博士学位論文審査要旨

２０１９年2月５日

論文題目： Structural polymorphism of alpha-synuclein fibrils
αシヌクレイン線維の構造多型

学位申請者： 田中　剛貴

審査委員：

主　査： 脳科学研究科　教授　御園生　裕明
副　査： 脳科学研究科　教授　藤山　文乃
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要　　旨：

この学位論文では、“シヌクレイノパチー”と呼ばれる神経変性疾患の原因因子である、αシヌクレインによって形成されるアミロイド繊維の構造多型について検討を行っている。シヌクレイノパチーは、パーキンソン病やレビー体型認知症など、変性が起こる脳部位やそこに蓄積するαシヌクレインの様態を異にする疾患群である。同じαシヌクレインを原因としながらどのように多様な疾患が生じるのかについては明らかにされていない。本学位研究は、各疾患に対応した点変異を持つαシヌクレインが、それぞれ特異的な構造の繊維を形成し、さらにそれが正常型シヌクレインを取り込んで増殖し、疾患特異的な蓄積物を生じるという仮説に立脚している。この論文では特に、各変異体が特異的な繊維を形成するかどうか、また一部の変異体に関して、その繊維の増殖が起こるかという点について、形態的、生化学的な検証を行っている。

申請者は、精製したリコンビナントαシヌクレインを試験管で線維化させ、電子顕微鏡による繊維形態の解析と、タンパク質分解酵素による繊維内タンパク質切断の解析を組み合わせ、繊維構造の違いを検討できる実験系を構築した。この系を用いて、ヒトとマウスのαシヌクレイン、および、シヌクレイノパチー変異体によって形成される繊維の構造を比較している。その結果、これらのαシヌクレインが異なる構造の繊維を形成することを明らかにした。また、パーキンソン型変異体によって形成された繊維が、正常型のヒトαシヌクレインを取り込んで増殖することを示唆する結果も示している。以上の結果は、今後、どのように疾患特異的な蓄積物が生じ、細胞種特異的に蓄積するのかを理解する上で重要であると考えられる。

口頭試問では、英語による適切な研究発表を行った。質疑応答では論文についての問題点がいくつか指摘されたが、それに対して満足のできる回答・討論を行い、学位論文の defense を行うことができたと判断できる。

よって、本論文は、博士（理学）（同志社大学）の学位を授与するにふさわしいものであると認める。

総合試験結果の要旨

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要　　　旨：

博士学位候補者の田中氏に対して、2019 年 1 月 30 日午後 3 時より総合試験を行った。

田中氏の学位研究は、αシヌクレインを原因とする一連の神経変性疾患“シヌクレイノパチー”に関して、疾患特異的なαシヌクレイン変異体が形成する異常繊維の構造を比較するものである。そこで総合試験では主に、生化学、タンパク質化学、神経病理学の基礎および専門的な知識についての試問をおこなった。なお、語学試験については、総合試験の前に行った公開口頭試問を英語で行ったため、試験では免除とした。

田中氏は、審査委員の質問について適切に答え、不明な点に関しては十分に論理的な考察を行った。関連分野の基礎知識は十分に備わっていると判断した。専門的な知識については、まだまだ不十分な点も認められるが、概して満足のできるレベルにあると判断した。

以上を踏まえて、審査委員一同の協議の結果、総合試験の結果は合格であると認める。

博 士 学 位 論 文 要 旨

論 文 題 目： Structural polymorphism of alpha-synuclein fibrils
αシヌクレイン線維の構造多型

氏　　　名： 田中　剛貴

要　　　旨：

Synucleinopathies comprise a diverse group of neurodegenerative diseases including Parkinson disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), those share a common pathological feature, the deposition of alpha-synuclein (a-syn) in neurons or oligodendroglias. Recent findings clearly suggested that distinct polymorphic a-syn affect the different pathogenicity in mouse brain, and distinct synucleinopathies are caused by distinct polymorphic a-syn fibrils. Namely, the existence of different structural characteristics in synucleinopathies suggests that synucleinopathies share commonalities with prion diseases and possibly explains how a single protein can result in different clinical phenotypes and pathology among synucleinopathies. However, the polymorphisms of a-syn fibrils are not clearly determined. In this thesis, we tackled the amyloid fibrils polymorphisms derived from a-syn to discuss the different disease phenotypes based on structural polymorphism of a-syn fibrils. To uncover them, we particularly focused on the substituted recombinant a-syn purified from E. coli. Though a-syn is highly conserved, single substitutions of disease mutations are mainly located in N-terminal region. Ala53, which is one of the PD-associated mutation sites, are not conserved even in mouse a-syn (Ala53 to Thr). In this thesis, we focused on the amyloid fibrils polymorphisms among human, mouse, and disease-associated mutant a-syn aggregates to uncover the structural polymorphisms of the aggregates and discuss the disease diversities in synuceinopathies.

