Form 3

Abstract of Thesis

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Title	A Viscoelasticity Evaluating System of Amyloid-β Peptide Fibrillation on Electrodeless Quartz Crystal Microbalance Biosensor (無電極水晶振動子バイオセンサーを用いたアミロイドβペプチト線維化の粘弾性評価システム)

Abstract of Thesis

Alzheimer's disease, one of the most devastating dementia, is an elderly suffered illness which involves neurodegenerative disorder. The disease pathogenesis is recently found that it is related to a structural-changing phenomenon: Amyloid β (Aβ) aggregation. In order to clarify the pathogenesis, researchers have made intensive efforts for studying the Aβ aggregation both *in vitro* and *in vivo* (or clinical). However, most of the previous *in vitro* studies were held in bulk solutions and which are different from the process in reality, where the aggregations take place on the cell membrane. In the present thesis, the aim is to develop an *in vitro* Aβ aggregation detection system on a surface, which more resembles to the *in vivo* Aβ aggregation, and to analyze the aggregation process from mechanical viewpoint.

The measurement is based on an electrodeless quartz-crystal-microbalance (QCM) biosensor. Here, Aβ aggregations is taken place on surfaces of the quartz crystal, which is similar to those of the actual Alzheimer's disease. Since sizes of amorphous-like Aβ aggregates were reported to be about 100 nm or less, a nanoscale sensing QCM should be established. To this end, a high frequency quartz crystal, whose fundamental resonant frequency is 58 MHz, is used and the resonance overtones up to 7^{th} modes is measured simultaneously. The quartz crystal is sealed in a package which is equipped with a small chamber to hold the quartz lightly in preventing energy loss by attachment. By connecting a microchannel embedded in the package, Aβ monomer solution can be introduced to the quartz crystal. Consequently, the aggregation can be monitored *via* acoustic overtones of QCM. Present experimental studies revealed that the overtone frequencies are shifted as progress of the Aβ aggregation, and that the aggregation process is classified into three stages. First is the binding stage where the frequencies have dropped as soon as the solution is injected. Second is the lag stage where frequencies are relatively stable for a long time after the binding stage. Finally, we have noticed the frequency ramp stage, where increasing in frequencies are found after the lag stage at around 20 hours.

In order to analyze the experimental results obtained by QCM, a two-dimensional viscoelastic model is introduced. It is consisted of three acoustic layers including quartz crystal, viscoelastic Aβ and surrounding liquid. This model relates the acoustic overtone frequencies to mechanical properties of Aβ aggregates on the quartz surface, *i.e.*, shear stiffness, viscosity, and thickness. This analysis revealed that the mechanical properties change with the stages in frequency response. In the binding stage, the three properties, shear stiffness, viscosity, and thickness, increased simultaneously due to the growth of the viscoelastic layer of Aβ. In the lag stage, the thickness decreases while other properties remained stable. It indicates that condensation of the monomers to Aβ aggregates are taken place on the QCM surface. In the frequency ramp stage, the stiffness increases with decreasing of thickness. In this stage, we have found the transformation of amorphous-like Aβ aggregates into the crystal-like fibrils.

In addition to the above mentioned QCM measurements, we conducted another observation of morphology of Aβ aggregates formed on the QCM by atomic force microscopy (AFM). In this experiment, QCM monitoring are interrupted in a pre-assigned stage and the surface of QCM is observed by AFM to clarify the relation between the mechanical properties and morphologies of Aβ. After the binding stage, small Aβ aggregates are confirmed on the surface of QCM. The aggregates are consisting of Aβ monomers and which cause the increasing in the three properties. After the lag stage, we confirmed the growth of the aggregates due to condensation and finally, after the frequency ramp stage, the rod-like fibrils, *i.e.*, fibrillation is verified. From the experimental and theoretical analysis, we conclude that the present viscoelasticity evaluation system can detect the sequential structural change of Aβ aggregates on QCM surfaces. Consequently, the thesis revealed the mechanical properties of Aβ in entire aggregation process. The system has promising usage in further applications such as discovering inhibitory medicines in drug development.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

Yen-Ting Lai君は、高感度水晶振動子バイオセンサーを用いて、その表面上で起こるアミロイドβペプチドの構造変態過程をモニタリングする新たな手法を開発した。アミロイドβペプチドは、アルツハイマー病の原因タンパク質として古くから認識されており、その構造は、単量体から不定形凝集体（オリゴマー）、そして、細胞毒性を示す線維へと変遷する。したがって、凝集体が線維へと構造変態を起こすメカニズムの解明は、アルツハイマー病の予防と創薬において重要な課題である。多くの研究者は、バルク溶液中においてこの過程を研究してきたが、実際の生体内における反応は、まず、細胞膜上に線維核が固定化され、そこに単量体のアミロイドβペプチドが沈着するという、核と単量体との相互作用により進行する。ところが、バルク溶液中においては、様々な凝集体と単量体との相互作用および凝集体同士の相互作用が起こり、実際の生体内での反応を再現することが難しい。そこで、Yen-Ting Lai君は、凝集核を自作の水晶振動子バイオセンサー表面に固体化し、そこに単量体をフローすることにより、生体内での反応に近い反応を再現して、振動子表面における凝集体の構造変態を捉えることに成功した。MEMSプロセスにより、高感度の薄型の水晶振動子バイオセンサーを作製し、さらに、複数の高次の共振モードを同時に計測するシステムを構築し、これにより、水晶振動子表面の凝集体の粘弾性特性をモニタリングすることに成功した。そして、線維化にともなって、凝集体の剛性率が増加する現象を見出し、剛直な線維形成の過程を、標識を用いることなくリアルタイムにモニタリングした。構造変化は、原子間力顕微鏡による詳細な表面観察により十分確認された。この成果は、例えば、アミロイドβペプチドの凝集抑制剤の効能評価において利用することが可能となる。つまり、薬剤候補物質を添加することにより、凝集体の剛性率変化を起こすことなく凝集反応が進行すれば、線維化を抑制すると判断することができる。

このように、Yen-Ting Lai君の研究成果は、タンパク質のアミロイド線維形成に対する学術的に重要な知見を与えるだけでなく、診断および創薬分野においての貢献も期待される。この研究成果は、米国化学会の雑誌 Langmuir において掲載された。以上より、Yen-Ting Lai君の研究論文は博士（工学）の学位論文として価値のあるものと認める。