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# Effect of saturation in phospholipid/fatty acid monolayers on interaction with amyloid β peptide

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**The effect of the saturation of fatty acid (FA) in 1,2-dimyristoyl-*sn*-glycero-3-phosphocoline (DMPC)/FA membrane on the interaction between lipid membrane and amyloid β monomer was investigated by using the Langmuir monolayer technique. The surface pressure (Π)-mean molecular area (*A*) isotherms and fluorescent measurements reveal that DMPC and octadecanoic acid (stearic acid, SA) molecules were somewhat miscible in the mixed membrane, which was maintained to homogeneous gel phase by enhance of the intermolecular hydrophobic interactions because of the all trans acyl chains. On the other hand, DMPC and 9Z,12Z-octadecadienoic acid (linoleic acid, LA) molecules were considered to be well miscible in the mixed membrane, where the membrane partially transferred from gel phase to liquid-crystalline phase. The Π-*A* isotherms of the monolayers on amyloid β-peptide (Aβ) solution indicated that Aβ monomers tend to be inserted into the saturated acyl chain region of monolayers at low surface pressure and that the Aβ monomers were then extruded from the monolayer at higher surface pressure. It was observed that behaviors of Aβ monomers at higher surface pressure depended on membrane microstructures. In the DMPC/SA monolayers, Aβ aggregated and then was extruded from monolayers at about 20 mN $m^{-1}$ of surface pressure irrespective of the SA proportion. On the other hand, in the DMPC/LA monolayers, Aβ, which favors to interact with DMPC, is dispersed in the monolayer even at high surface pressure because DMPC and LA molecules were well miscible in the monolayer.**



[**Key words:** Saturation of lipid; Amyloid β-peptide; Lipid membrane–peptide interaction; Membrane microstructure; Langmuir monolayer technique]

A possible role of the biomembrane in the conformational diseases, e.g., Alzheimer's disease (AD), has gradually been an attractive issue for many researchers. It is considered that the assembly of monomeric amyloid β into β-sheet aggregates initiates a sequence of pathological responses in AD, because the fibril of amyloid β-peptide (Aβ) is the predominant component of the extracellular plaques on brains of AD patients (1,2). Aβ and its aggregation have been widely studied to elucidate pathological events and identify therapeutic targets (3–6). Although the majority of these studies are carried out in aqueous solutions and there have been much interest for the behavior of Aβ itself (7–11), a significant role of membrane through its cooperative interaction with Aβ molecule has recently been reported (12–19).

The cell membrane is one of the most important constituents in biological systems, since it is not only a physical boundary against the environment but also a functional unit that senses environmental conditions and recognizes other molecules (nucleic acids, proteins, etc.) or other self-assemblies (viruses, cells, etc.). In studies using phospholipid bilayer membrane vesicles (liposomes), which are highly structured supramolecules and analogous to microbial cell membranes, it has been found that the lipid bilayer acts as a functional boundary, which recognizes biomolecules. For instance, the membrane shows highly selective permeability for ions and amino acids (20). Further, it has been reported that, under some conditions, liposomes interact with partly denatured proteins, e.g., assisting in the refolding of proteins (21–23) and inducing the translocation of proteins (24,25). Such molecular recognition of the lipid membrane are also utilized to separation (26) and sensing techniques of proteins (27).

It has been widely accepted that biological membranes show lateral phase separation and domain formation (28–30). The rafts have been implicated in a number of important cellular processes such as signal transduction, membrane trafficking, and so on (31,32). The multi-component lipid bilayer membranes have also been reported to exhibit segregated lipid phases coexisting over a given temperature (33–37). In such model membranes, the lipid raft phase is typically associated with a so-called liquid-ordered ($l_o$) phase, while the non-raft phase has been identified as the liquid-disordered ($l_d$) phase based on differences in the short-ranged lipid translational and conformational order (38). How such a microstructure of the lipid membrane is involved in expression of functions such as molecular recognition on the membrane surface is an interesting issue.

