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Endo- and Exocytic Budding Transformation of Slow-Diffusing Membrane Domains Induced by Alzheimer's Amyloid Beta.

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

Masamune Morita, Tsutomu Hamada*, Mun'delanji C. Vestergaard, Masahiro Takagi.

DOI: 10.1039/x0xx00000x

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Cell-sized liposomes are powerful tool for clarifying physicochemical mechanisms that govern molecular interactions. Herein, budding transformation of membrane domains were induced by amyloid beta peptides. The peptides increased membrane viscosity as demonstrated by Brownian motion of membrane domains. These results could aid in understanding the physicochemical mechanism of Alzheimer's disease.

It is important that we understand the physicochemical mechanisms that govern the structural dynamics of cell membranes in response to external molecules. Within cell membranes, laterally-segregated domains, called rafts^[1], are formed to function as platforms for molecular signalling and endocytic trafficking^[1]. This membrane heterogeneity was produced in cell-sized model systems with a ternary mixture of saturated and unsaturated phospholipids and cholesterol^[2]. This ternary system is characterized by two-liquid phase separation between liquid-ordered (Lo) and liquid-disordered (Ld) phases, where each phase corresponds to rafts and a surrounding fluid bilayer, respectively. Raft model membranes are widely used as a tool for studying the physicochemical properties of micro-domains. Several studies have been performed on the dynamics of membrane domains (thermodynamic stability^[2], domain diffusion^[2], domain growth^[2], and budding formation of domains^[3]). We have also developed biomimetic model membranes^[4], i.e., cell-sized liposomes with biological heterogeneity, to investigate the dynamical response of the membrane structure to external molecules^[5].

Amyloid beta (A β) peptides, A β , which consist of 40 or 42 amino acid residues, have been implicated in the death of neural cells in Alzheimer's disease (AD)^[6]. Recently, using model membrane systems, several studies have reported on the interactions between amyloidogenic peptides including A β and lipid bilayers^[7]. A β monomers spontaneously aggregate into fibrils via oligomers. Since these oligomers are reportedly the most toxic species of amyloid related to neurodegenerative diseases^[8], the cellular toxicity of A β oligomers has received much attention^[9]. However, the physicochemical mechanisms that underlie A β toxicity, such as the interaction between A β oligomers and cell membrane surfaces, are

still poorly understood. Previously, we reported that A β peptides induced the membrane transformation of homogeneous one-phase liposomes^[10].

In the present study, we investigated the interaction between A β -42 and raft model membranes (two-phase liposomes), and found that A β -42 induced endo- and exocytic budding transformations of rafts. To the best of our knowledge, this is the first report on the direct observation of the dynamical behavior of membrane domains induced by A β peptides. We believe that an elucidation of the physicochemical mechanism, as demonstrated clearly in this work aids in increasing our understanding, and opens new approaches to further the research.

First, we conducted the real-time observation of changes in membrane morphology induced by 5 μ M A β -42 oligomers. Oligomeric species of A β -42 were prepared by incubation for 12 h^[10]. The degree of A β -42 aggregation was confirmed using atomic force microscopy^[9] (Supporting Figure S1). Lo/Ld phase-separated vesicles (final conc. 200 μ M) were formed from saturated and unsaturated lipids, such as dipalmitoyl phosphatidylcholine (DPPC) and dioleoylphosphatidylcholine (DOPC), together with cholesterol (Chol). The membranes were stained with rhodamine-DHPE (Rho-PE) and NBD-DPPE (NBD-PE), which preferentially partition into the Ld and Lo phases, respectively. Figure 1A shows snapshots of the morphological transformation of raft-exhibiting giant vesicles induced by A β -42 oligomers. Interestingly, A β -42 oligomers caused membrane fluctuation, and the raft domains budded toward the exterior or interior of the fluctuating membrane (Figure 1A, B). The formation of endo- or exocytic daughter vesicles proceeded until the Lo-phase region on the mother vesicles disappeared (Figure 1C). These A β -induced domain dynamics, including the coexistence of exo- and endo-buds from Lo domains together with the enhanced fluctuation of the Ld matrix, are different from those reported previously. An increase in reduced volume due to external stimuli, such as osmotic pressure, has been shown to result in a budding transformation of domains^[3, 11]. During osmosis-induced Lo budding, the remaining Ld part of the membrane maintained an essentially spherical morphology, and buds were formed only on the outside of the vesicle (Figure S2). When liposomes interacted with A β -42 oligomers, the percentage of membrane transformations was 84 % (n=26). Approximately 38 % of the observed transformations

(Figure S3) were a combination of both exo- and endo-buds, as shown in Figure 1A. Careful observations confirmed that the membrane area gradually increased during the A β -induced vesicle transformation. The increase in surface area was $5.3 \pm 2.5\%$ ($n=18$). The addition of A β monomers seldom led to vesicle transformation ($<10\%$) ($n > 10$).

