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Interaction of amphipathic model lipopeptides with phospholipid bilayers

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ABSTRACT

In order to investigate the conformation and localization of lipopeptides in lipid bilayers, a basic model peptide with a long alkyl chain, Ac–Ser–Val–Lys–Amy–Ser–Trp–Lys–Val–$NHCH_3$ (Amy-1; Amy = α-aminomyristic acid) was synthesized. Its interaction with neutral and acidic phospholipid bilayers was studied by circular dichroism (CD) spectroscopy, dye leakage and fluorescence measurements. Another peptide, Ac–Leu–Ala–Arg–Leu–Trp–Amy–Arg–Leu–Leu–Ala–Arg–Leu–$NHCH_3$ (Amy-2), which was prepared previously, was used for comparison. The CD data indicated that Amy-1 took a β-turn and/or a β-structure in the absence and presence of liposomes. Amy-2 formed a β-structure in aqueous solution and an α-helical structure in liposomes. The dye leakage ability of Amy-1 was much weaker than that of Amy-2. Fluorescence spectroscopic data suggest that the peptides are immersed in lipid bilayers. Based on these results, discussion is made in terms of localization of the peptides in lipid bilayers.

INTRODUCTION

Studies on anchoring and localization of lipoproteins in biomembranes have become of interest from the standpoint of their functional importance. An alkyl chain of lipoproteins is linked to the N- or C-terminus, or a side-chain of proteins after protein biosynthesis, and plays a role in the localization of proteins in biomembranes [1,2]. However, the structural change of proteins in anchoring and localization is not well understood at the molecular level.

We found previously that Ac–(Ser–Val–Lys–Val)$_2$–$NHCH_3$ (**1**) and Ac–(Leu–Ala–Arg–Leu)$_3$–$NHCH_3$ (**2**) are good models of an amphipathic β-structure and an amphipathic α-helix, respectively, to study the peptide–lipid interaction [3,4]. In order to obtain further information, we designed two new model peptides which contain an amino acid with a long alkyl chain, Ac–Ser–Val–Lys–Amy–Ser–Trp–Lys–Val–$NHCH_3$ (Amy-1; Amy = α-aminomyristic acid) and Ac–Leu–Ala–Arg–Leu–Trp–Amy–Arg–Leu–Leu–Ala–Arg–Leu–$NHCH_3$ (Amy-2). These peptides are tentatively named model lipopeptides in this study, although they are not lipopeptides according to the strict definition. As reported previously [5], circular dichroism (CD) spectroscopy, dye leakage and fluorescence measurements of Amy-2 indicated that the alkyl chain anchors the peptide to liposomes and the anchoring causes the conformational changes of Amy-2 to a greater extent.

In this study, we synthesized Amy-1 and examined its conformation in the absence and presence of liposomes. Discussion is made of the effect of a long alkyl chain on conformational changes of the peptide backbone and on anchoring to lipid bilayers. Amy-2 was also used for comparison.