Hitherto several studies about lipid monolayer-Aβ interactions have been reported. Ambroggio et al. (16) examined the association ability of Aβ in the membrane using premixed peptide-lipid monolayer. Electrostatic effects (17) and effect of acyl chain length of phosphoethanolamine (18) on the adsorption and orientation of Aβ in the membrane were discussed. It was reported that cholesterol

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influenced the adsorption of Aβ to unstable monolayer such as containing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (19). In present study, the effect of saturation of lipid monolayers on membrane microstructure and on the interaction between lipid membrane and Aβ monomer was investigated by Langmuir monolayer technique. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocoline (DMPC) was used as representative component of the model membrane. Saturation of lipid monolayers was varied by adding of octadecanoic acid (stearic acid, SA) or 9Z,12Z-octadecadienoic acid (linoleic acid, LA). In order to examine a lipid-lipid and lipid-Aβ interactions, the surface pressure (Π)-mean molecular area (*A*) for binary monolayers composed of DMPC and fatty acids in the absence and the presence of Aβ in subphase solution were measured.

## MATERIALS AND METHODS

**Materials** 1,2-Dimyristoyl-*sn*-glycero-3-phosphocoline (DMPC) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Octadecanoic acid (stearic acid, SA) and 9Z,12Z-octadecadienoic acid (linoleic acid, LA) were purchased from Olbracht Serdary Research Laboratories (Toronto, Canada). Amyloid β(1–40)-peptide (Peptide Institute, Inc., Mino, Japan) was used for the model peptide. 1,6-Diphenyl-1,3,5-hexatriene (DPH, Sigma–Aldrich, Co. Ltd., St. Louis, MO, USA), 1-(4-trimetylammoniumphenyl)-6-phenyl-1,3,5-hexatriene iodide (TMA-DPH, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan, LifeTechnologies, Eugene, OR, USA) were used for evaluations of membrane propertied of liposome. All other reagents and solvents were purchased from Wako at analytical grade and used as supplied.

**Compression isotherm of monolayer** The monolayer measurement was performed using a Langmuir balance (Minitrough 2, KSV Instrument, Helsinki, Finland). The surface pressure was measured using a Wilhelmy plate. The teflon trough (375 mm × 75 mm × 5 mm) was equipped with two symmetrically moving barriers. Before each measurement, the subphase surface was cleaned by sweeping and suction. Stock solutions of DMPC, SA and LA were each prepared in chloroform at concentration of 0.5 mM, and were mixed with a given molar fraction before spreading. Sample solutions were spread with Hamilton syringe on the subphase solution, and left for 10 min to allow solvent evaporation and to reach an equilibrium state of monolayer. All isotherms were recorded upon symmetric compression of the monolayer at a constant speed of 10 mm $min^{-1}$ $barrier^{-1}$ at 25.0 °C. In the case of Aβ coexistence system, a stock solution of Aβ (1 mM) was added in the subphase solution (final concentration in subphase: 80 nM) just before spreading of lipids.

**Preparation of liposome** Liposome was prepared by extrusion method (39). DMPC was mixed with fatty acid in chloroform ($x_{FA} = 0.286$). The lipid mixture was dried onto the wall of a round-bottom flask *in vacuo* and then left overnight to ensure the removal of all of the solvent. The dried thin lipid film was hydrated with 0.1 M Tris-HCl buffer (pH 8.0) to form multilamellar vesicles (MLVs). Large unilamellar vesicles (LUVs) were formed from the MLVs with five freeze–thaw cycles which consisted of freezing the samples at −80 °C and warming the samples at 40 °C, which was above the liquid-crystalline phase transition temperature of DMPC. Alternatively, monodisperse LUVs were prepared using an extruder (LiposoFast, Avestin Inc., Ottawa, Canada) equipped with polycarbonate filters possessing pore sizes of 100 nm.

**Evaluations of membrane properties of liposome** The polarizations of fluorescent probes, DPH and TMA-DPH, were measured as index of membrane fluidity (40–42). Fluorescent probe DPH was added to a liposome suspension in a molar ratio of 1/250 to lipid and the final concentration of DPH was 0.4 μM. The fluorescence polarization of DPH ($\lambda_{ex}$ = 357 nm, $\lambda_{em}$ = 432 nm) was measured using a fluorescence spectrophotometer (Fluoro Max 4, Horiba, Kyoto, Japan) at the range of 20–30 °C after incubation at 35 °C for 60 min. The sample was excited with vertically polarized light (357 nm), and both of vertical intensity ($I_{VV}$, parallel to the excitation light) and horizontal intensity ($I_{VH}$, perpendicular) of emission light were recorded at 432 nm. The polarization (*P*) of DPH was then calculated from the following equations:

$$P = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}} \quad (1)$$

where *G* is the correction factor defined from vertical intensity ($I_{VH}$) and horizontal intensity ($I_{HH}$) of emission light to the horizontally polarized excitation light as following.

$$G = \frac{I_{HV}}{I_{HH}} \quad (2)$$

The fluorescence polarization of TMA-DPH ($\lambda_{ex}$ = 355 nm, $\lambda_{em}$ = 430 nm) was also measured as described above at the final concentration of probe of 2 μM.

