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Formation of Substrate-Induced Amphiphilic Sequential Peptide Assembly in a Lipid Monolayer

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Amphiphilic sequential peptides, $Leu_8GluLeu_7$, having functional groups (Asp, Trp, and Tyr) that bind to the specific site on a target substrate, acetylcholine, at the *N*-terminal were prepared. Dipalmitoylphosphatidylcholine monolayers containing the peptides were formed on buffered subphase solutions with and without the substrate, and were transferred onto gold-deposited glass plates. The amphiphilic peptides took a β-sheet conformation in the lipid monolayer. Furthermore, the peptides re-arranged to form on β-sheet assembly, owing to the interaction between the terminal functional groups and acetylcholine. Hydrogen-bonding network was formed between the –COOH of Glu residues in the substrate-induced amphiphilic sequential peptide assembly.

Key words: amphiphilic sequential peptide, substrate-induced peptide assembly, β-sheet assembly, lipid monolayer, FTIR-RAS

1. INTRODUCTION

The cellular response to a particular extracellular signaling molecule depends on its binding to a specific receptor protein located on the surface of a target cell. The protein contains a unique distribution of functional groups, such as charged, hydrogen bonding, and hydrophobic amino acids, on its exterior surface. The specific location of these amino acids on the protein surface yields the specific binding site of the particular signaling molecule.[1] In a nicotinic acetylcholine receptor, which is the longest known and best studied neuroreceptor, a specific arrangement of tyrosine and tryptophan residues of α-subunit, and tryptophan and asparatate residues of γ-subunit, form an acetylcholine binding site.[2-5] However, antibodies can recognize a great number of molecules owing to highly variable amino acid sequences in the hypervariable region in those binding sites.[6,7] Studies on synthetic molecular recognition systems having an ordered assembly of functional groups in the binding site may be important not only in contributing to the understanding of a simple and/or essential mechanism for signal reception and transduction through biological interfaces, but also may provide the basis of a molecular device capable of receiving and transferring information. It has been reported that artificial receptors composed of the ordered assembly of functional groups exhibit a specific binding ability of a particular substrate.[8,9] In a previous study, we have proposed a novel approach to preparing artificial receptors consisting of a peptide assembly in a lipid monolayer. In this system, the peptides can re-arrange in the lipid monolayer owing to the interaction between the terminal functional groups of peptides and the target substrate.[10]

In this paper, we report a structural study of substrate-induced amphiphilic sequential peptide assembly, which can convert the chemo-information of the substrate to an electrochemical response owing to on/off switching an ion pore that formed amphiphilic peptides in a lipid membrane. In this system, the recognition of the target substrate by the peptide assembly, that is to say, a blocking of the substrate-induced ion permeable pore by itself, can be expected to induce the changes of the electrochemical properties, such as an ion permeability and membrane potential.

2. EXPERIMENTAL

2.1 Peptides

$Leu_8GluLeu_7$ sequence was chosen as an ion pore forming element. And Tyr, Trp, and Asp residue for acetylcholine binding site[5] and Ser and Tyr[11] residue for γ-aminobutyric acid binding site were introduced at the amino terminal of the amphiphilic peptides,

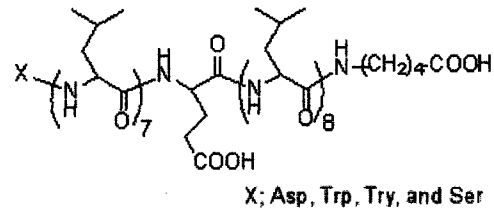

Chart 1. Amphiphilic Sequential peptides

respectively (Chart 1). Peptides, $Leu_8GluLeu_7$, having Tyr, Trp, Asp, and Ser at the *N*-terminal were synthesized by the conventional solid-phase method[12], respectively. A 5-aminovaleric acid loaded CLEAR-Acid-Resin (0.4 meq/g, Peptide Institute Inc.) was used for the resin of peptide synthesis. A 0.5 g of the resin was swelled by 5 mL of dichloromethane for 1 day in the reaction vessel and then the resin was rinsed by 5 mL of pure dimethylformamide (DMF) 3 times. A solution of Fmoc-amino acid (0.7 mmol) in 3 mL of

