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ARTICLE TYPE

Selective Localization of Alzheimer's Amyloid Beta in Membrane Lateral Compartments.

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Model membrane systems revealed that lateral heterogeneity of the membrane mediates the localization of amyloid beta peptides in a petide aggregation-dependent manner.

10 The interaction between amyloid beta (Aβ) peptides and a cell membrane surface is one of the key steps in the neurotoxicity of Alzheimer's disease (AD).[1] Aβ peptides with 40 or 42 amino acid residues, Aβ-40 or -42, are produced from an amyloid precursor protein embedded in plasma membranes.[1] Aβ
15 monomers aggregate into fibrils via oligomers, leading to the formation of senile (amyloid) plaques in the brains of individuals with AD.[2] The mechanism of the association of Aβ on a cell membrane surface is poorly understood and needs to be elucidated. Plasma membranes have been reported to form
20 microdomains, called rafts,[3] that are laterally segregated with high lipid order; the raft concept was derived from studies on detergent-resistant-membranes (DRM) which are rich in cholesterol and sphingolipids, isolated from cells.[3] Aβ was found to be enriched in DRM,[4] and several studies have examined the
25 specific interactions between a raft-constituent lipid, such as ganglioside (GM1), and Aβ.[5] However, there is still a large gap in our understanding of the association of Aβ within the heterogeneous membrane interface that needs to be bridged.

Rafts are considered to be a form of order-disorder phase
30 separation that develops due to the interaction between membrane lipids.[3] The phase behavior of lipid bilayers has been investigated using model membrane systems such as cell-sized liposomes.[6] Simple ternary systems composed of saturated and unsaturated lipids and cholesterol show order-disorder phase
35 separation between the liquid-order (Lo) and liquid-disorder (Ld) phases or solid-order (So) and Ld phases.[6] The type of ordered phase (Lo or So) is determined by the ternary mixing fractions; cholesterol-rich membranes tend to produce Lo domains, whereas membranes with lower cholesterol fractions exhibit So
40 domains.[6] Although rafts are considered to be the Lo phase membrane that is rich in cholesterol,[3, 6] DRM experiments cannot distinguish Lo and So phases.

We have developed biomimetic model membranes[7] and investigated interactions with external molecules,[8] including Aβ
45 peptides.[9] Previously, we found that Aβ-40 induced the endocytic transformations of a lipid membrane,[9] and Aβ-40 monomers and oligomers were localized in the Ld phase of Lo/Ld phase-separated membranes.[10] In the present study, to advance our understanding of the interaction between Aβ and
50 heterogeneous membranes, we used three aggregation species of

Aβ-42 (oligomers, pre-fibrils and fibrils) and two model membrane systems (Lo/Ld and So/Ld phase separation).

Briefly, we prepared each aggregation species of Aβ-42 as follows. Aβ-42 and fluorescent-labeled Aβ-42 (Hylite Fluor 488)
55 were mixed and allowed to spontaneously aggregate. We selected three incubation periods, 0 h, 12 h, and 48 h, which essentially correspond to oligomers, prefibrils and fibrils, respectively. The degree of aggregation was confirmed using total internal reflection fluorescence microscopy (TIRFM) (Fig.
60 S1A), atomic force microscopy (AFM) (Fig. S1B) and a thioflavin T (ThT) fluorescence assay[11] (Fig. S1C).

First, we studied the interaction between Aβ-42 and Lo/Ld phase-separated membranes. We used saturated and unsaturated lipids, such as dipalmitoyl phosphatidylcholine (DPPC) and
65 dioleoyl phosphatidylcholine (DOPC), together with cholesterol (Chol) to show two-liquid Lo (DPPC and Chol rich)/Ld (DOPC rich) phase separation (Fig. S2). The membranes were stained with a red fluorescent lipid, rhodamine-DHPE (rho-PE), which was partitioned in the Ld phase.[10, 12] We also tested the effect of
70 GM1 on the membrane association of Aβ. Fig. 1A-D shows typical fluorescent images of Lo/Ld phase-separated liposomes that interact with cholera toxin B subunit (CTB) and each Aβ-42 assembly with 1 mol% GM1. As a control, we used CTB, since cholera toxin is known to be a raft-associating protein, which
75 binds to glyco chains of GM1.[13] While CTB did not bind the membrane without GM1 (Fig. S3A), CTB was localized in the Lo phase of the GM1-containing membrane (Fig. 1A). Oligomers and pre-fibrils of Aβ-42 were partitioned in the Ld phase (Fig. 1B, C), whereas fibrils did not localize in the
80 membrane but rather floated in an aqueous solution (Fig. 1D). Notably, the presence of 1 mol% GM1 did not affect the localization preference of Aβ on the Lo/Ld membrane (Fig. S3B-D).

