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学 位 論 文 題 目	Study of Sphingomyelin/Cholesterol/Ganglioside GM1-SPB Domain Structures Which Accelerate Amyloid Beta Aggregation Reactions
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論文内容の要旨

The pathological hallmark of Alzheimer's disease (AD) is the form of senile plaque in the gray matter of the brain. Among the senile plaques of AD patient brain tissue, the primary component of the plaque is amyloid beta (Aβ). Although it is popularly regarded that the toxicity of Aβ is derived from small or larger oligomeric Aβ, the formation mechanism of the Aβ aggregation is not clear. The Aβ binding to several photopolymerized oligosaccharides containing sialic acid was investigated in an effort to develop new ways to prevent Aβ toxicity. From the primary pathological change in AD, the structure of Aβ combined with ganglioside GM1 (GM1), a glycosphingolipid, was indentified by fractionation and immunochemical Aβ detection. Several recent reports revealed that GM1 forms a seed complex with Aβ and accelerates the formation of toxic Aβ oligomers and/or Aβ fibrils, which have been implicated as a cause of neuronal death in AD. In order to investigate the interaction between GM1 and Aβ in detail, the author studied the polymerizing reaction of Aβ40 on GM1-containing supported planar lipid bilayers (SPBs) on mica and SiO_2 substrates using atomic force microscope (AFM) and fluorescence microscope (FM), and examined the GM1 structure in the SPBs active for the Aβ40 aggregation by molecular dynamics (MD) computer simulations. These SPBs contained various compositions of sphingomyelin (SM), cholesterol (CHOL) and GM1, and were treated at physiological salt concentrations.

In the present study, a unique structure of GM1, which accelerates the formation of Aβ oligomers, was investigated. Previous liposome studies showed that Aβ fibrils form when Aβ40 monomer and liposome containing GM1, CHOL and SM at the ratio of 20:40:40 are incubated at 37°C and that GM1 plays the crucial role for the initial step of the Aβ fibrils formation. It is well known that the Aβ fibril does not grow from Aβ40 monomer solution without a specific "seed", for which fragments of Aβ fibril are generally used. GM1 is one of major gangliosides distributing at the gray matter of the brain, thus it is quite important to clarify the mechanism how the GM1 works as the seed of the Aβ fibril formation on the molecular level. The author prepared SPBs consisting on GM1, SM and CHOL on mica and

SiO_2/Si(100) as model cell membranes, and investigated the phase separation behavior, molecular distribution and the activity of Aβ fibril formation in the GM1-containing SPBs. The author found that the phase separation and the domain structure of the SPB highly depend on the substrate material. Unique triangular domains are often observed only on the mica surface, and the Aβ40 fibril formation proceeds on the phase-separated SPB on mica, while no phase separation occurs and no fiber-like structure grows on the SPB on the SiO_2 surface. In order to investigate the detailed structures and orientation of the lipid molecules and the sugar chains of the GM1 head group, the author performed MD simulation of lipid bilayers consisting of ions, water boxes and lipid molecules. The models with various lipid ratios were constructed and structural optimization was performed by using NAMD program. The author included the effects of the substrates by arranging immobile water molecules at one side of the bilayer to mimic the "structured water layer" at the solid-water interface. The author proposes interdigitated lipid disorder domain (ILDD) model for the GM1-rich region in the SPB on mica, in which the hydrocarbon tails of the lipids are inserted into the opposite leaflet partially and have disordered orientation. Clustering of the sugar chains at the GM1 head groups occurred at the ILDD domains, and this GM1 cluster will be the active site for the formation of the seed of Aβ40 fibrils.

In the former half of the doctoral thesis, the author described the formation of SPBs composed of SM/CHOL/GM1 on mica and SiO_2/Si(100), their phase separation behavior and Aβ40 reaction on the SPBs. These SPBs were formed by vesicle fusion method on the SiO_2 and mica surfaces. On mica substrate, triangular domains were often observed in a 40:40:20 SM/CHOL/GM1 SPB after the SPB was incubated at 37 °C for 24 h. The size of the triangle was different from sample to sample. Even in the triangle domains, further phase separation is observed after longer incubation time. The morphology and height of these SPBs were measured with AFM, and the distribution of SM and GM1 was identified by using FM and CTX-B assay, respectively. After adding the Aβ40 solution to the SPB on mica, Aβ fibrils are formed in the GM1-rich area. Moreover, after adding Aβ40 to a 50:50 GM1/CHOL SPBs on mica, the Aβ fibrils are also observed. But on the SiO_2 substrate, any phase separation was

not observed and globular Aβ40 agglomerates were formed instead of the fibrils.

MD was used to simulate the detailed structure of GM1 molecules in the SPBs on mica and SiO_2 substrates. In order to simulate the effect of mica substrate, a fixed water layer was set under the lipid bilayer models. The oxygen atoms of the water molecule were set at the same level and arranged in a hexagonal lattice. The distance of each oxygen atom in the fixed water layer was 0.52 nm corresponding to the unit cell size of mica. Because of the distribution of GM1 and SM is different between in and out of the triangle domains, the ratio of GM1/SM/CHOL is varied in different lipid bilayer model. After equilibration for 10 nano-seconds, the thickness of each model was determined so that the simulation data were consistent with experimental results.

The experiment and simulation results show that the Aβ40 fibrils observed at the all GM1 concentrations examined on the mica surface are attributed to the molecular conformation of GM1 clusters induced by the clustering effect of GM1 molecules, and by the strong interactions between the GM1 head group and the water molecules bound closely to the ditrigonal cavities in the mica surface. These effects cause the ILDD structure in the GM1-rich areas. The detailed molecular conformation of GM1 in each domain in the SPBs on SiO_2 and mica surfaces has been determined, and these results represent an important step in the precise molecular characterization of Aβ-GM1 interactions.