dimethylformamide (DMF), 1,3-diisopropylcarbodi -imide (0.7 mmol) in 1 mL of DMF, and 1-hydroxy-7-azabenzotriazole (0.7 mmol) in 1 mL of DMF was added to the resin, and then the suspension was shaken for 2 hours to attach the Fmoc-amino acid to the amino group on the resin. After the reaction, the resin was rinsed by pure DMF and then the DMF solution containing 20 vol% piperidine was added to the vessel to remove the amino terminal Fmoc-protecting group for 1 hour. After the reaction, the resin was rinsed by pure DMF until the piperidine was completely removed. This reaction cycle were repeated successively to obtain the desired sequence. After the coupling reactions were completed, the peptide-resin was dried under vacuum and then cooled in an ice bath. A 10 mL of cooled aqueous solution containing 95 vol% trifluoroacetic acid was added to the cooled peptide-resin to remove the peptide from the resin support for 1 hour at room temperature. After the reaction, the reaction mixture was filtrated to separate the peptide solution from the resin support. The TFA solution of the peptide was concentrated to a volume of approximately 1-2 mL and then a 100 mL of cooled ether was added to the TFA solution to precipitate the peptide. Identification of the peptide was made by MALDI-TOF mass spectroscopy (SHIMADZU /KRATOS KOMPACT II Kratos Analytical).

2.2 Surface Pressure - Area Measurements

Surface pressure - area (π-A) isotherms of a dipalmitoylphosphatidylcholine (DPPC) monolayer containing the peptides were measured with a multi-trough type Langmuir film balance (Nippon Laser & Electronics Lab., NL-LB-200-MTC). The subphase water used was Milli-Q treated water. DPPC and the peptides were each dissolved in DMF. These solutions were mixed. The molar ratio of the peptides to DPPC was 0.01. Then, this solution poured into a glass flask, forming a thin film on the inner surface of the flask after solvent evaporation. Spectroscopic grade chloroform as a spreading solvent was added to the flask to dissolve the thin film. The concentration of DPPC was 0.6 mg/mL. A measured small amount of the solution was delivered to the water surface from a Termo micro syringe. π-A isotherms of the monolayer on a buffer solution (50 mM HEPES-NaOH, pH 7.2) and on a buffer solution containing 0.1 mM acetylcholine were taken at a compression rate of 5 mm/min and a subphase temperature of 25°C, respectively.

2.3 Spectroscopic Measurements

The circular dichroism (CD) spectra of the peptides in the trifluoroethanol were measured with a JASCO J-820 CD spectrometer.

The Fourier transform infrared reflection-absorption spectra (FTIR-RAS) of the DPPC monolayers containing the peptides were measured with a Bio-Rad FTS-6000-Mid-IR equipped with a HARRICK reflectance accessory. The monolayer transfer onto a gold-deposited glass plate was carried out using the horizontal lifting method from the buffer solution, and from the buffer solution containing the 0.1 mM acetylcholine at a surface pressure of 15 mN/m and 40 mN/m, respectively

3. RESULTS AND DISCUSSION

3.1 Conformational Study of Amphiphilic Sequential Peptides in Solution

The amphiphilic sequential peptides having functional group at the *N*-terminal obtained by conventional solid-phase method were identified by MALDI-TOF mass spectroscopy. Molecular weights of $Leu_8GluLeu_7$, having Tyr, Trp, Asp, and Ser at the *N*-terminal were 2107.0, 2031.6, 2058.6, and 2032.5, respectively. These values were in fair agreement with calculated values ($TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$: 2106.9, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$: 2129.9, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$: 2058.6, $SerLeu_8GluLeu_7$-$(CH_2)_4COOH$: 2032.5). Leu 16-mer was also synthesized by the solid-phase method.

