氏　　名	呂　　明
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第1015号
学位授与の日付	平成１８年９月２９日
学位授与の要件	先導科学研究科　光科学専攻 学位規則第６条第１項該当
学位論文題目	Structural Studies of Amyloid Fibril of β2-Microglobulin : Application of IR Micro-spectroscopy
論文審査委員	主　査　助教授　佃　達哉 教授　松本　吉泰 特任教授　安藤　正海 特任教授　北川　禎三 教授　桑島　邦博（東京大学）

論文内容の要旨

Amyloid fibril has been found more than one hundred years ago (1854) in relation to several diseases. It is an aggregation of proteins and/or peptides. It has attracted multiple interests from clinical study and fundamental research. Because it is possible that partially denatured protein in amyloid fibril escapes from the cellular quality control and propagates by itself, the amyloid fibril is expected to be dangerous on clinical studies of several diseases. Such a character is also investigated in the field of structural biology with regards to self-assembly of macromolecules and protein folding.

To date, much effort has been paid on the protein structure in an amyloid fibril, because of importance for understanding the mechanism of protein folding and fibril formation. However, it is still a challenging issue to determine the whole protein structure in the fibril.

Another approach on this matter is to limit a scope. For example, one of the important aspects of the fibril is a property to replicate by itself. In this case, a nature of intermolecular interactions would be an indispensable information to understand what it is. As another example, it is of interest to know why the morphology of amyloid fibril is unique and exhibit similar dimension to each other regardless of precursor proteins. In this case, a width of β-sheet core (as a common feature) should be verified and such a factor should be discussed in relation to the dimension of the aggregation. Thus, partial character of the structure may give a hint to explain several properties of the amyloid fibril.

In this thesis, an amyloid fibril of β_2 -microglobulin (β_2m), which is related to the dialysis-related amyloidosis, has been treated. IR spectroscopy and IR microscope is adopted as main technique. Truncated peptide fragment of b2m is chosen as a probe to search the structural information of the interacting segment.

My aims at this point. First, a novel procedure is applied to clarify the structure of the interacting segment of β_2m amyloid fibril (fA[β_2m]) by using IR spectroscopy. Even though the structural information brought from IR spectroscopy is rather obscure than those of others including X-ray diffraction or NMR spectroscopy, it brings practically essential information when it is applied in an appropriate way. Mechanical structure will be clarified.

Second aim of my thesis is the elucidation of chemical property of the interacting segment. For this purpose, several fragment peptides derived from the sequence of β_2m is examined. Fibrilization efficiency and the structure are discussed under various pH conditions, and the experimental results will be discussed in terms of the pH dependence of side chains and terminal charges.

This thesis contains four chapters: Chapter 1 reviews the general background of amyloid fibril, character of β_2m as well as its fibril state, the remaining problems in this field, and the purpose of this thesis. Chapter 2 reports the novel protocol to search the position and to explore the conformation of the interacting segment along the primary structure of β_2m. In this chapter, the #21-31 fragment of β_2m was chosen as probe, and its fibril was prepared with the aid of the seeding property of the protein fibril (fA[#21-31]-on-fA[β_2m]). The formation of this species was detected by ThT fluorescence method.

Possibility of spontaneous fibril formation of the #21-31 fragment was eliminated by designing the fibril preparation condition adequately. It is considered that an attached tip that consists of the fragment peptide molds the structure of the interacting segment of protein fibril. The result confirms that one of the interacting segments is located at F22~V27 region with planar parallel β-sheet structure. This feature is different from the spontaneous fibril on which the energetically stable structure is accompanied by moderate curl of β-sheet. This difference has been assigned to the planar β-sheet structure of the interacting segment of fA[β_2m] and the molding property of the amyloid fibril. Chapter 3 treats amyloid formed by truncated peptides of β_2m and consisted of two parts (I and II); I focuses on side chain effect on fibril formation and II discusses the terminal charge effect on the structure. Both focuses on the chemical character of interacting segment and the factors that appears as the critical interaction therein. In this chapter, a series of fragment peptide of β_2m around the interacting segment (e.g. around #21-31 region) was chosen as sample, and the fibril formation property has been examined under various pH conditions. The result has strongly indicated that the F22~V27 part possesses the inherent propensity to form the b-sheet. In addition, it has been shown that (i) the aromatic -aromatic interaction is important, (ii) aliphatic -aliphatic interaction is not strong enough, and (iii) electrostatic interaction between charged side chains depends upon the sign of charge with regard to the stabilization of fibril structure. Chapter 4 is the conclusion drawn out from this thesis. The nature of interacting segment of fA[β_2m] will be discussed by taking account of the mechanical and chemical properties deduced from this study.

論文の審査結果の要旨

本論文は英文 80 ページ，実質的には 3 章（形式的には 4 章）から成る．第 2 章は既に J. Mol. Biol.に掲載され，第 3 章が現在投稿中のものである．

第 1 章は本研究の背景を述べたもので，アミロイド線維は 150 年以上も前（1854 年）に病気との関連で見つけられているが，結晶化しないためにその正確な構造が未だに明らかにされていない．それ故に，各種分光学的研究が非常に重要である．アミロイド構造は蛋白質のネイティブ構造とは異なる安定形であるが，会合体を形成しているため不溶性の堅固な線維となる．アミロイド現象は，ヒトではアルツハイマー症等約 20 種の病気の原因となっている．各々の場合の原因となる蛋白質は異なるが，できてくるアミロイド線維は全てよく似た形態をとり，β シート構造ペプチドが 10 残基程度分子間結合で会合体のコアをつくっている．本研究ではそのコアとなる分子間相互作用をつくり出す部分のペプチド構造をフーリエ変換顕微赤外分光法で解明せんとするもので，材料としてはヒト腎臓透析アミロイド病の原因となる β_2 ミクログロブリン（β_2m）そのものと，そのペプチド断片を用いている．

第 2 章は β_2m とその#21-31 ペプチドを用いてアミロイド線維をつくって，FTIR 法で調べたものである． β_2m は酵母菌でつくらせた．ペプチドは化学合成で得たもので，望みの位置に ^{13}C ラベルアミノ酸を導入する事ができた．アミロイド線維ができたかどうかは ThT 蛍光で見た．#21-31 ペプチドだけでも濃度が高ければアミロイドはできるが， β_2m アミロイドを種として少し混入させると非常に薄い濃度でもアミロイドは形成される．本論文は両者のアミロイド構造の違いを議論している．^{13}C=O 伸縮振動数から，その位置の 2 次構造を推定した．それより β_2m の#21-27 部分が平面型平行β構造をとり，会合の原因となる分子間相互作用をしていると推定された．種のない条件でできたペプチドのアミロイド線維は少しカールした逆平行 β シート構造をとるので，その違いが種効果として説明されている．平行か逆平行かは端末基の電荷によって決まる．

第 3 章は短縮した β_2m のアミロイド線維の研究で，アミノ酸側鎖と末端電荷の影響が主テーマである．相互作用領域（#21-31）を色々な風に切りとって，それよりできるアミロイド線維の構造を pH を変えて調べている．#22-27 シークエンスは β 構造を好む本質的な性質を持っているように思われる．側鎖間相互作用では芳香族—芳香族相互作用は重要だが，脂肪族—脂肪族相互作用はそれほどではない．ペプチド末端の電荷の効果は電荷の符号によって異なることがわかった．

第 4 章は 2 ページの短いもので，第 2 章，第 3 章を統合した本論文の結論がまとめられている．

以上に基づき，本論文は，学位論文として充分な内容を持つと審査委員全員一致で判断した．