cdot 4C_2H_4O_2\cdot 3H_2O$ (1659.1)	81	0.41	0.81
Ac-LAKKLAKLLKKLLKAL-$NHCH_3\cdot$6AcOH (5·6AcOH)	$C_{90}H_{171}O_{17}N_{23}\cdot 6C_2H_4O_2\cdot 7H_2O$ (2333.9)	84	0.33	0.81
Ac-LAKKLAKLLKALLKAL-$NHCH_3\cdot$5AcOH (6·5AcOH)	$C_{87}H_{164}O_{17}N_{22}\cdot 5C_2H_4O_2\cdot 5H_2O$ (2180.7)	79	0.39	0.77
Ac-LAKALAKLLKKLLKAL-$NHCH_3\cdot$5AcOH (7·5AcOH)	$C_{87}H_{164}O_{17}N_{22}\cdot 5C_2H_4O_2\cdot 6H_2O$ (2198.6)	89	0.45	0.79
Ac-LAAKLAKLLKKLLKAL-$NHCH_3\cdot$5AcOH (8·5AcOH)	$C_{87}H_{164}O_{17}N_{22}\cdot 5C_2H_4O_2\cdot 5H_2O$ (2180.7)	78	0.44	0.75
Ac-LAKLLAKLKAKLLAKL-$NHCH_3\cdot$5AcOH (9·5AcOH)	$C_{87}H_{164}O_{17}N_{22}\cdot 5C_2H_4O_2\cdot 3H_2O$ (2147.7)	73	0.38	0.76
Ac-LAKLKAKLKAKLLAKL-$NHCH_3\cdot$6AcOH (10·6AcOH)	$C_{87}H_{165}O_{17}N_{23}\cdot 6C_2H_4O_2\cdot 5H_2O$ (2255.8)	81	0.36	0.81
Ac-LAKALLKKLLAKLLAL-$NHCH_3\cdot$4AcOH (11·4AcOH)	$C_{87}H_{163}O_{17}N_{21}\cdot 4C_2H_4O_2\cdot 8H_2O$ (2159.7)	84	0.48	0.70
Ac-LAKALAKLAKALAKLA-$NHCH_3\cdot$4AcOH (12·4AcOH)	$C_{78}H_{145}O_{17}N_{21}\cdot 4C_2H_4O_2\cdot 8H_2O$ (2033.5)	94	0.46	0.70
Ac-$(LLKL)_3$-$NHCH_3\cdot$3AcOH (L-$4_3\cdot$3AcOH)	$C_{66}H_{124}O_{13}N_{22}\cdot 3C_2H_4O_2\cdot 3H_2O$ (1688.0)	92	0.48	0.67
Fmoc-$(LKKLLKL)_2$-OH·6AcOH (F·6AcOH)	$C_{99}H_{172}O_{17}N_{20}\cdot 6C_2H_4O_2\cdot 10H_2O$ (2455.1)	90	0.34	0.86

a) Amino acid analysis of the peptides gave satisfactory results of the amino acid components. b) The reasonable compositions were considered from the results of elemental analysis. c) *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2, v/v). d) Relative value to Lys.

carried out by both agar dilution[19] and serial dilution method in solution medium.[20] The results of antibacterial assay of the peptides are listed in terms of minimum inhibitory concentration of growth (MIC) in Table 3 compared with that of peptide $\mathbf{4}_3$, $\mathbf{4}_4$, GS, [D-Dpr$^{4,4'}$]GS and polymyxin B (PM). In agar dilution method, present model peptides (**1**—**12** and L-$\mathbf{4}_3$) revealed weak activity against Gram-positive bacteria as compared with peptide $\mathbf{4}_3$ and GS. On the other hand, peptides with the increased number of the Lys residues (**1** and **9**) showed weak but substantial activity against Gram-negative bacteria such as *E. coli*. On the contrary, by means of serial dilution method in solution medium, all the peptides examined were active against both Gram-positive and -negative bacteria. Especially peptides **1** and **5** showed stronger activity against *E. coli* than GS did. These results indicate that increase in the number of the Lys residues leads to decrease in the activity against Gram-positive bacteria and increase in that against Gram-negative bacteria. It is noteworthy that the decrease in activity of the peptides against *S. aureus* was more marked than that against *B. subtilis*, this fact being comparable to the result that PM was inactive against *S. aureus* although strongly active against Gram-negative bacteria. Non-amphiphilic peptides (**9** and **10**) showed fairly high activity, suggesting that amphiphilic character is indeed important, but not essential for the activity against Gram-positive bacteria, especially *B. subtilis*. Deviation in distribution of the Lys residues from pep-