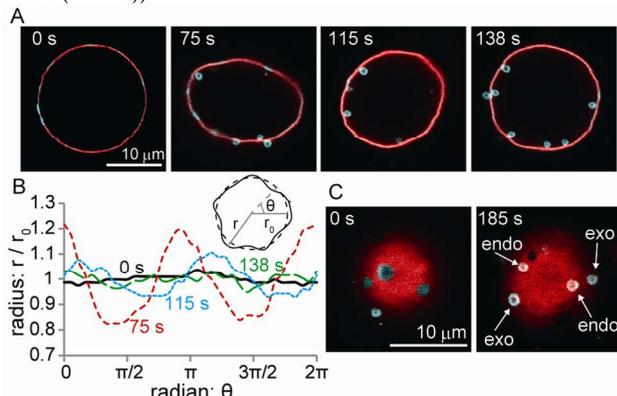


Figure 1. (A) Sectional images of the transformation of heterogeneous membranes after the addition of A β -42 oligomers. Time elapsed after treatment. (Red: Ld-phase, Cyan: Lo-phase). (B) Change in membrane fluctuation. The radius r of the mother vesicle is shown for each θ ($\theta=2\pi/n$, $n=1, 2, \dots, 36$). (C) Typical microscopic images of a vesicle surface before (0 s) and after (185 s) the budding of domains.

To clarify the physicochemical mechanism of the change in the morphology of membrane rafts induced by A β -42 oligomers, we analyzed the behavior of lateral domains. First, we confirmed the localization preference of A β -42 on a Lo/Ld phase-separated membrane surface. Previously, we reported that A β -40 and -42 monomers and oligomers were localized in the Ld-phase region of a Lo/Ld membranes at ambient room temperature^[10]. Figure 2 shows the membrane surface of a single liposome with A β -42 oligomers under a change in temperature. At 46 °C, which is above the miscibility transition temperature (~ 30 °C)^[5, 12], the membrane was homogeneous without domains. The A β -42 oligomers (Figure 2A) and monomers (Figure S4) were distributed fairly evenly over the membrane surface. When we decreased the temperature (at -10 °C/min) to induce phase separation^[2, 5], the A β -42 oligomers (Figure 2B) and monomers (Figure S4) selectively associated in the Ld-phase region.

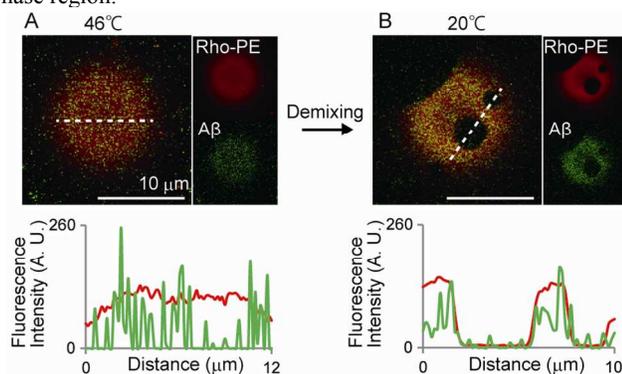


Figure 2. Selective association of A β -42 oligomers during the mixing(A)/demixing(B) transition. As the membrane phase-separates, A β -42 oligomers localize in the Ld phase.

Next, we captured the Brownian motion of each domain^[2] on a liposome surface without (Black dot circle 1 in Figure 3A) or