EXPERIMENTAL

Materials

Egg yolk phosphatidylcholine (EYPC) and egg

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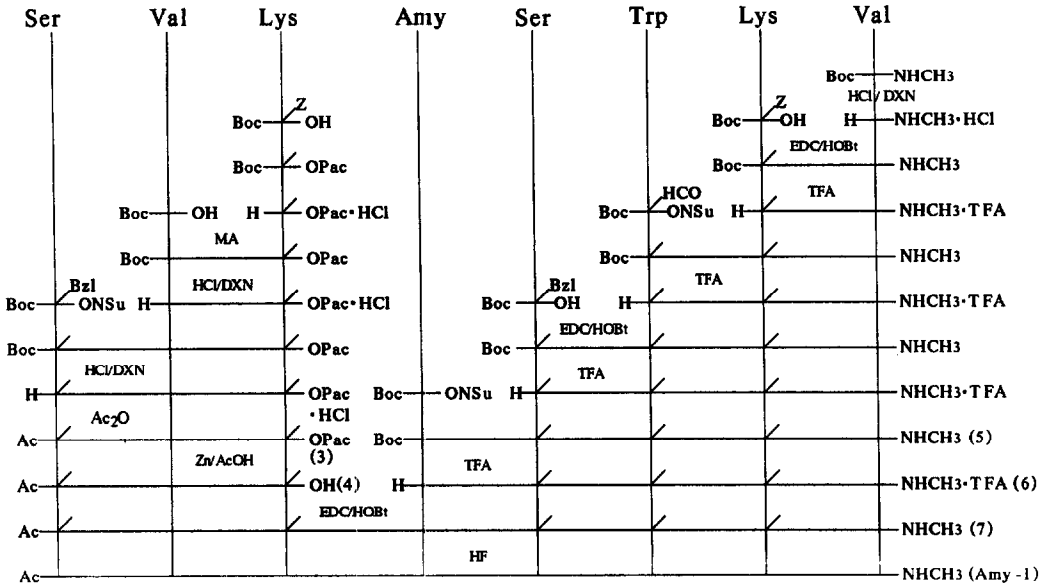

Fig. 1. Synthetic route for Amy-1. Boc = *tert.*-butyloxycarbonyl; Z = benzyloxycarbonyl; DXN = dioxane; OPac = phenacyl ester; ONSu = succinimidyl ester; EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt = N-hydroxybenzotriazole.

yolk phosphatidylglycerol (EYPG) were purchased from Sigma (St. Louis, MO, USA). Carboxyfluorescein obtained from Eastman Kodak (Rochester, NY, USA) was purified by recrystallization from ethanol. All other reagents were of analytical-reagent grade.

Peptide synthesis

Fig. 1 shows the synthetic route for Amy-1. The protected peptide **7** was prepared by the solution method. Short fragments (**3** and **5**) were obtained by stepwise condensation from the C-terminus. Fragment **4** obtained by treatment of **3** with zine powder in 90% acetic acid, was condensed with **6** to give **7**. The crude peptide obtained by treatment of **7** with HF was chromatographed on a Sephadex G-15 column with 30% acetic aicd. The product was purified by reversed-phase high-performance liquid chromatography (HPLC) (μBondasphere C_4) using an acetonitrile gradient in an aqueous phase containing 0.1% trifluoroacetic acid (TFA). The elution pattern of the purified product is shown in Fig. 2. Amino acid analysis of the acid hydrolyate gave Ser 2.20 (2), Val 2.00 (2) and Lys 1.98 (2). Amy was not eluted under the conditions of the standard amino acid analysis.

Spectroscopic measurements

CD data were recorded on a JASCO J-500 spectropolarimeter (Japan Spectroscopic, Tokyo, Japan). Small vesicles (*ca.* 50 nm in diameter) were prepared as described previously [3]. The peptides

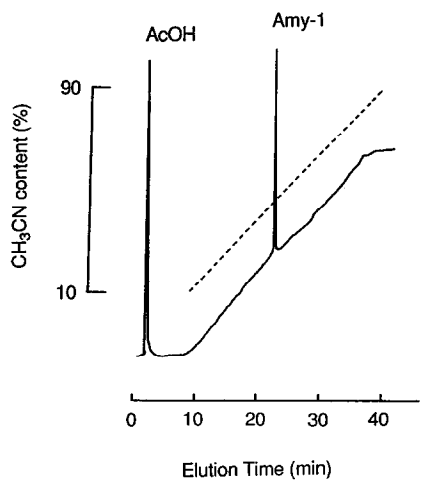

Fig. 2. Reversed-phase HPLC of purified Amy-1. Column, μBondasphere C_4 (150 × 3.9 mm I.D.); solvent, (A) acetonitrile–water (90:10) containing 0.1% TFA and (B) acetonitrile–water (10:90) containing 0.1% TFA, with a gradient from B to A as shown by the dashed line; detection, 220 nm.

were dissolved at a concentration of 10–15 μM in 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) buffer (pH 7.4) containing 0.9 mM EYPC or EYPC–EYPG (3:1) liposome solution. The solution was equilibrated for 10 min at 22°C. All measurements were performed at 22°C and the data are expressed in mean residue ellipticities. The peptide conformation was evaluated according to the Greenfield and Fasman method [6].