Table 3. Antibacterial Activity of the Peptides

Peptide	Minimum inhibitory concentration/μg ml^{-1}			
	S.aureus FDA 209P	*B.subtilis* PCI 219	*E.coli* NIHJ JC-2	*S.flexneri* EW-10
1	100	25 (0.5)[a]	100 (1.25)[a]	6.25
2	100	12.5	>100	12.5
3	50	25	>100	12.5
4	>100	25	>100	25
5	>100	50 (1.25)[a]	>100 (2.5)[a]	50
6	100	50	>100	100
7	100	25	>100	50
8	>100	25	>100	50
9	100	12.5	100	6.25
10	>100	50	>100	12.5
11	25	12.5 (0.5)[a]	>100 (5.0)[a]	12.5
12	>100	50	>100	>100
L-**4**$_3$	25	25	>100	>100
F	>100	100 (2.5)[a]	>100 (5.0)[a]	25
4$_3$	6.25	3.13 (0.5)[a]	>100 (6.0)[a]	6.25
4$_4$	25	12.5	>100	>100
GS	3.13	3.13 (0.5)[a]	>100 (5.0)[a]	6.25
[D-Dpr$^{4,4'}$]GS[b]	100	12.5	25	25
PM	>50	6.25	0.78	0.39

a) Against *B.subtilis* IFO 3007 and *E. coli* B by serial dilution method in solution medium. b) Data are derived from the Ref. 15.

tide **4**$_4$ resulted in decreased activity **12**, indicating that the specific sequence and secondary structure in **4**$_3$ (Figs. 1 and 2) are important to show strong activity against Gram-positive bacteria. It is interesting that the [D-Dpr$^{4,4'}$]GS-resembling peptide (**11**) showed the same activity against Gram-positive and -negative bacteria as peptide **4**$_4$, also indicating the significance of the specific structure. Replacement of the Ala residues in peptide **4**$_3$ by the Leu residues (L-**4**$_3$) caused marked decrease in the activity, indicating that increase of the hydrophobicity in the peptide is not important to show the activity, but the Ala residues are important to show the activity. Generally, the peptides with the length of 12 residues showed higher activity against Gram-positive bacteria than those with 16 residues. This finding is comparable to the fact that peptide **4**$_3$ (n=12) was more active than **4**$_4$ (n=16).[12] The chain length of a peptide is important for its activity as described previously.[12] Peptide F did not show strong activity against Gram-positive and -negative bacteria on our assay systems contrary to DeGrado's results.[13] This might be due to the different bacterial assay systems, e.g., difference in media and conditions of bacteria.