The secondary structure of the peptides was estimated from the CD spectra. In the trifluoroethanol solution, the CD spectrum of $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$ -$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$-$(CH_2)_4COOH$ and Leu_{16} showed a double minimum profile at 208 nm and 222 nm, typical of a stable, right-handed α-helix[13] (Figure 1). The molar ratio of $TyrLeu_8GluLeu_7$

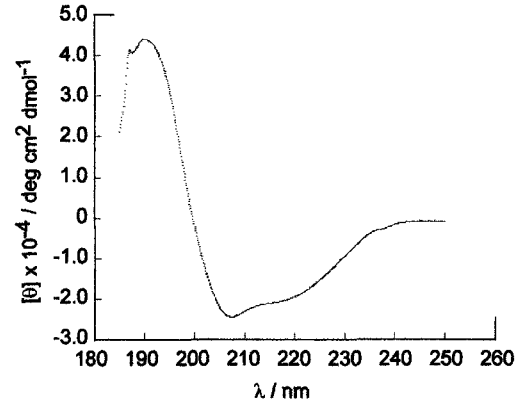

Figure 1. CD spectra of $TyrLeu_8GluLeu_7$ -$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$ -$(CH_2)_4COOH$ and Leu_{16} in trifluoroethanol.

-$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$ -$(CH_2)_4COOH$ and Leu_{16} was fixed to 1:1:1:1:4. The helicity of the peptides was estimated to be above 70% from the observed molar ellipticity at 208 nm.

3.2 Formation of Substrate-induced Amphiphilic Sequential Peptide Assemblies in Lipid Monolayer

We investigated the acetylcholine induced structural changes of the DPPC monolayer containing amphiphilic sequential peptides and Leu_{16} by the π-A isotherm and FTIR-RAS measurements. The molar ratio of $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$ -$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$-$(CH_2)_4COOH$ and Leu_{16} was fixed to 1:1:1:1:4. The Leu_{16} was added as a matrix of amphiphilic peptide assembly in the monolayer to fix the substrate-induced specific location of the functional groups of the amphiphilic peptides. The molar ratio of

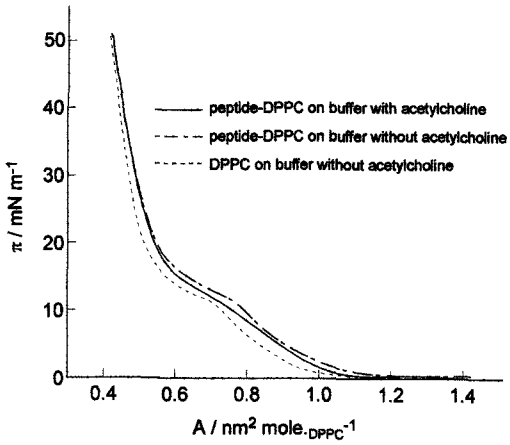

Figure 2. π-A isotherms of DPPC monolayer containing $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$-$(CH_2)_4COOH$ and Leu_{16} on the buffered subphase solutions without acetylcholine at 25°C.. And π-A isotherms of pure DPPC monolayer on the buffered subphase solutions without acetylcholine at 25°C. The molar ratio of $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$-$(CH_2)_4COOH$ and Leu_{16} was fixed to 1:1:1:1:4. And the molar ratio of the peptides to DPPC was 0.01.

the peptides to DPPC was 0.01. Figure 2 shows the π-A isotherms of the DPPC monolayer containing these peptides on the buffered subphase solutions with and without 0.1 mM acetylcholine, conducted at a constant temperature of 25°C. The π-A isotherm of pure DPPC monolayer on the buffered subphase solutions without acetylcholine are also shown in this figure. The abscissa indicates the area per DPPC molecule. The surface area of DPPC monolayer containing peptides was larger than that of pure DPPC monolayer over the range of examined surface pressures on the buffered subphase solutions without acetylcholine. This implies that the peptides were incorporated into the DPPC monolayer. The π-A isotherm of the DPPC monolayer containing the peptides on the buffer solution with 0.1 mM acetylcholine displayed a significant condensation at the surface pressure below ca. 20 mN/m. This result suggested that the peptides, $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, and $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, re-arrange to form a compact, high-order structure owing to the interaction between the acetylcholine and terminal amino acids, Tyr, Trp, and Asp, of the peptides.

