

Title	アミロイドベータ (A $\beta$ -42) のエンドサイトーシスによる膜輸送に必要な因子について
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Citation	
Issue Date	2017-06
Type	Thesis or Dissertation
Text version	ETD
URL	<a href="http://hdl.handle.net/10119/14752">http://hdl.handle.net/10119/14752</a>
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学 位 の 種 類	博士(マテリアルサイエンス)			
学 位 記 番 号	博材第 429 号			
学 位 授 与 年 月 日	平成 29 年 6 月 23 日			
論 文 題 目	Researches about important factors for the endocytic transport of amyloid beta(A $\beta$ -42) through biomimetic and biological membranes (アミロイドベータ (A $\beta$ -42) のエンドサイトーシスによる膜輸送に必要な因子について)			
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## 論文の内容の要旨

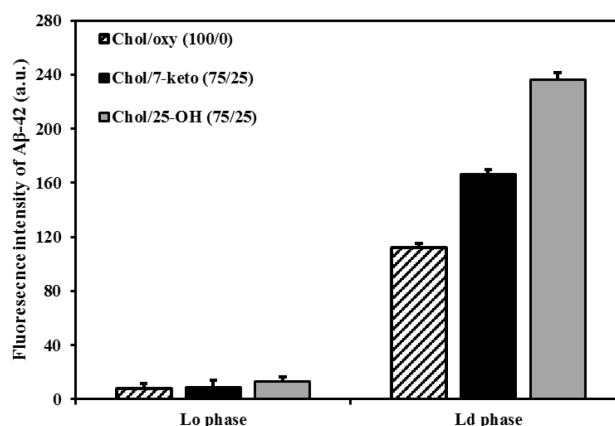
**Background:** Alzheimer's disease (AD) is an age related dementia affecting millions of life globally. Deposition of amyloid beta (A $\beta$ ) peptides into extracellular plaques and formation of intracellular neurofibrillary tangles (NFT) are the pathological hallmarks of this neurodegenerative disorder. Aggregation, accumulation, misfolding and cytotoxicity of A $\beta$  peptide are the key events in the pathogenesis of AD. Accumulation of misfolded proteins induces stress to endoplasmic reticulum (ER). This phenomenon is generally known as ER stress which is linked with other cellular responses such as release of calcium ions, inflammation and apoptosis. It has been reported that ER stress may occur by externally added A $\beta$  which would affect the cellular processes. In my study, I have speculated that exogenous A $\beta$  may internalize into the cell via endocytic pathway, then causes stress to the organelle. Thus, I have focused on the factors affecting the peptide transport, there is a critical link between AD and diabetes where oxidative stress, AGEs etc. are the common features between these two ailments. Oxidative stress causes lipid oxidation which generates oxygenated derivatives of cholesterol, known as oxysterols. Recently, oxysterols have been recognized as risk factors for Alzheimer's disease which can be generated by enzymatic oxidation of cholesterol.

Accumulating evidence have suggested that interaction of A $\beta$  with cell membranes has important role in these processes. 7-ketocholesterol (7-KC) and 25-hydroxycholesterol (25-OHC) were the two oxysterols used to substitute the membrane cholesterol level among which latter is produced by reactive oxygen species (ROS) and former gets generated on the enzymatic oxidation to make the transport of cholesterol from brain in form of its derivatives. Notably, toxicity of A $\beta$  depends upon its isoforms and

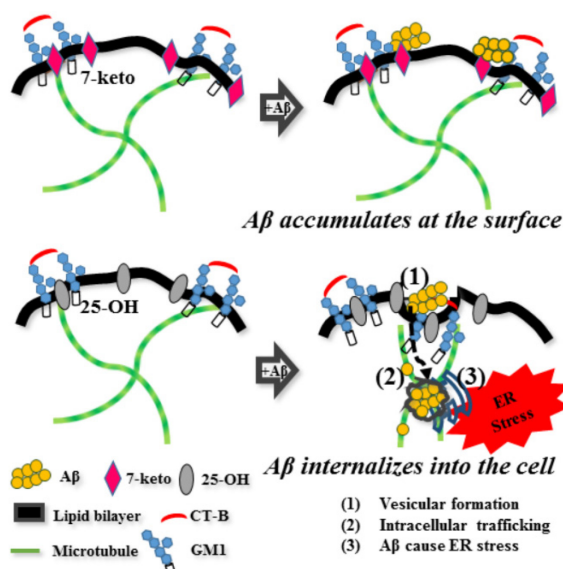
their state of aggregation, thus based on the current understanding, protofibrillar A $\beta$ -42 was used throughout the study.