with A β -42 oligomers (Black dot circle 2 in Figure 3A). Typical fluorescent microscopic images of domains are shown in Figure 3A, where the two phases are distinguished as bright (Ld phase) and dark (Lo phase) regions. Figure 3B shows the average of vertical and horizontal mean square displacements $\langle l^2 \rangle$ with time t . The linear slope of the fitted data gives us diffusion coefficients D for each domain with a radius of r as $\langle l^2 \rangle = 4D(r)t$. Figure 3C shows the resulting diffusion coefficients as a function of r without (White Circles) and with A β -42 oligomers (Gray Triangles). Association with A β -42 oligomers decreased the diffusion coefficients of domains approximately 2-fold, when domains with a nearly equal radius were compared. Notably, under the presence of A β -42 monomers, the diffusion coefficients of domains were slightly reduced (Figure S4B). The diffusion coefficient^[13] of an object in the membrane was originally described by the Saffman-Delbrück equation^[13] (see Supporting Information 5), assuming that $r < \lambda_0 = h\eta_{3D}^*/\eta_w$ (where h is the membrane thickness, η_{3D}^* is the membrane viscosity, and η_w is the bulk viscosity of the surrounding aqueous phase). The typical length-scale λ_0 of domains can be calculated to be 400 nm, where h is 4 nm, η_{3D}^* is 10^{-1} Ns/m², and η_w is 10^{-3} Ns/m².^[14] Hughes and co-workers then developed an equation for diffusing domains larger than μm ($r > \lambda_0$), which can be observed by optical microscopy^[13] (see SI 5). $D(r)$ without A β -42 oligomers show a good fit with the Hughes equation (solid line in Figure 3C), where the membrane viscosity is $\eta_{3D}^* \approx 10^{-1}$ Ns/m².^[14] Similar results have been reported regarding the isothermal diffusion of micron-scale domains within membranes.^[2, 13] On the other hand, $D(r)$ with A β -42 oligomers appear below the line given by the Hughes equation, indicating that the assumption of $r > \lambda_0$ is not appropriate for A β -associated membranes. To characterize the effect of A β peptides on diffusing domains, we adopted an approximation provided by Petrov and Schwille^[13] (see SI 5), which describes an intermediate region between the Saffman-Delbrück and Hughes equations. When the substituted membrane viscosity is increased, the curve given by the Petrov-Schwille equation approaches the experimental data (Figure 3D). This indicates that the association of A β -42 peptides on a lipid bilayer leads to an increase in membrane viscosity, i.e. slow domain dynamics.

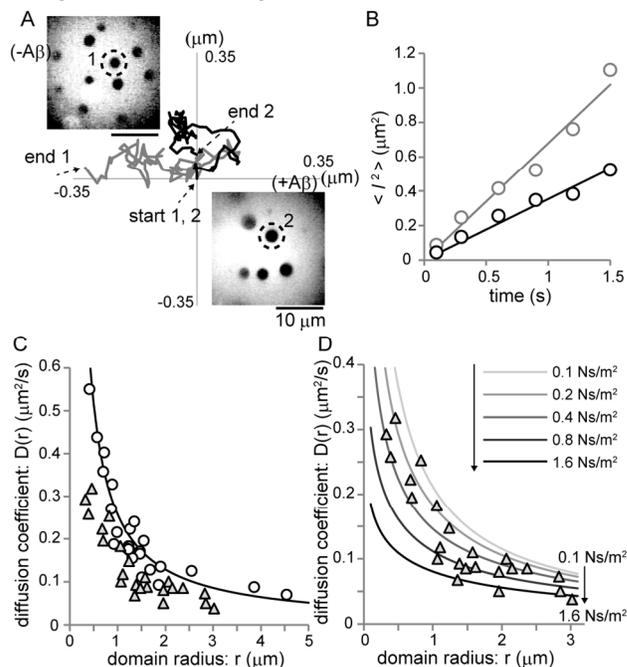


Figure 3. (A) Tracks of a domain for 3.4 s on each vesicle without (Gray) and with (Black) A β -42 oligomers. (B) Mean square

displacement of domains without (Gray) and with (Black) A β -42 oligomers. (Black circle: $r = 1.5 \mu\text{m}$, Gray circle: $r = 1.6 \mu\text{m}$). (C) Diffusion coefficients as a function of the domain radius without (White Circles) and with (Gray Triangles) A β -42 oligomers. The solid line shows the theoretical curve given by the Hughes equation. (D) Diffusion coefficients of domains with A β -42 oligomers. Several lines show the theoretical curve given by the Petrov-Schwille equation with increased membrane viscosities.

We next focused on the fusion of two domains that exhibit random thermal motion (Figure 4). The domains become larger through collision and fusion during thermal agitation^[2, 13]. Within a lipid membrane without A β , the time-scale of domain fusion was typically 10^{-1} s (Figure 4A). The fused domain immediately recovered a spherical shape because of line tension. In contrast, when the domains collided in an A β -associated membrane, it took several seconds for two domains to fuse into one large spherical domain (Figure 4B). Figure 4C exemplifies time-dependent changes in the periphery length of domains during the fusion event (see SI 6). The presence of A β -oligomers slowed domain dynamics by one to two orders of magnitude.