The leakage experiment was carried out by monitoring the release of 5(6)-carboxyfluorescein trapped in EYPC or EYPC–EYPG (3:1) liposomes, which fluoresced at 515 nm when excited at 470 nm as described previously [3]. The liposomes (2 ml, *ca.* 70 μM) containing 100 mM 5(6)-carboxyfluorescein were incubated with increasing concentrations of the peptide and the fluoresence data were collected 3 min after incubation.

Fluorescence spectra were recorded on a JASCO FP-550A spectrometer. A peptide solution (1 ml) at a concentration of 26 μM was added to the appropriately diluted liposome solution (1 ml) and mixed for 10 min for equilibration. The tryptophan fluorescence was recorded at 22°C with excitation at 250 nm in order to remove any effect of the Raman scattering beam on the tryptophan fluorescence region caused by water.

RESULTS AND DISCUSSION

Fig. 3 shows the CD spectra of Amy-1 in the absence and presence of neutral and acidic liposomes. Amy-1 had a positive band at 230 nm and a negative band at 216 nm both in buffer and in the presence of liposomes, indicating that it has a restricted structure under these conditions. Amy-1 may contain a β-turn conformation, because its CD pattern is similar to that of some model peptides which contain a β-turn [7]. The presence of the negative band at 216 nm suggests that it also contains a β-structure. Peptide **1**, the original peptide of Amy-1, adopts a random structure in buffer [3], whereas Amy-1 adopts a restricted structure such as a β-structure and/or a β-turn. As reported previously [5], CD spectra of Amy-2 indicated the presence of a β-structure (*ca.* 60%) in buffer and an α-helical structure (40–50%) in liposomes. As the hydrophobicity of Trp is much smaller than that of Amy, the strong intermolecular hydrophobic interactions

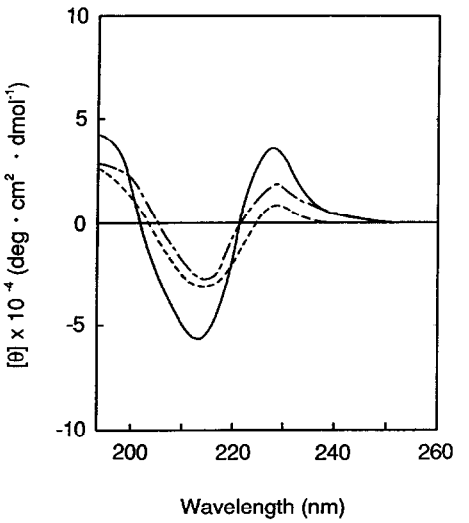

Fig. 3. CD spectra of Amy-1. The spectra were measured in (———) 50 mM HEPES buffer (pH 7.4) and in the presence of (— · —) 0.9 mM EYPC and (·····) EYPC-EYPG liposomes. Peptide concentration, 10–15 μM.

of the side-chains of Amy in Amy-1 and Amy-2 in buffer seem to contribute mainly to the formation of a β-structure.

In order to study the action of the peptides on phospholipid membranes, the ability of the peptide to leak encapsulated carboxyfluorescein from EYPC–EYPG (3:1) vesicles was studied. Fig. 4 shows the release profile as a function of peptide concentration. The dye release increased with increase in peptide concentration. Amy-1 showed

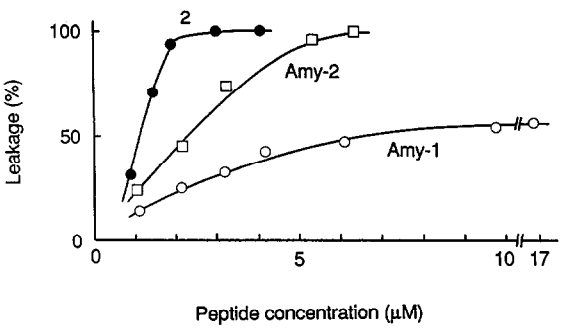

Fig. 4. Peptide-induced release of carboxyfluorescein encapsulated in EYPC–EYPG (3:1) liposomes as a function of the peptide concentration. The data were collected 3 min after incubation of the peptide in liposomes. ○ = Amy-1; □ = Amy-2; ● = **2**.