The DPPC monolayer containing $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$-$(CH_2)_4COOH$ and Leu_{16} was transferred onto a gold-deposited glass plate from the subphase to the air phase both on the buffer solution and the buffer solution containing 0.1 mM acetylcholine at 15 mN/m and 40 mN/m by the horizontal lifting method, respectively. The conformation of the peptides in the DPPC monolayer was confirmed by the position of the amide I band[14] in the FTIR-RAS of the monolayer on the gold-deposited glass plate (Figure 3). In the amide I region of the monolayers, which transferred at 15 mN/m

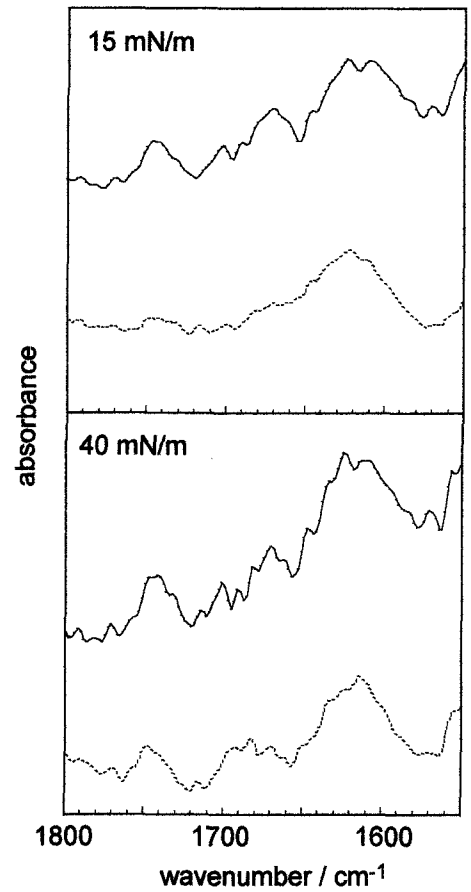

Figure 3. FTIR-RAS of amide I band for DPPC monolayers containing $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$-$(CH_2)_4COOH$ and Leu_{16} transferred onto gold-deposited glass plats at 15 and 40 mN/m from the buffered subphase with (solid line) and without (doted line) 0.1 mM acetylcholine. The molar ratio of $TyrLeu_8GluLeu_7$-$(CH_2)_4COOH$, $TrpLeu_8GluLeu_7$-$(CH_2)_4COOH$, $AspLeu_8GluLeu_7$-$(CH_2)_4COOH$, $SerLeu_8GluLeu_7$-$(CH_2)_4COOH$ and Leu_{16} was fixed to 1:1:1:1:4. And the molar ratio of the peptides to DPPC was 0.01.

and 40 mN/m from the buffered subphase with and without acetylcholine, characteristic absorptions with β-sheet structure at 1632 cm^{-1} were observed. It is noting that the peptides took stable α-helical conformation in trifluoroethanol solution (Figure 1). However, the peptides took mainly the β-sheet structure in the DPPC monolayers. This result suggested that the peptides formed assembly owing to tight packing of hydrophobic Leu moieties in the DPPC monolayers and then the intramolecular hydrogen bonding is likely to be rearranged to the intermolecular one, which induced the structural change of the peptide from α-helix to β-sheet.