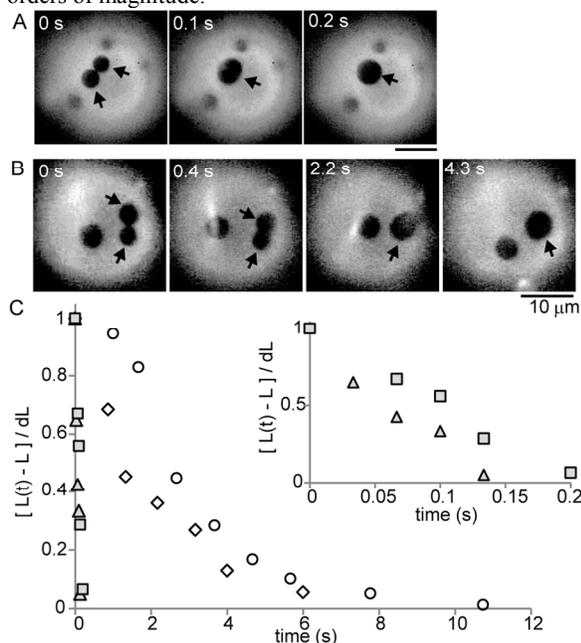


Figure 4. Time evolution of domain fusion on a vesicle without (A) and with (B) A β -42 oligomers (Arrows show domains that exhibit fusion). (C) Time-dependent change in periphery length L (see SI 6) during domain fusion. The white circles and diamonds denote domains with A β -42 oligomers, and the gray squares and triangles show domains without A β -42 oligomers.

In this study, we found that A β localized in the Ld phase (Figure 2), induced a change in the motion of Lo domains that float within the Ld phase (Figures 3, 4), and caused the budding of Lo domains (Figure 1). The observed slow dynamics of the Lo domains could possibly be attributed to an increase in membrane viscosity, which was revealed by an analysis of the diffusion coefficients of Lo domains (Figure 3). The slow relaxation of domain fusion implies a decrease in line tension of the domain boundary.

The budding dynamics of Lo domains induced by A β (Figure 1) are different from those previously reported for the budding of phase-separated vesicles^[3]. The transformation of vesicles is known to be attributed to a gain of excess surface area in response to stimuli, such as osmotic stress. We also tested the vesicle

dynamics in response to osmotic stress in this study (Figure S2). Since the line energy of phase boundaries is generally greater than the bending curvature energy^[3, 11], vesicles decrease the length of the phase boundary to form buds of Lo domains as the excess surface area increases. Therefore, the remaining Ld part of the membrane maintained an essentially spherical morphology induced by osmotic stress (Figure S2). In contrast, under interaction with A β , the Ld-phase membranes fluctuated during the budding of Lo domains (Figure 1). The fluctuation of Ld-phase membranes may be related to the particular transformation with endo- and exocytic budding. Although the detailed mechanism of fluctuating membranes is beyond the scope of this paper, a possible factor can be considered. First, the membranes are under a nonequilibrium conditions, where they acquire excess surface area by A β -induced membrane fusion^[10]. Second, the reduction in line tension by the association of A β possibly weakens the contribution of the line energy in comparison to the bending energy (Figure 4).

Recently, cellular toxicity has been reported to be mostly caused by oligomeric A β ^[9], which agrees well with our results. Although both A β monomers and oligomers localized on the Ld phase (Figure 2), monomers did not induce membrane transformations. The decrease in the diffusion coefficient of Lo domains was also less, when the membrane was treated with A β monomers (Figures 3, S4). The slight decrease in diffusion coefficient of Lo domains may be due to the oligomerization of several monomers that occurs on the Ld phase membrane surface. The difference in the membrane response between monomers and oligomers is caused by peptide-membrane interaction. With the use of computational simulation studies, Strodel *et al.* reported that A β monomers absorbed on or hooked into lipid bilayers, while oligomers inserted deeply into bilayers^[15].

The insertion of oligomers into the membrane may be associated with an increase in membrane viscosity. The height of oligomers (6.1 ± 0.15 nm, Figure S1C) is greater than the membrane thickness (4 nm). The insertion of oligomers increases the membrane thickness, which leads to an increase in membrane viscosity according to the Saffman-Delbrück equation. In addition, under our experimental conditions (lipid : peptide = 40:1), many oligomers were suspended in the Ld-phase bilayer (Figure 2B). It is known that the viscosity of colloidal suspensions increases with an increase in colloid density^[16]. Thus, inserted oligomers could increase the viscosity of membranes. Recently, α -synuclein, another amyloidogenic peptide, was reported to insert into the bilayers^[17]. The insertion induced the lateral expansion of lipid molecules, which progresses to further bilayer remodelling. These results suggest that amyloidogenic peptides tend to insert in the bilayers to change the physicochemical properties of the membrane.

