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研究課題名（和文）モデル膜による分子ストレスセンシング

研究課題名（英文）Molecular sensing using model membranes

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研究成果の概要（和文）：我々は、（i）アミロイドβ(Aβ)は、その種類や膜組成に依存し相互作用することを示してきた。その結果、膜の変形、崩壊、粘度変化を引き起こす。これは、毒性メカニズム解明に貢献すると思われる。（ii）酸化コレステロールの影響で膜流動性が温度上昇により上昇した。細胞を用いても同様の結果を得た。この結果は、Aβ誘導毒性におけるコレステロールの影響を明確にする為有用だった。（iii）酸化した膜が抗体との結合を強めた。（iv）神経保護剤は、構造依存的に膜と相互作用する。官能基レベルで化合物の重要性を解明することは生理活性薬剤候補のデザインへとつながる。我々は、Aβ検出のため免疫チップを発展させた。

研究成果の概要（英文）：We have (i) shown that amyloid beta (Ab) interacts with membranes in a species-dependent and membrane composition manner. They cause membrane transformation, breach and viscosity. These may contribute towards understanding toxicity mechanisms; (ii) studied how oxidation of cholesterol influences membrane dynamics, demonstrating that temperature increase renders oxidized membranes more fluid. We have similar findings using biological cells. The findings are useful for clarifying the impact of cholesterol in Ab-induced toxicity; (iv) oxidized membranes enhance Ab's association with membranes; (v) potential neuro-protective agents interact with membranes in a structure-dependent manner as does their ability to inhibit Ab aggregation. Unraveling the importance of these compounds, at a functional group level opens the key to tailored design of potential bioactive drug candidates. We have developed an immunosensor chip for Ab.

研究分野：工学

科研費の分科・細目：プロセス工学　生物機能　バイオプロセス

キーワード：免疫センサー　流動性　毒性　神経保護　アミロイド-β　アルツハイマー病　電気化学　生体模倣センサー

１．研究開始当初の背景

Scientific Background of Research: Life-style diseases such as type 2 diabetes, heart and neurodegenerative diseases appear to increase in frequency as people live longer. My vision is to develop nano-based sensing technologies that closely mimic the physiological environment, and utilize them for understanding molecular mechanisms behind the pathologies.

I started this research, using Kiban C grant in June, 2011.

２．研究の目的

Purpose of the Research (Outline) To develop and further the current understanding of molecular mechanisms governing some patho-physiological situations related to neurodegenerative diseases and life-style related illnesses. My first target was Alzheimer's disease, with focus to amyloid beta peptide. To realize my goal, I planned to develop and/or utilize sensing technologies that closely mimic the physiological environment. I planned to utilize two main types of technologies: (1) model membrane systems and (2) biosensing technology.

Biosensing technology is one of the most promising platforms (*Anker et al., Nat. Materials, 2008*) for studying proteins. Biosensors are devices that combine a biological component (*a recognition layer)* and a physico-chemical detector component (*a transducer).* My past research has involved development of simple methodologies for real-time detection of protein mis-folding/aggregation *(Vestergaard et al., J. Am. Chem. Soc., 2005)*, and biosensor technologies for application to the medical field, using surface-mobilized molecular recognition elements *(Kerman, Vestergaard et al., Anal. Chem. 2006).* Most current technologies have some short-comings in particular, when targeting low-abundant molecular species in complex sample matrices and biological samples *(Greenough at al., Proteomics, 2004).*