3.2 Structural Studies of Substrate-induced Amphiphilic Sequential Peptide Assemblies in Lipid Monolayer

To elucidate the structure of substrate-induced amphiphilic sequential peptide assemblies, FTIR-RAS for Glu residue of the amphiphilic sequential peptides in the carbonyl stretching regions were measured (Figure

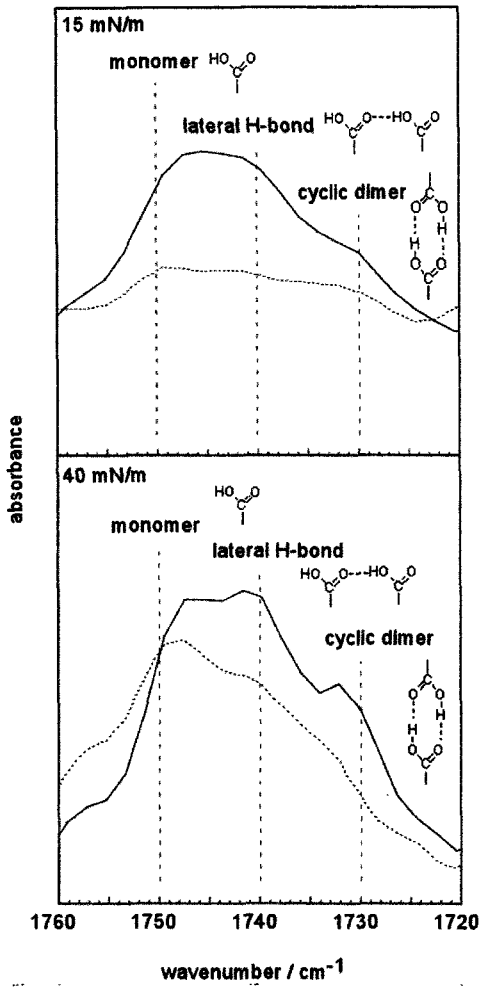

Figure 4. FTIR-RAS of carboxylic band for amphiphilic sequential peptides in the DPPC monolayers transferred onto gold-deposited glass plats at 15 and 40 mN/m from the buffered subphase with (solid line) and without (doted line) 0.1 mM acetylcholine.

4). FTIR-RAS analysis of the peptides in the DPPC monolayer that transferred from the buffered subphase without acetylcholine at 15 mN/m showed very week absorption band around 1740 cm^{-1} that assigned to be –COOH. This imply that the Glu residue in the amphiphilic sequential peptide was in carboxylate anion that orientated to the aqueous phase, that is to say, the amphiphilic peptide were laid at the air-water interface in the liquid state DPPC monolayer. On the other hand, the absorption band of –COOH for Glu residue were apparently observed in the DPPC monolayer containing amphiphilic sequential peptides, which transferred at 40 mN/m. This means that the carboxylate anion of Glu residue are gradually protonated, perhaps by the perpendicular orientation[15] in the monolayer to avoid the contact between the Glu residue and aqueous phase in the solid condensed state.

In the DPPC monolayer transferred from the buffered subphase with acetylcholine at 15 and 40 mN/m, -COOH band clearly observed. This suggested that the amphiphilic sequential peptides arranged perpendicular to the monolayer to form a compact, high-order structure owing to the specific interaction between the acetylcholine and terminal amino acids, Tyr, Trp, and Asp, of the amphiphilic sequential peptides even in liquid state monolayer. Furthermore, the –COOH band for the Glu residue of the amphiphilic sequential peptides in the DPPC monolayer, that transferred at 40 mN/m, shifted lower wavenumber that assigned to be lateral hydrogen -bond (1740 cm^{-1}) and cyclic dimmer (1730 cm^{-1}), respectively.[16] This suggested that the intermolecular acid/acid hydrogen bonds were formed in the substrate-induced β-sheet assembly like a β-barrel structure composed of amphiphilic sequential peptides in the solid state DPPC monolayer.

4. CONCLUTION

The amphiphilic sequential peptides having functional groups at the terminal re-arranged to form on β-sheet assembly like a β-barrel, owing to the interaction between the terminal functional groups and target substrate, and hydrogen-bonding network formation between Glu residues in the solid state lipid monolayer. Further studies, the channel activity and that regulation by the substrate binging to the substrate-induced β-barrel like assembly are under investigation.

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