論文 / 著書情報
Article / Book Information

題目(和文)	42残基βアミロイドの凝集とクロスシーディングによる 凝集についての固体NMR解析
Title(English)	Solid-State NMR Studies of Misfolding and Cross-Seeding for 42-Residue β-Amyloid
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種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
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論 文 要 旨

THESIS SUMMARY

専攻： Department of	分子生命科学　専攻	申請学位（専攻分野）： Academic Degree Requested	博士　（　理学　） Doctor of
学生氏名： Student's Name	松田　勇	指導教員（主）： Academic Supervisor(main)	石井　佳誉　教授
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Protein misfolding is an important topic to understand the mechanisms of variety of disorders. Alzheimer's disease (AD) is one common disorder caused by accumulation of misfolded proteins and peptides, known as amyloids. This thesis focuses on the misfolding of an amyloid peptide, called 42-residue β-amyloid (Aβ) or Aβ42, and its structural transition from an oligomer to a fibril. This thesis also focuses on the misfolding of Aβ42 under the presence of a different isoform of Aβ. Solid-state nuclear magnetic resonance (SSNMR) spectroscopy was used as the primary tool for structural characterization in both studies.

This thesis comprises three chapters. Chapter 1 presents a brief introduction of AD. As the aging of society becomes a greater social issue worldwide and especially in Japan, understanding of age-related diseases such as AD, for which age is a major risk factor, is critical. Two major hypotheses related to the research of AD and roles of Aβ peptides within those hypotheses are discussed.

In Chapter 2, we examined the properties of a spherical oligomer of Aβ42 and its structural transition using SSNMR spectroscopy and other biological methods. The leading hypothesis of AD states that the deposition of fibrillar aggregates of Aβ in the brain as senile plaques is the direct cause of AD. Increasing evidence, however, indicates higher toxicity of Aβ oligomers than Aβ fibrils. Therefore, those neurotoxic oligomers may play a central role in the pathology of AD. However, isolation and characterization of those oligomers has been difficult due to their instability in a long term. In this project, a spherical oligomer of Aβ42, called a spherical amyloid assembly (SPA), was successfully captured and isolated. We characterized the site-specific structure of SPA and its structural transition from the spherical oligomer state to a fibril state using SSNMR spectroscopy. The transmission electron microscopy analyses indicate that SPA is spherical in shape. SPA has a diameter in a range of 11–21 nm with the average diameter of 15.6 ± 2.1 nm. The immunological assay and SSNMR results show similarity of SPA to an AD patient-derived toxic Aβ oligomer, called amylospheroid (ASPD), in its morphology and structure, suggesting possible pathological relevance of SPA in AD. The SSNMR analyses show that SPA is likely to have the first case of an off-register

parallel β-sheet arrangement, unlike any other forms of Aβ oligomers and fibrils. The SSNMR results also show a difference in the structural details between SPA and a fibril obtained at the end of the SPA incubation condition. The results suggest that as SPA is converted to the fibril, SPA undergoes a large conformational change to form the fibril. The study provides an insight in the misfolding pathway of Aβ42, establishing SPA with relatively well-ordered structure and stability as an excellent model system of a pathologically relevant spherical oligomer ASPD.

In Chapter 3, we studied the interaction between Aβ42 and a closely related isoform of Aβ using kinetic assay and SSNMR spectroscopy. Aβ peptides exist as 39–43 residue peptides with varying C-terminus. The most abundant isoforms are Aβ42 and Aβ40. Although Aβ42 fibril is more toxic than Aβ40 fibril, Aβ40 is much more abundant than Aβ42. Misfolding and structures of Aβ42 and Aβ40 fibrils has been studied by many research groups separately to investigate the mechanism and pathology of AD. However, because different isoforms of Aβ coexist in the brain of an AD patient, it is important to study how fibril of one Aβ isoform affects the misfolding of another Aβ isoform. It has been recently shown that E22G Aβ40 misfolding followed a template structure-specific polymerization with both Aβ40 and Aβ42 seed fibrils. In this study, we present the significance of the tertiary structure of seed fibril in the misfolding of Aβ42 monomer. SSNMR analyses and thioflavin T fluorescence assay show that the misfolding of Aβ42 is promoted by the addition of Aβ40 seed fibril in a concentration-dependent manner at 0%, 5%, and 10%. However, our previous study suggested that misfolding of Aβ40 monomer in the presence of Aβ42 seed fibril was unaffected even at 20% Aβ42 seed fibril concentration. Such asymmetric prion-like behavior between Aβ42 and Aβ40 is associated with the compatibility of Aβ monomers to the tertiary structures of the seed fibrils. This study provides an insight in the association between cross-seeding behavior of Aβ isoforms and structures of the seed fibrils in the development of AD and other amyloid diseases, such as prion disease.

SSNMR spectroscopy employed in our studies has been used as the primary structural characterization of amyloid proteins and peptides. Recent rapid theoretical and technological developments in SSNMR allowed application of this technique to complex systems, including Aβs. Basic concepts of NMR spectroscopy and techniques specific to SSNMR spectroscopy are introduced in Appendices.