In the latter half of the doctoral thesis, the formation mechanism of the ILDD, which is observed in the GM1/SM/CHOL bilayers on a mica surface and accelerates the formation of Aβ fibrils, is investigated by MD simulation. GM1 molecules have the tendency to form clusters via the intermolecular hydrogen bond at their hydrophilic headgroups. Under the influence of the mica surface and the fixed water layer, the GM1 cluster forms the ILDD structure. In order to investigate the effect of GM1 concentration in the formation process of the ILDD, the ratio of GM1/SM in the lipid bilayer model was varied. The effect of SiO_2 surface was simulated by setting a fixed water layer with random water arrangement under the lipid bilayer model. The MD simulation results show that the ILDD structure is stable both on mica and SiO_2 surface. But it was not observed on the SiO_2 surface experimentally,

that is, the phase separation to the SM- and GM1-rich micro-domains is not induced on the SiO_2 surface. This indicates that not only clustering effects but also the surface effects on the mica are essentially necessary to induce the phase separation. On mica surface, the ILDD structure is formed and each size increased through the coalescence during incubation. Because the coalescence process is sensitive to the slight uncontrollable change in the experimental conditions, sometimes the triangular domains were not observed.

Briefly, in the GM1-containing SPBs, the activity for the Aβ40 aggregation and the shape of the agglomerates depend on the molecular conformation of GM1, which varies depending on the substrate materials. On mica substrate, the GM1 molecules at the GM1-rich area are significantly active for the Aβ40 fibril growth, thus work as the seed of the Aβ aggregation. According to the MD results, the author proposes that ILDD structure is essential in the GM1-rich area in the SPBs on mica. The detailed knowledge about the GM1 molecular conformation obtained in this work will be valuable for the future researches on the detailed mechanisms of interaction between Aβ and GM1.

博士論文の審査結果の要旨

Zhiguo Shang 氏の学位論文では、シリコン酸化膜（SiO_2）およびマイカ表面上に形成した、ガングリオシド GM1(GM1)/スフィンゴミエリン(SM)/コレステロール(Chol)からなる脂質二重膜のドメイン構造と脂質分子の分子構造を調べ、これらの構造がアルツハイマー病の原因蛋白質であるアミロイドベータ(Aβ)との相互作用にどのような影響を及ぼすかについて述べられている。GM1 は Aβの凝集をもたらす分子として近年特に注目されており、本研究はアルツハイマー病発症の分子機構に関する基礎的知見を与えるものである。先行研究において、マイカ表面上では脂質二重膜の相分離により三角形状のドメイン構造が形成され、このドメイン構造の外側では非常に速い速度で線維状の Aβ凝集体が形成されるのに対し、SiO_2 表面では相分離が起こらず、Aβの球状凝集体が非常に遅く形成されるという明瞭な違いが観測されていた。しかし、以下の基本的な疑問点については、未解決であった：(1) マイカ表面における GM1 を中心とする脂質分子の分子構造はどのようになっているのか、(2) なぜ高速で Aβの凝集反応が起こるのか、(3) なぜマイカ表面では相分離が起こり SiO_2 表面では起こらないのか。Shang 氏はこれらの疑問について、実験と計算機シミュレーションにより明確な回答を得ることに成功した。

第 1 章では、Aβ凝集反応とアルツハイマー病に関する現在までの知見を紹介した後、上記の未解決であった問題も含め本研究の背景ついて記述している。第 2 章および第 3 章に主たる研究成果が記述されている。第 2 章では、上記の疑問(1) と(2) に答えるための実験と計算機シミュレーションの結果が記されている。実験により、三角形ドメインの外側は GM1 リッチな液相、内側は SM リッチ（ただし少量の GM1 は含まれる）な液相であり、いずれも単一の脂質二重膜からなることがわかった。三角形ドメイン内外の二重膜の厚みと SiO_2 上の二重膜の厚みを正確に計測し、これらの情報をもとに、計算機シミュレーションによって、それぞれの相における二重膜の分子構造を決定することに成功した。特にマイカ表面では、三角形ドメインの外側でアシル鎖が相互に入り組んだ不規則液相ドメイン(Interdigitated liquid disordered domain: ILDD)が形成されており、そこでは大きな GM1 分子の頭部が分子内自由回転によって折れ曲がった構造をとっていることが明らかになった。このユニークな相分離と ILDD 構造の安定化には、マイカ表面第 1 層の三角対称性を有する吸着水との相互作用が本質的な役割を果たしていることを明らかにした。GM1 分子が Aβの凝集に関わることは知られていたが、本研究では、凝集速度が GM1 分子の構造に大きく依存することを、初めて明らかにした。第 3 章では、このような相分離が、マイカ表面の特異な吸着水と脂質二重膜との相互作用に加え、GM1 分子のもつクラスター形成特性によって誘起されていることを、マイカ表面の吸着水層の影響を考慮した脂質二重膜の安定性の解析と、脂質分子の拡散速度の解析により明らかにした。

以上、本論文は、マイカ表面の GM1/SM/Chol 脂質二重膜の構造と Aβ凝集反応に関する未解決の問題を解明し、アルツハイマー病発症に深く関わる Aβ凝集反応に関して重要な新知見をもたらすものであり、十分意義ある研究成果である。審査委員会は出願論文が博士（理学）の授与に値すると全員一致で判断した。