**Aim:** In this dissertation, I aimed to investigate the important factors mediating the endocytic transport of A $\beta$ -42. These factors are associated with the risk factors for Alzheimer's disease such as aging, oxidative stress, advanced glycation end products (AGEs), diabetes, genetic or hereditary factors. Thus, in this study, role of oxysterols in AD was emphasized with other factors involved in the endocytic transport of the peptide. Biomimetic and biological membranes possessing different levels of complexities were employed to achieve the aim of this dissertation.

**Experimental results and discussion:** In chapter 2, after substituting the cholesterol level with 25-OHC in the heterogeneous biomimetic membranes, I have found that the localization of protofibrillar A $\beta$ -42 was influenced in comparison to the membrane without substitution. Predominantly, it occurred in the liquid-disordered (Ld) phase of lateral compartments of the membrane as shown in the figure 1. Next, I assess the aggregation kinetics of the peptide in the presence and absence of oxysterol. As oxysterols are cytotoxic in nature, thus presumably, they would enhance the toxicity induced by A $\beta$  peptide. So as happened after the addition of 25-OHC, the fibrillation of A $\beta$ -42 was restricted to form oligomers and protofibrils as compared to less toxic monomers and mature fibrils.



**Figure 1.** Localization of A $\beta$ -42 in the lateral compartments of membranes in the presence of cholesterol and oxysterols (25-OHC and 7-KC).

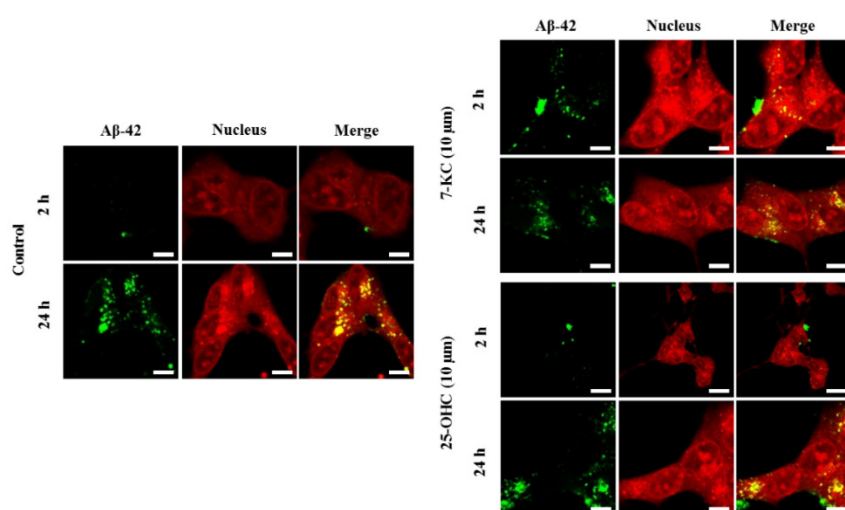


**Figure 2.** Illustration of the intracellular transport of A $\beta$ -42 in the 25-hydroxycholesterol and cholera toxin B subunit added cells and not in 7-ketocholesterol.

Chapter 3, studies on biological membranes, using Jurkat T cells (a leukemic cell line) were performed to assess the effect of oxysterols on the interaction of the peptide with cell membranes. With previous understanding about the oxysterols which suggested induction of peptide insertion into the membrane, increase in excess surface area, enhance surface interaction of A $\beta$  with the membranes. This dissertation have clearly shown that 25-OHC mediates the endocytic transport of

protofibrillar A $\beta$ -42 in the cell in correspondence to negative curvature induction by cholera toxin B subunit and monosialoganglioside GM1 interaction. After internalizing into the cells, peptide was transported to ER via microtubules as depicted in the figure 2. As a result, intracellular calcium ions release was observed which induced after disruption of calcium homeostasis by the action of A $\beta$ -42 and oxysterols in a concentration dependent manner. Although A $\beta$ -42 and oxysterols are cytotoxic in nature but they could not cause severe damage to the Jurkat cells under used conditions.