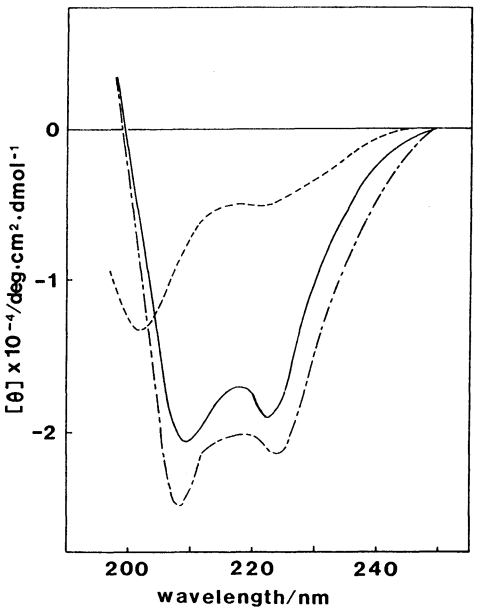

Fig. 4. CD spectra of peptide **5** in H_2O (----) and in the presence of DPPC (——) and DPPC-DPPG (3: 1) (-·-·-) liposomes.

CD Study. CD spectra of peptide **5** in H_2O and in the presence of phospholipid liposomes are shown in Fig. 4, representatively. Although peptide **5** showed the negative band at 200 nm in H_2O attributable to disordered structure, the spectra in the presence of liposomes showed marked increase in ellipticities of the negative bands at 208 and 222 nm, indicating the formation of α-helical structure in the presence of liposomes. A slight difference was observed between the spectra in the presence of neutral DPPC and acidic DPPC-DPPG (3 : 1) liposomes. α-Helicity of the peptides under various conditions was calculated from the ellipticity at 222 nm[21] and is shown in Table 4. All the peptides took the α-helical structure which was amphiphilic (except for peptides **9** and **10**) along the axis of helix in the presence of liposomes in contrast with the disordered structure in H_2O. These findings are similar to those described for peptides **4**$_3$ and **4**$_4$.[12]

Phase-Transition Release. In order to examine further the interaction of the peptides with model

Table 4. α-Helical Content and Effect on Fluorescein Leakage of the Peptides

Peptide	α-Helical content/%				Fluorescein leakage/%[a]	
	H_2O	TFE	DPPC[b]	DPPC-DPPG[b]	DPPC[b]	DPPC-DPPG[b]
1	R[c]	40	40	55	70	100 (85)
2	R[c]	40	37		85	
3	R[c]	40	45		90	
4	R[c]	37	45		90	
5	R[c]	30	55	63	55	100 (75)
6	20	45	58		55	
7	25	45	60		45	
8	25	40	58		60	
9	28	45	60		60	
10	R[c]	45	40	55	60	100 (100)
11	R[c]	35	50	55	85	100 (65)
12	R[c]	30	40	40	50	100 (65)
L-$\mathbf{4}_3$	25		50	40		60
F	R[c]		30	27		100 (70)
$\mathbf{4}_3$	20	40	60	55	100	100 (50)
$\mathbf{4}_4$	30	40	60		100	
GS	—	—	—	—	[80][d]	25
[D-Dpr$^{4,4'}$]GS	—	—	—	—		25
PM	—	—	—	—	[10][d]	20

a) Values are intensities of fluorescence at scanning time of 5 min (see Fig. 5 and Experimental). Concentrations of peptides are 0.2 μg ml^{-1} (0.1 μg ml^{-1} in parentheses). b) In the presence of DPPC or DPPC-DPPG (3:1). c) Disordered structure. d) Concentrations are 1.0 μg ml^{-1}.

membrane and the ability of the peptides to perturb membrane, the effect on phase-transition release of carboxyfluorescein from phospholipid vesicles was measured.[22,23] Profiles of fluorescence leakage from the DPPC vesicles by the action of peptides are shown in Fig. 5. The degrees of the leakage of the dye by the peptides are listed in terms of a relative value in Table 4. The peptides leaked the dye effectively at the phase-transition temperature of DPPC vesicles, but the degree of the leakage was lower as compared with $\mathbf{4}_3$. These results indicate that the peptides taking the α-helical structure in the presence of the phospholipid have the ability to perturb the phospholipid bilayer structure at the liquid-crystalline state of the membrane. The perturbing effect of the peptides on acidic DPPC-DPPG (3:1) vesicles was larger than that on neutral DPPC vesicles, indicating the electrostatic interaction of the cationic peptides with the acidic vesicles. It should be noted that cyclic peptides GS and PM have only the low degree of the leakage in contrast with the α-helical peptides.