Next, we examined the effect of a change in membrane phase
85 properties, from two-liquid (Lo/Ld) to solid-liquid (So/Ld) phase separation, on the interaction of Aβ-42. We prepared So/Ld liposomes composed of DOPC/DPPC/Chol = 50/50/0 in the presence (Fig. 1E-H) and absence (Fig. S3E-H) of 1 mol% GM1. Fig. 1E-H shows typical fluorescence images of So/Ld phase-
90 separated liposomes interacting with CTB and each aggregation species of Aβ-42 with 1 mol% GM1. While CTB did not bind to the membrane without GM1 (Fig. S3E), CTB was localized in the So phase of membrane that contained GM1 (Fig. 1E). While oligomers of Aβ-42 were distributed randomly on both the So
95 and Ld phases (Fig. 1F), pre-fibrils and fibrils showed a partitioning preference into the So phase (Fig. 1G, H). Again, the
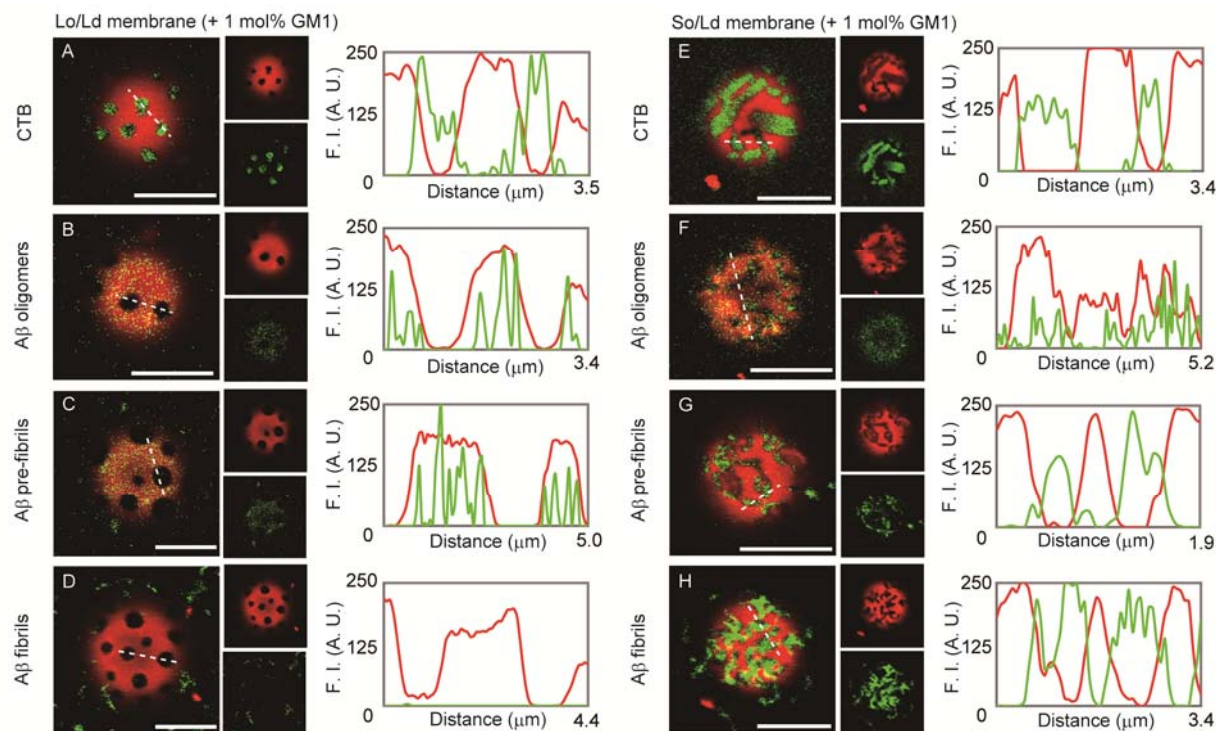
Fig. 1 Typical fluorescence images of the lateral localization of Aβ-42 on cell-sized liposome surfaces that show Lo/Ld and So/Ld phase separation. The liposomes were composed of DOPC/DPPC/Cholesterol = 40/40/20 (A-D) and 50/50/0 (E-H) with 1 mol% GM1. The images show (A,E) cholera toxin B subunit (CTB), (B, F) Aβ-42 oligomers, (C, G) Aβ-42 pre-fibrils, and (D, H) Aβ-42 fibrils. Red and green indicate fluorescence from rho-PE and CTB or Aβ-42, respectively. Fluorescence intensities (F. I.) for each dye along the white dashed line are shown at the right of the images. The scale bar is 5 μm.
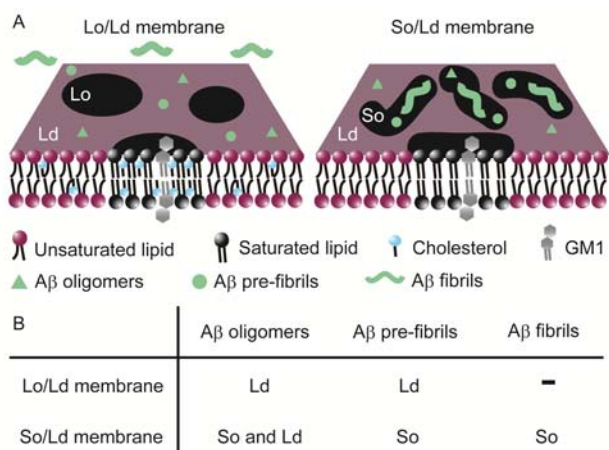
Fig. 2 (A) Schematic illustration of the selective localization of each Aβ aggregation species within Lo/Ld and So/Ld membranes. (B) Summary of the localization preference.