Laurdan is sensitive to the polarity around itself, which allows the surface polarity of lipid membranes to be determined (43–47). Laurdan emission spectra exhibit a red shift caused by dielectric relaxation. Thus, the general polarization (*GP*) are calculated from observed emission spectra as follows:

$$GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}} \quad (3)$$

where $I_{440}$ and $I_{490}$ are the emission intensities at 440 nm and 490 nm of Laurdan excited with 340 nm light. Laurdan was mixed when liposome was prepared, and the final concentrations of lipid and Laurdan in the sample solution were 100 and 2 μM, respectively. The emission spectra were recorded at 20–30 °C.

## RESULTS AND DISCUSSION

**Compression isotherms of DMPC/FA monolayers** The surface pressure (Π)-mean molecular area (*A*) isotherms of DMPC/SA and DMPC/LA monolayers were measured on 0.1 M Tris-HCl buffer (pH 8.0) at 25 °C (Fig. 1). Collapse pressure (maximal pressures) $\Pi_c$ of DMPC monolayer was hardly changed by the addition of SA. DMPC and SA were therefore considered to be immiscible in the

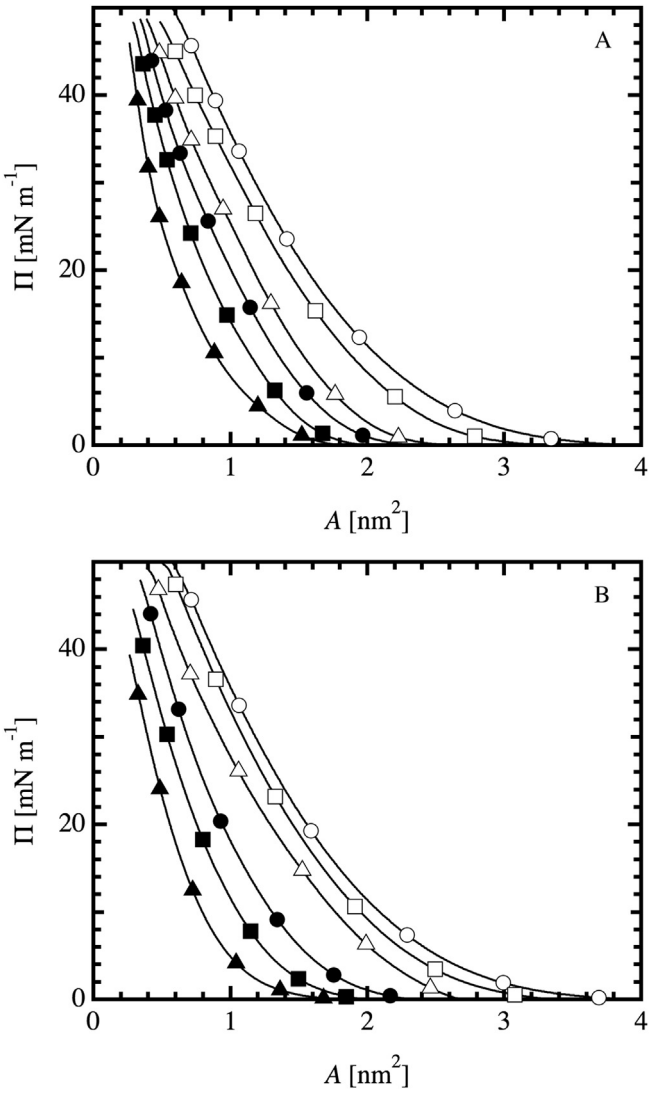


FIG. 1. Π-*A* isotherms of binary mixtures DMPC/SA (A) and DMPC/LA (B) spread on 0.1 M tris-HCl buffer (pH 8.0) at 25 °C. Open circles, $x_{FA} = 0.0$; open squares, $x_{FA} = 0.2$; open triangles, $x_{FA} = 0.4$; closed circles, $x_{FA} = 0.6$; closed squares, $x_{FA} = 0.8$; closed triangles, $x_{FA} = 1.0$.

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