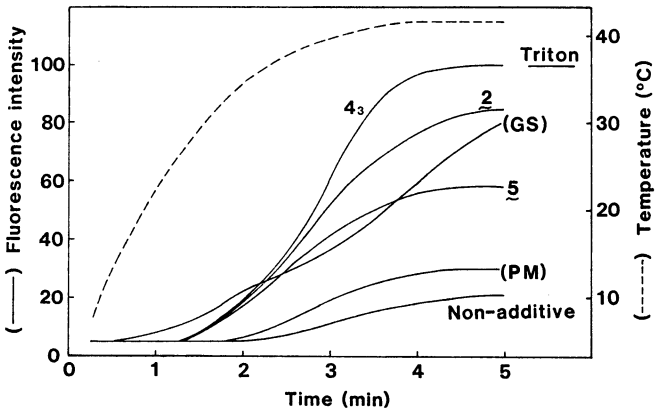

Fig. 5. Profiles of carboxyfluorescein leakage from DPPC vesicles induced by the peptides, GS and PM. Individual peptides were incubated in 2 ml of 0.1 M NaCl/20 mM phosphate buffer (pH 7.4) containing 20 μM DPPC vesicles at temperatures from 0 to 42°C for the indicated scanning time. Peptide concentration: peptide $\mathbf{4}_3$, **2**, and **5** (0.2 μg ml^{-1}); GS and PM (1.0 μg ml^{-1}).

Discussion

Previously,[12] we proposed that α-helical amphiphilicity of the whole peptide molecule, i.e., appropriate positioning of cationic and hydrophobic groups constructed in the stable α-helical structure as shown in the peptides $\mathbf{4}_3$ and $\mathbf{4}_4$, is a significant factor requisite to perturb the membrane of Gram-positive bacteria and to exhibit antibacterial activity against Gram-positive bacteria. We have postulated[12] that the peptide $\mathbf{4}_3$ takes an amphiphilic α-helix on the surface of the membrane of Gram-positive bacteria and the hydrophilic (cationic) side in the helix interacts with the acidic moiety of phospholipid in the membrane and the hydrophobic surface penetrates into the membrane to reveal the antibiosis. Here, the peptides **1**—**12** showed high ability to take α-helical structure in the presence of model membrane (Fig. 4 and Table 4) and to perturb the membrane (Fig. 5 and Table 4), though the abilities were slightly weaker than those of $\mathbf{4}_3$. Antimicrobial activity of the peptides against Gram-positive bacteria was also weaker than that of peptide $\mathbf{4}_3$ (Table 3). The ability of the peptides

and $\mathbf{4}_3$ to interact with model membrane is parallel to the activity against Gram-positive bacteria.

The increase of one or two Lys residues into $\mathbf{4}_3$ and $\mathbf{4}_4$ leads to the increase in the activity against Gram-negative bacteria, and to the decrease in the activity against Gram-positive bacteria, especially against *S. aureus* (Table 3). Polylysine and cecropins are active against both Gram-positive and -negative bacteria.[7,8,24] Polylysine capable to take a helical structure in the presence of phosphatidylserine[25] revealed antimicrobial activity against Gram-negative bacteria, though it has no amphiphilic character. These results indicate that the amphiphilic character of the peptides is not important for the activity against Gram-negative bacteria, but important for the strong activity against Gram-positive bacteria.