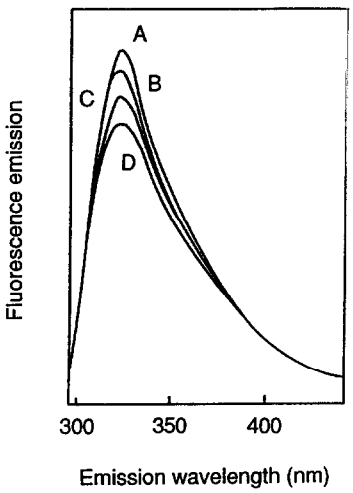

Fig. 5. Fluoresence emission spectra of Amy-1 in EYPC–EYPG (3:1) liposomes as a function of the lipid concentration. Peptide concentration, 26 μM. Lipid/peptide ratio: A, peptide only; B, 1; C, 4; D, 15.

about 60% leakage at a peptide concentration of 17 μM. On the other hand, Amy-2 released 100% of the dye at 6 μM, which was a three times higher concentration than that observed for **2**, the original peptide of Amy-2. We have found that α-helical peptides induce a large release of fluorescent dye from both neutral and acidic liposomes, whereas the ability of **1** to release the dye from acidic liposomes is about 100 times less than that of **2** [3]. It could be inferred that Amy in Amy-1 enhances the hydrophobic interaction between Amy-1 and lipid and stabilizes the bilayers.

A fluorescence study of Trp in Amy-1 was carried out in absence and presence of phospholipid liposomes. The result is shown in Fig. 5. The emission spectrum of Amy-1 in buffer (pH 7.4) showed a maximum at 320 nm. No change in the maximum wavelength occurred on addition of EYPC–EYPG (3:1) liposomes, although the intensity decreased slightly with increase in the concentration of liposomes. It was found that the fluorescence spectrum of Ac–Ser–Val–Lys–Val–Ser–Trp–Lys–Val–$NHCH_3$ in buffer showed an emission maximum at 357 nm, which shifted 12 nm to shorter wavelength on addition of dipalmitoyl-DL-phosphatidylcholine–dipalmitoyl-DL-phosphatidylglycerol (3:1) liposomes [3]. The blue shift can be explained in terms of the translocation of the Trp residue from a hydrophilic to a hydrophobic region. The magnitude of the blue shift, however, indicates that the Trp residue is not deeply embedded in liposomes. The present result indicates that the Trp residue in Amy-1 exists in a hydrophobic environment in buffer. The side-chain of the Amy residue in Amy-1 may contribute to inducing such a hydrophobic environment, because the side-chains of Trp and Amy in Amy-1 lie on the same side of the plane of the peptide backbone formed by the β-structure and/or β-turn. We assume that the Amy-1 molecules aggregate in buffer to keep the Trp residue in the hydrophobic environment. On addition of liposomes, Amy-1 may interact with the liposomes in such a manner that the side-chain of Amy penetrates into lipid bilayers. The emission maximum at 320 nm means that the Trp residue of Amy-1 penetrates deeply into lipid bilayers. The decrease in the maximum intensity with increase in lipid concentration may be due to the quenching of tryptophan fluorescence by the lipid head groups.

The previous fluorescence study indicated that the Trp residue in Amy-2, which exists in a hydrophilic environment in buffer, is incorporated into lipid bilayers on addition of liposomes [5]. As shown in the dye leakage experiment, the leakage ability of Amy-1 and Amy-2 was weaker than that of **2**, suggesting that the long alkyl chain acts as a constituent of the bilayers to make them stable. This work again showed that the alkyl chain anchors the peptides to liposomes and the anchoring causes the conformational change of the peptides; such work will be helpful in analysing the molecular recognition between lipoproteins and biomembranes.

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