Another major problem is the utilization of environments that, at times, are far-removed from physiological situations such as analysis carried out in aqueous or buffer solutions. For example, fibril formation and defibrilization of amyloid beta is greatly influenced by the lipid composition *(Martins et al., Eur. Mol. Biol. Org, 2008).* This is where employing biomimetic membrane systems is important. We understand that mis-folding of amyloid beta playes a role in alzheimer's disease and thath it does interact with membrane. Biomimetic membranes have as their main advantage, the ability to simulate biological conditions in a controlled environment. Another important aspect is that the vesicles >10 μm allow direct visual observation (*Vestergaard et al., Biotech. Bioeng., 2008).* They are prepared from lipid compositions similar to biological membranes and I can easily change the composition and relative concentrations of the model membranes to suit the desired goal. Model membranes have found wide application as bioreactors (*Chen et al., J. Am. Chem. Soc., 2005)* and platforms for studying disease mechanisms (*Engel et al., Proc. Natl. Acad. Sci. USA., 2008)*. My recent research has centered on construction, and utilization of model membranes to study, in real-time, Alzheimer's A β -induce neurotoxicity *Morita et al., Biophys. Chem., 2010; Hamada et al., J. Phys. Chem. Lett., 2010.* Previously, we observed, in real-time, membrane morphological changes in the presence of amyloid beta. I will study how this may relate to neurotoxicity. The composition of the membrane is very important *Vestergaard et al., Curr. Alzheimer Res., 2010*).

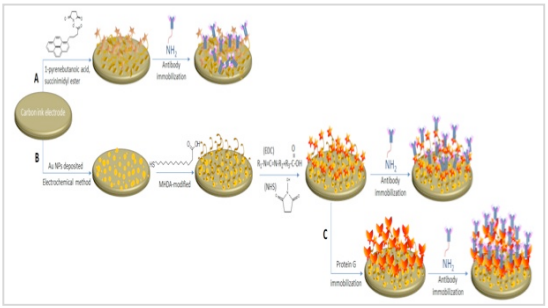
３．研究の方法

Research Plan and Method (Outline)

(1) Using biomimetic membrane systems I planned to study (i) the interaction of amyloid beta with the membrane systems, interms of localization of the peptide, membrane instability as reflected by membrane fluctuation, and morphological changes; (ii) how the peptide:membrane interaction is influenced by membrane composition; (iii) the effect of membrane oxidation (oxidative) stress on peptide:membrane interaction; and how all this relates to neurotoxicity. In future studies, I would develop the research further by looking at naturally-occurring compounds, such as polyphenols, for their potential as neuro-protective agents. Some polyphenols have been reported as potential neuro-protective agents because

of their anti-oxidant abilities. I used various types of microscopy systems (AFM, Confocal, and simple optical microscopes); fluorescence spectroscopy, and Langmuir monolayer as the main analytical instruments.

(2) Using electrochemistry in conjunction with biosensing principles, I planned to develop (i) a 'quantitative' sensitive and selective detection of amyloid beta in buffer as well as in the presence of biological lipids. I also used gold nanoparticles for fabrication of an impedimetric immunosensor chip in order to enhance detection sensitivity of amyloid beta; (ii) a biomimetic sensing platform following the first two. Simple electrochmistry such as voltammetry would be used for understanding the oxidative potentials of the polyphenols.



4．研究成果

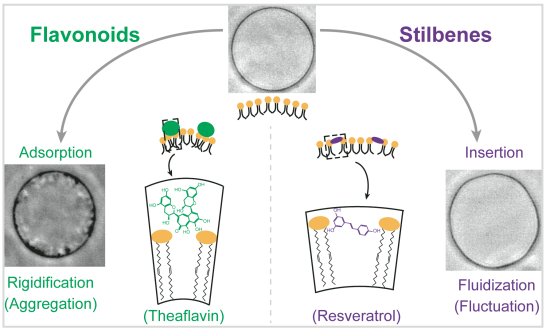
Research Results and Discussion

Main Research Achievements in 2011

The research conduced so far has increased our understanding of how Alzheimer's amyloid beta may cause neurotoxicity through its interaction with cell membranes. Using biomimetic membranes, we have shown that there are differences in the localization of amyloid beta (Aβ) peptide on the membrane surface, and that the localization is dependent on Aβ aggregated species and the composition of the membrane (*Morita et al. Soft Matter, 2012*). The concentration of cholesterol in the membrane surface is important in influencing where the peptide localizes. We have also shown that Aβ breaches the membrane and have established a clear correlation between the membrane breach and the peptide aggregation (mis-folded) states. The smaller oligomeric aggregated species of the peptide breach the membrane more readily than the fibrillar species. Previous work has shown that the small oligomeric species are more toxic than the fibrillar species. Towards studying the influence of oxidized membranes on Alzheimer's amyloid beta-induced toxicity, we first looked at how oxidation of cholesterol affects membrane dynamics (*Yoda et al., Chem. Lett., 2010, Vestergaard et al. BBA-Biomembranes, 2011).*