The peptide **11** resembling [D-Dpr$^{4,4'}$]GS has four Lys residues in the hexadecapeptide sequence in which distribution of the Lys residues is different from that of peptide $\mathbf{4}_4$ (Fig. 2) and showed the same activity against Gram-positive bacteria as peptide $\mathbf{4}_4$. In view of the low activity of peptide **12** having four Lys residues, the sequence of peptides $\mathbf{4}_3$ and $\mathbf{4}_4$ and another one of peptide **11** are important for the strong activity. However, peptide **11** did not show the activity against Gram-negative bacteria different from [D-Dpr$^{4,4'}$]GS.[15] It is noteworthy that GS has cyclic β-structure which is more rigid than the α-helical structure.

Peptide **12** was designed for the Lys and Leu residues to arrange straight along the axis of helix (Fig. 2) according to repeating heptad sequences (a-b-c-d-e-f-g)$_n$ in which a and e are hydrophobic, and c and g are hydrophilic residues.[26] This peptide showed the low activity against Gram-positive bacteria. Lau et al.[27] reported that the peptides with repeating heptad sequences were inclined to aggregate by forming double-stranded α-helical coiled-coils. The fact that the ability of the fluorescence leakage of peptide **12** was relatively low (Table 4) suggests the presence of such a self-aggregation which weakens the degree of the interaction of the peptide with bacterial membrane resulting in low activity.

It was surprising that replacement of the Ala residues in peptide $\mathbf{4}_3$ by the Leu residue (L-$\mathbf{4}_3$) resulted in 4 and 8 times decrease in activity against *S. aureus* and *B. subtilis*, respectively. This indicates the significance of the Ala residues in the sequence of $\mathbf{4}_3$. α-Helical content also decreased by substituting Leu for Ala (Table 4). These results suggest that the Ala residues in $\mathbf{4}_3$ contribute to the stabilization of α-helix.

It was found that peptide $\mathbf{4}_3$ with specific structure in which the Lys residues twist along the helix (Fig. 2) showed the strongest activity against Gram-positive bacteria, the highest helicity in the presence of liposomes and ability to leak the dye from phospholipid vesicles among the peptides examined.

Experimental

Synthesis of Peptides. TLC was carried out on Merck silica gel G with the following solvent systems: R_f^1, $CHCl_3$-MeOH (5:1); R_f^2, *n*-BuOH-pyridine-AcOH-H_2O (4:1:1:2). Paper electrophoresis was performed on a Toyo Roshi No. 52 paper with the solvent system of HCOOH-AcOH-MeOH-H_2O (1:3:6:10, v/v, pH 1.8) for 3 h at 600 V, the mobility of the peptides being given relative to Lys (R_{Lys}). Optical rotations were measured with a Union high sensitivity polarimeter PM-71. Amino acid analyses were performed on a Hitachi KLA-5 amino acid analyzer after the hydrolysis in 6 M HCl (1 M=1 mol dm^{-3}) in sealed tubes at 110 °C for 24 h.

Representative procedures are described here, and yields and physical constants are summarized in Tables 1 and 2.

Boc-Ala-Lys(Z)-OPac (14). To a mixed anhydride prepared from Boc-Ala-OH (1.52 g, 8.0 mmol), isobutyl chloroformate (1.04 ml, 8.0 mmol) and Et_3N (1.12 ml, 8.0 mmol) in THF (20 ml) was added a solution of H-Lys(Z)-OPac (3.48 g, 8.0 ml) and Et_3N (1.12 ml) in $CHCl_3$ (20 ml) at −10 °C. The mixture was stirred at room temperature for 4 h and evaporated in vacuo. The residue was dissolved in EtOAc, and the solution was washed with 10% citric acid, 4% $NaHCO_3$ and water, and dried over Na_2SO_4. After evaporation, the residue was recrystallized from EtOAc-ether-petroleum ether.

Boc-Ala-Leu-NHCH$_3$ (17). To a chilled solution of H-Leu-NHCH$_3$·HCl (3.05 g, 17 mmol) and Et_3N (2.38 ml, 17 mmol) in DMF (20 ml) was added Boc-Ala-ONSu (4.86 g, 17 mmol). The mixture was stirred at room temperature for 6 h. After evaporation, the residue was dissolved in EtOAc, and washed with 10% citric acid, 4% $NaHCO_3$ and water, and dried over Na_2SO_4. After removal of the solvent, the residue was recrystallized from EtOAc-petroleum ether.