presence of 1 mol% GM1 did not change the localization of Aβ in the So/Ld membranes (Fig. S3F-H). Moreover, the detection of a time-dependent change in Aβ-membrane interaction supported the observed localization preferences (Fig. S4). Aβ-42 oligomers (0 h incubation) and So/Ld liposomes were mixed and incubated to induce Aβ aggregation in the presence of the liposomes. As the aggregation of Aβ-42 proceeded with time, a localization preference into the So phase was observed (Fig. S4), which agrees with the results of the interaction with pre-incubated Aβ aggregations (Fig. 1G, H).

The present results show that membrane phase heterogeneity plays an important role in the localization of Aβ-42 aggregation species. The selective localization of each Aβ-42 aggregation species is summarized in Fig. 2A (schematics) and B (table). This is the first report of a systematic analysis of the interaction between several aggregation species of Aβ-42 and biomimetic Lo/Ld and So/Ld phase-separated liposomes.

We now discuss the mechanism of the observed localization preference of each Aβ aggregation species by considering the difference in membrane fluidity between the two coexisting phases. Although the detailed molecular conformation of the associating Aβ is beyond the scope of this paper, the membrane association of Aβ peptides essentially results from two possible interactions: hydrophobic-driven insertion[14] and van der Waals-driven adsorption.[15]

For Lo/Ld fluid membranes, we should consider hydrophobic-driven insertion.[14] When peptides insert into a lipid bilayer, hydrophobic interaction creates a vacancy within the bilayer.[16] The free energy cost is proportional to the area expansion modulus of the membrane.[16] It has been reported that a higher fraction of cholesterol inhibits the bilayer insertion of Aβ.[17] The area expansion modulus of the cholesterol-rich Lo phase is greater than that of the Ld phase.[12] Therefore, Aβ tends to insert in the Ld phase when interacting with Lo/Ld membranes (Fig. S5). Recently, another amyloid peptide, islet amyloid polypeptide (IAPP), was reported to localize into the Ld phase in an Lo/Ld membrane.[18] We also reported that Aβ-40 monomers and oligomers localized in the Ld phase of an Lo/Ld membrane,[10] similar to the Aβ-42. This result suggest that amyloid-like peptides tend to insert in the Ld phase in two-liquid (Lo/Ld) heterogeneous membranes. This insertion may lead to the penetration of Aβ into the vesicular space (Fig. S6), which is expected to be one of the mechanisms of the cytotoxicity of Aβ.[19] It is noteworthy that fibrils did not localize on the membrane surfaces. This may be attributed to geometric

constraints; i.e., large (>μm) aggregations cannot insert into a thin 5-nm bilayer.

In contrast, when Aβ interacts with So/Ld (solid-liquid phase-separated) membranes, peptides cannot insert into but adsorb on the highly rigid So phase region. Aβ oligomers show both insertion in the Ld phase and adsorption on the So phase, whereas large pre-fibrils and fibrils, which exhibit weak insertion characteristics, interact only with the So region through adsorption (Fig. S5). Recently, Choucair *et al.* reported the accumulation of Aβ-42 aggregation on So phase domains of a supported bilayer system, using AFM and TIRFM,[20] which agrees with our data.

Our results showed that the presence of 1 mol% GM1 did not affect Aβ-42 association, indicating that the observed selective localization is mediated simply by non-specific mechanical properties of the membrane. However, brain cell membranes were reported to contain 10-20 % of GM1.[21] The concentration of GM1 possibly influences its interaction with Aβ. It should be also noted that a high fraction of GM1 causes a change in membrane phase properties.[22] Further experimental developments intended to unravel the possible effect of GM1 on the lipid phase organization together with Aβ-membrane interaction are underway.

Moreover, in AD patients, senile plaques containing fibril and aggregated Aβ have been shown to be deposited on the surface of brain cells.[2] Our results show that fibril Aβ was adsorbed on the So region of So/Ld membranes, but not on Lo/Ld membranes. The existence of So phase domains would accelerate the deposition of Aβ on the membrane surface. Differences in repulsive forces between fibril Aβ and each membrane, such as electrostatic interactions and thermal undulation, should be taken into consideration and investigated.[23]

In summary, we clarified the localization of Aβ-42 aggregation species within phase-separated heterogeneous membranes. Lateral heterogeneity of the membrane mediates the localization of Aβ-42 in a peptide aggregation-dependent manner. Lo/Ld and So/Ld separated membranes showed different partitioning preferences. This indicates that the mechanical properties of the membrane play an important role in the interaction with membrane-associating peptides. A change in the fluidity of membrane domains may be a key factor in onset of AD.

Notes and references

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