A-syn is highly conserved in vertebrates, but the primary sequence of mouse a-syn differs from that of human at seven positions (A53T, S87N, L100M, N103G, A107Y, D121G, and N122S). However, structural differences of their aggregates remains to be fully characterized. At first, we studied the human and mouse a-syn aggregates generated in vitro, and found that they showed morphologically distinct amyloid fibrils, the twisted and straight structures, respectively. Furthermore, we identified distinct protease-resistant core regions, long and short cores, in human and mouse a-syn aggregates, respectively. To identify the amino acid(s) responsible for the structural differences between human and mouse a-syn fibrils, we generated the mutants of human a-syn containing each substitution of the different amino acid between human and mouse a-syn. Interestingly, among the seven unconserved amino acids, only A53T substitution, which is one of the familial PD mutations, is responsible to this structural dimorphism. On the other hand, S87N, L100M, N122S formed predominantly twisted type of fibrils, and A107Y, N103G, and D121G formed twisted fibrils and bundles of two or more straight fibrils. Besides, we also checked protease-resistant core of those mutant a-syn fibrils, indicating that the mouse type structural core determined by protease digestions is also explained by a single substitution of A53T. Thus, the results, for the first time, showed that the interspecies single substitutions used in this study can regulate distinct biochemical and morphological properties in different mutant a-syn aggregates. Additionally, focusing on the interspecies difference, these results strongly suggested that a single substitution of A53T mainly regulated those morphological and biochemical differences.

Morphology and molecular structure of amyloid fibrils are sensitive to subtle differences in fibril forming conditions in de novo preparations, but both morphology and molecular structure are transmissible in seedingexperiment. To examine the structural transmissibility of sequence-dependent fibril polymorphism, we performed seeding experiments and investigated whether seeds could induce structures different from the spontaneously-formed aggregates. We found that human a-syn seeded with mouse a-syn aggregates formed straight-type fibrils with short protease-resistant cores. As same in the single substitution experiments, only A53T was responsible for the transmissible structures of mouse a-syn fibrils. Furthermore, we found that bundles types of fibrils were also transmitted to human WT a-syn by seeding reactions, that was shown using agarose gel-electrophoresis for resolving aggregates (AGERA). These data revealed seed-induced structural amplification, suggesting that although the structure of spontaneous aggregates depends on their sequence, the seeds change this spontaneous tendency. Particularly, a single interspecies substitution, A53T, which is one of the familial PD mutations, is responsible for the mouse-type structures including straight morphology and short protease-resistant core, which was completely transmitted to human a-syn by seeding.

Disease-associated mutations in a-syn (namely A30P, E46K, H50Q, G51D, A53T, and A53E) are known to cause familial PD, DLB, and MSA. So, we also performed the biochemical and morphological methods to classify the aggregated forms of disease-associated a-syn mutants. By electron microscopy we observed variations of amyloid fibrillar morphologies among the aggregates of a-syn mutants, mainly categorized into two groups; twisted fibrils observed for WT and E46K and straight fibrils for the other mutants, including A53T. Guanidine hydrochloride (GdnHCl) denaturation experiment revealed that the a-syn mutants except of E46K were more resistant than WT against the denaturation. Mass spectrometry analysis of protease-treated aggregates showed a variety of protease-resistant cores (WT and E46K; long, A30P, G51D, and A53T; short, and H50Q and A53E; short and long). According to a previous review article, the disease-associated mutants may be classified into PD (A30P, H50Q), PD and MSA (A53T, G51D, and A53E), and PD and DLB (E46K) based on clinical and pathological phenotypes. According to the classification, we could differentiate the biochemical and morphological properties of those mutants. Mutants associated with PD or PD with MSA formed straight amyloid fibrils, which were more resistant to denaturation by GdnHCl. PD or PD with MSA showed short core or short and long core demonstrated by the proteinase K-resistant core experiments. In contrast, in DLB type, E46K mutant formed twisted amyloid fibrils, with low resistance to denaturation by GdnHCl, similar to WT, which showed the long core. Using electron microscopy, GdnHCl denaturation assay, and protease-resistant experiments, we successfully classified the disease-associated mutants based on the biochemical and morphological properties of fibrils generated under in vitro condition.