Deprotection of Boc Group in Boc-Ala-Lys(Z)-OPac (14). Compound **14** (5.7 g, 10 mmol) was dissolved in 4 M HCl in dioxane (25 ml) at room temperature for 2 h or in TFA (10 ml) at 0 °C for 30 min. After evaporation, the residue was crystallized by the addition of ether. This was used for the next step without further purification.

Boc-Leu-Ala-Ala-Lys(Z)-OH (41). To a solution of Boc-Leu-Ala-Ala-Lys(Z)-OPac (**32**) (640 mg, 0.85 mmol) in 90% AcOH (10 ml) was added Zn powder (0.6 g) at room temperature. The mixture was stirred at room temperature for 6 h. After filtration, the filtrate was evaporated. To the residue was added 10% citric acid, and the separated oil was extracted with EtOAc and washed with water, and dried over Na_2SO_4. After evaporation, the residue was recrystallized from EtOAc-ether.

Boc-Leu-Ala-Ala-Lys(Z)-Leu-Ala-Lys(Z)-Leu-OPac (59). To a chilled solution of Boc-Leu-Ala-Ala-Lys(Z)-OH (**41**) (636 mg, 1.0 mmol), TFA·H-Leu-Ala-Lys(Z)-Leu-OPac (810 mg, 1.0 mmol), HOBt (270 mg, 2.0 mmol) and Et_3N (0.14 ml, 1.0 mmol) in DMF (10 ml) was added EDC·HCl (288 mg, 1.5 mmol). The mixture was stirred at 0 °C overnight. After evaporation, the residue was solidified by the addition of water. The solid was collected and washed 10% citric acid, 4% $NaHCO_3$ and water, and dried in vacuo.

Ac-Leu-Ala-Lys(Z)-Lys(Z)-Leu-Ala-Lys(Z)-Leu-Leu-Lys(Z)-Lys(Z)-Leu-NHCH$_3$ (93). Boc group in Boc-dodecapeptide-NHCH$_3$ (**79**) (450 mg, 0.21 mmol) was deprotected with TFA (3 ml) at 0 °C for 40 min. To a solution of the TFA salt (430

mg, 0.2 mmol) and Et_3N (0.028 ml, 0.2 mmol) in the mixture of DMF (5 ml) and DMSO (5 ml) was added Ac-ONSu (63 mg, 0.4 mmol) at room temperature. The mixture was stirred at room temperature for 1 d. After removal of the solvent, the residue was solidified by the addition of water. The solid was collected and washed with water, and dried in vacuo.

Ac-Leu-Ala-Lys-Lys-Leu-Ala-Lys-Leu-Leu-Lys-Lys-Leu-$NHCH_3$·5AcOH (1·5AcOH). Compound **93** (300 mg, 0.14 mmol) was dissolved in 25% HBr in AcOH (8 ml) at room temperature. The solution was allowed to stand at room temperature for 4 h. After evaporation, the residue was solidified by the addition of ether and decantated several times with ether. The HBr salt was dissolved in water (5 ml) and passed through a column (0.9×10 cm) of Amberlite IR-45 (AcO^- form), and eluted with water. The fraction was lyophilyzed to give a white powder.

Antimicrobial Assay. Agar dilution method.[19] The minimum inhibitory concentration (MIC) was determined by the standard agar dilution method using Trypticase soy agar. About 5 μl of bacterial suspension containing about 10^7 colony forming units ml^{-1} was inoculated with a multiple inoculator onto agar plates containing twofold serial dilution of each antibiotic. The plates were incubated at 37 °C for 18 h, and the MIC was defined as the lowest concentration of the peptide on which there was either no visible growth or less than four colonies per spot.