Main Research Achievements in 2012

Following on from establishing how amyloid beta (Aβ) associates with the membrane, I have now established that membrane transformation induced amyloid beta is caused at least in part, by membrane fusion and/or recruitment of smaller lipid vesicles to the mother vesicle. The findings have been summarized, peer-reviewed and are now disseminated (*Vestergaard et al. BBA-Biomembranes, 2013)*; and presented at an international conference in 2013. Our studies into the effect of temperature on membrane dynamics of model-membranes containing natural and artificial cholesterol derivatives established the oxidation of cholesterol renders membranes more fluid and this is effect is increased with ncreased temperature (*Yoda et al., Lipids, 2012).*

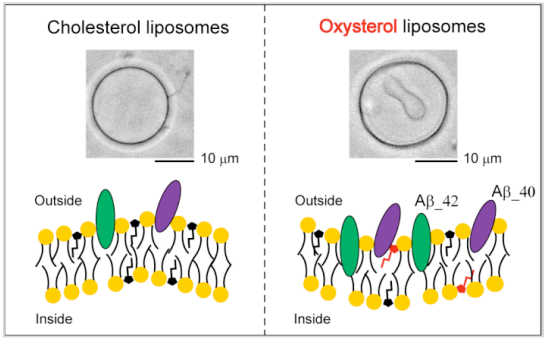


We have also started studying the role of oxidized cholesterol in Aβ-induced membrane dynamics. Preliminary results show that oxidized cholesterol membranes associate with Aβ more readily and are destabilized more readily (*Phan et al, BBA-Biomembranes, 2013).* In our planned studies on potential neuro-protective agents, we have investigated the interaction of naturally occurring secondary plant metabolites (polyphenols) with membranes. We have profiled the interaction of membranes with our chosen polyphenols, and found that changes in membrane structure/properties are structure dependent. The results are now being summarized for submission to an international journal (*Phan et al., Submitted)*; and presented at an

international conference in 2013. Unraveling the importance of these polyphenols, at a functional group level further opens the key to tailored design of bioactive compounds as potential drug candidates.

Main Research Achievements in 2013

Using two classes of polyphenols, we studies how they affect self-assembly of Aβ. We have found that these polyphenols inhibit the aggregation of the peptides in a structural-dependent manner (*Samarat et al., In preparation)*. Using electrochemistry, we have just finished investigating if following interaction with the peptides, (i) there are changes in the redox potential of these compounds and how these are related to the functional groups attached to their aromatic backbone. Bio-physical chemical membrane studies have shown that amyloid beta cause membrane viscosity (*Morita et al., Phys Chem Chem Phys, 2014)*. Following on from oxidized membrane systems and amyloid beta interactions with membranes, we have shown that cholesterol affects the localization of protofibrillar A β in all lateral membrane compartments by modulating membrane fluidity. The presence of 7keto majorly promoted the protofibrils to partition into Ld phase because of its ability to induce a significant increase



in fluidity of this phase. We also used biological (T cells) cells and the results have shown that A β association with cell membrane was increased by changes in cholesterol level and 7keto has a higher effect on this association compared to cholesterol. These findings are useful for clarifying the impact of cholesterol in A β -induced toxicity (*Phan et al., In Preparation*).

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

Publications

〔雑誌論文〕（計 13 件）

Journal Articles

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〔学会発表〕(計６件)

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〔図書〕(計３件)

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〔産業財産権〕

○ 出願状況（計０件）

Patents

〔その他〕

ホームページ等

Others

６．研究組織

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