In chapter 4, undifferentiated human neuroblastoma SH-SY5Y cell line which is an analogue of neuronal cells was used to investigate the A $\beta$ -induced toxicity in the progression of Alzheimer's disease. To assess the effect of concentration of peptide, I used different concentrations of protofibrillar A $\beta$ -42 and observed that toxicity of the peptide was dependent upon the concentration of A $\beta$ -42 protofibrils. This was in accordance with a previous study which proposed that the aggregation of peptide varies with its density. At higher concentrations, A $\beta$ -42 was lethal to cells whose effect was increased in the presence of both



**Figure 3.** Internalization of A $\beta$ -42 in the human neuroblastoma SH-SY5Y cells in absence and presence of 25-OHC and 7-KC after 2 and 24 h incubation with the peptide. Confocal microscopy was used, A $\beta$ -42 and CT-B are represented by green and red fluorescence, respectively. Scale bars =10  $\mu$ m.

oxysterols in time-dependent experiments. Lower

concentration of the peptide was used to analyze the internalization affected by oxysterols at different incubation times. Higher amount of protofibrillar A $\beta$ -42 was internalized into the cells in the presence of oxysterols as shown in the

figure 3. After internalizing into the cell, protofibrillar A $\beta$ -42 was localized into the ER which indicates the

induction of ER stress after accumulation of misfolded proteins in the lumen of ER which was the inspiration and speculation of this study.

In conclusion, use of biomimetic and biological membranes provided an advantage to further the understanding about the mechanism behind changes induced by A $\beta$  in AD. Through the findings of this study, a clear view about the risk factors for the disease was represented. Oxysterols, glycosyl chains in the membrane, nano-structures of the peptide were the risk factors studied here. Thus, prevention of oxidation of cholesterol, fibrillation of A $\beta$ -42 and avoiding glycation may be a substantial approach in the

treatment of the illness.

**Keywords:** Amyloid beta, oxysterols, glycation, internalization, membranes.

## 論文審査の結果の要旨

**This dissertation consist with the following four chapters.**

### **(Chapter 1: Introduction and Abstract)**

Alzheimer's disease (AD) is an age-related neurodegenerative disorder. The aggregation of misfolded amyloid beta (A $\beta$ ) peptide is one of the main causes of AD. There are multiple risk factors including impaired lipid metabolism, oxidative stress and advanced glycation. In this study, some of the risk factors involved in the intracellular transport of A $\beta$ -42 were investigated using biomimetic and biological membranes. Previously, Prof. Takagi's group demonstrated that the effect of oxysterols was more prominent than cholesterol. Therefore, cholesterol, 25-hydroxycholesterol (25-OH) and 7-ketocholesterol (7-keto) were chosen and compared to investigate neurotoxicity by A $\beta$ .

### **(Chapter 2)**

Using model membranes, the fibrillation of the A $\beta$  peptide was examined. It was shown that fibrillation was hindered in the presence of oxysterols.

### **(Chapter 3)**

Jurkat cells were used to clarify the essential factors in the transport of A $\beta$ 42. A $\beta$  was found to be internalized in the presence of 25-OH and CT-B-GM1. After internalization, A $\beta$ 42 was localized in the endoplasmic reticulum (ER). Moreover, pathway of the transport of peptide was microtubule-dependent. This suggests that the peptide induced ER stress which has been reported in neural cells after external addition of peptide.

### **(Chapter 4)**

Human neuroblastoma SH-SY5Y cell line was used to show that even sub  $\mu$ M concentration of A $\beta$ 42 could induce toxicity, and the effect was enhanced at longer A  $\beta$  cell incubation. More number of cells were observed to contain A $\beta$ 42 after pre-treatment of cells with both, 25-OH and 7-keto; and internalization of peptide in cells was increased after longer incubation period.

These findings will further the understanding about the AD and the mechanism involved in the transport of the peptide in the pathogenesis of the illness. Therefore, the committee concluded that this dissertation is suitable for the Degree (Material Science).