Serial dilution method in solution medium.[20] The cell suspension was diluted with the medium to 2×10^6 cells ml^{-1}. Various concentrations of the peptide solution were placed in the test tubes, made up to 1 ml with medium, and the cell suspension (1 ml) was added. After incubation at 30 °C for 17 h, distilled water (1 ml) was added, and the absorbance at 620 nm was measured.

Results of both assays are shown in terms of MIC in Table 3.

Preparation of Phospholipid Liposomes. DPPC and DPPG were purchased from Sigma. Phospholipid (37 mg, 50 μmol) was dissolved in chloroform (2 ml) and dried by breathing of nitrogen in a conical glass tube. The dried lipid was hydrated in 3 ml of 20 mM Tris·HCl buffer (pH 7.4) with repeated vortexed-mixing at 50 °C for 30 min. The suspension was sonicated at 50 °C for 60 min using a TOMY SEIKO ultrasonic disruptor model UR-200P and diluted to 50 ml with the same buffer (lipid concentration, 1.0 mM). The mixture of uni- and multilamellar vesicles was used for the CD measurement without further purification. When the unilamellar vesicles trapping carboxyfluorescein were prepared, the same method as described above was used except that the dried lipid (27 μmol) was hydrated in 2.0 ml of 0.1 M NaCl/20 mM phosphate buffer (pH 7.4) containing 100 mM carboxyfluorescein. The mixture of uni- and multilamellar vesicles trapping carboxyfluorescein was subjected to gel-filtration through a Sephadex 4B column (1×20 cm) in 0.1 M NaCl/20 mM phosphate buffer (pH 7.4). The separated small unilamellar vesicles were utilized in phase-transition release measurements.

CD Measurements. CD spectra were recorded on a JASCO J-40A spectropolarimeter with a JASCO data processor Model J-DPY using quartz cell of 1 mm pathlength. Spectra in water and 2,2,2-trifluoroethanol were measured at a peptide concentration of 100 μM. Spectra in the presence of phospholipid liposomes were measured at a peptide concentration of 10 μM in 20 mM Tris·HCl buffer (pH 7.4) solution containing 0.9 mM vesicles. All the measurements were carried out at 20 °C. The CD data were expressed as mean residue ellipticities as shown in Fig. 3.

Phase-Transition Release. The phase-transition release experiment was carried out according to the procedure of Utsumi et al.[22] Phospholipid vesicles, the peptides and 0.1 M NaCl/20 mM phosphate buffer (pH 7.4) were individually pre-chilled on ice. To 2 ml of phosphate buffer in the cuvette on ice was added 50 μl of the vesicles containing 100 mM carboxyfluorescein to give a final concentration of 20 μM lipid. To the mixture was added 20 μl of an appropriate dilution of the peptide in phosphate buffer. The cuvette was placed in the heated (42 °C) cuvette holder of the fluorometer, and both the fluorescence intensity and the temperature were continuously recorded. For determination of the fluorescence intensity derived from 100% dye-release, 10 μl of Triton X-100 solution (20% in phosphate buffer) was added to dissolve the vesicles. The percentage of dye-release caused by the peptide was evaluated by equation, $100\ (F-F_o)/(F_t-F_o)$, where F is the fluorescence intensity achieved by the peptides, F_o and F_t are intensities of post-transition fluorescence without the peptides and post-Triton X-100 treatment, respectively. Results are shown in Fig. 5 and Table 4.

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viations: DPPC, dipalmitoyl-DL-α-phosphatidylcholine; DPPG, dipalmitoyl-DL-α-phosphatidylglycerol; Dpr, 2,3-diaminopropionic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Fmoc, 9-fluorenylmethyloxycarbonyl; GS, gramicidin S; HOBt, 1-hydroxybenzotriazole; -ONSu, succinimidooxy; Pac, phenacyl; PM, polymyxin B; TFA, trifluoroacetic acid.

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