In vivo, it has also been reported that vesicle endocytosis was caused by A β (senile plaques)^[18]. The interaction between A β and a specific lipid (ganglioside GM1) is considered to be an endocytic mechanism for cell membranes^[19]. Previously, we observed the endocytic movement of model membrane systems under the interaction between GM1 and cholera toxin B subunit^[3, 20]. The existence of GM1 may accelerate the interaction of A β with a membrane^[10], leading to vesicle endocytosis^[19]. These interactions should be taken into consideration and investigated.

Conclusions

In this communication, we observed the changes in raft model membranes after the application of A β -42 peptides. We found that oligomeric species of A β induced both exo- and endo-buds from Lo domains together with enhanced fluctuation of the Ld membrane. The analysis of moving domains revealed that the

association of A β -42 peptides leads to an increase in membrane viscosity, i.e. slow domain dynamics. The present results could help us to better understand the toxicity of A β -oligomers in terms of changes in the physical properties of membranes.

Acknowledgements

We thank Prof. Shigeyuki Komura (Tokyo Metropolitan University) and Prof. Masayuki Imai (Tohoku University) for their fruitful discussions regarding diffusion coefficients of the membrane domain. This work was supported by KAKENHI Grants-in-Aid for Scientific Research (B) and (C) and Young Scientists (B) from JSPS and on Priority Areas ‘‘Soft Interfaces’’, ‘‘Molecular Robotics’’ and ‘‘Spying Minority in Biological Phenomena’’ from MEXT of Japan, by a Kurata Grant from the Kurata Memorial Hitachi Science and Technology Foundation, and by a Sunbor Grant from the Suntory Institute for Bioorganic Research. M.M. is supported by a research fellowship from JSPS (2310735).

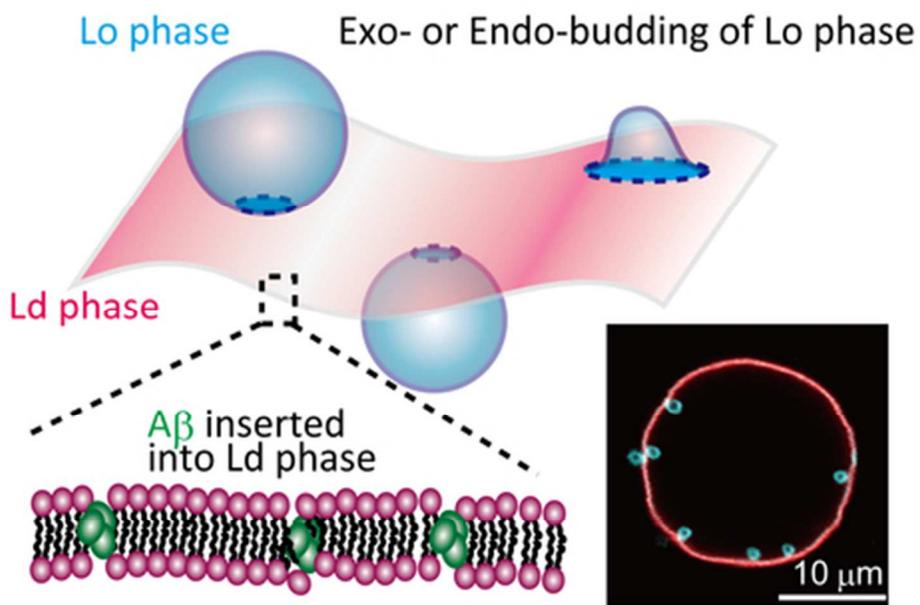
Notes and references

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1, Asahidai, Nomi City, Ishikawa, 923-1292, Japan.

E-mail: t-hamada@jaist.ac.jp; Tel: +81 761-51-1657

† Electronic Supplementary Information (ESI) available: Materials and methods, characterization of each A β -42 aggregation species, membrane transformation induced by glucose osmotic pressure, localization of A β -42 monomers and diffusion coefficients of domains in the presence of A β -42 monomers, domain diffusion coefficients of membrane, insertion model of A β -42 monomers and oligomers. See DOI: 10.1039/c000000x/

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