様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Encapsulation of alpha-synuclein oligomers into a protein cage to elucidate the dynamic behavior.

研究課題名（英文）Encapsulation of alpha-synuclein oligomers into a protein cage to elucidate the dynamic behavior.

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：フェリチンケージのC末端でアミロイドベータ（1-42）やα-シヌクレイン（61-95）などのアミロイド形成ペプチドを融合させ、正確な数の外来ペプチドをインビボでケージにカプセル化しました。 分析アッセイにより、ケージ内にベータシート構造が存在することが確認されました。 HS-AFMを使用して、フェリチンを分解することにより、カプセル化されたペプチドオリゴマーを視覚化しました。 アミロイドコアは、時間の経過とともに球状の形状を線形に変化させる動的な挙動を示しました。 全体として、私たちの研究は、通常は溶液中で研究するのが難しい制限された空間へのアミロゲンペプチドの正確なカプセル化を示しました。

研究成果の学術的意義や社会的意義
Since the role of amylogenic peptides in brain disease is not clear yet, our current study is expected to be a useful from the view point of oligomer specific drug discovery because our system can isolate a precise oligomer and study the dynamic behavior of the oligomer in a confined environment.

研究成果の概要（英文）：We fused the amylogenic peptides such as Amyloid beta (1-42) and Alpha-synuclein (61-95) at the C-terminal of the ferritin cage and thus, encapsulated a precise number of foreign peptides in vivo into the cage. Analytic assays like ThT and FT-IR confirmed the presence of beta-sheet structure inside the cage. We used high-speed AFM to visualize the encapsulated peptide oligomers by disassembling the ferritin cage at pH2.0. We observed that the amyloid core was surrounded by the ferritin subunits. The amyloid core showed dynamic behavior which changing the globular shape to linear over time. Unlike amyloid beta peptide, the alpha-synuclein oligomer dissociates with time. Overall, our studies demonstrated the precise encapsulation of amylogenic peptides into a restricted space which usually difficult to study in solution.

研究分野：Chemical biology

キーワード：Ferritin　Amyloid beta oligomer　High-speed AFM

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

Amylogenic peptides are recognized as the main cause of brain disease like Parkinson's, Alzhaimer's disease etc. Oligomeric forms of such amylogenic peptides were found to be the most toxic species. Current researches are mostly focusing on the molecular mechanism of oligomerization and fibril formation. However, the transient nature, heterogeneity and difficulty in isolation of defined oligomers complicates the detail analysis including dynamic features in solution. Although cross-link treatment and encapsulation into micelle were employed to study the properties of oligomers, it remained a challenge to isolate a precisely defined and structurally characterized homogeneous oligomers to understand its role in the disease and to understand the fibrillization process. Therefore, a suitable methodology is necessary to overcome the difficulties.

２．研究の目的

The purpose of the proposed research was to explore the following

(a) Encapsulation of amylogenic peptides such as α-syn oligomer and amyloid-β peptide into the ferritin cage through genetic fusion.

(b) Characterization of the encapsulated amyloid oligomers including dynamic behavior study by high-speed FAM.

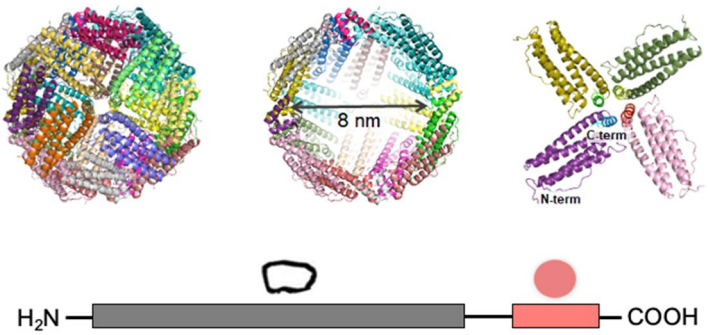

Figure 1: Ferritin cage structure and scheme for the fusion of amylogenic peptide into ferritin cage.

３．研究の方法

We fused the α-synuclein (61-95) and amyloid-β (1-42) peptide at the C-terminal of the recombinant horse spleen apo-ferritin which are located inside the cage. The methodology enabled to encapsulate a precise number of peptides into the cage in vivo. The hybrid cage has been characterized by MALDI, X-ray crystal structure analysis, TEM. The beta-sheet structure was evaluated by ThT assay and FT-IR. In addition, we studied the effect of metal, pH etc. on the beta-sheet content. We studied the dynamic behavior of the amyloid oligomer by high-speed AFM.

４．研究成果

We successfully fused the amylogenic peptides such as Amyloid beta (1-42) and Alpha-synuclein

(61-95) at the C-terminal of the ferritin cage and thus, encapsulated a precise number of foreign peptides in vivo into the cage. The cage structure remained unaltered as evaluated by X-ray crystal structure and TEM. The encapsulated peptides form beta-sheet structure inside the cage as evident from ThT assay and FT-IR. We used high-speed AFM to visualize the encapsulated peptide oligomers by disassembling the ferritin cage at pH2.0. We observed that the amyloid core was surrounded by the ferritin subunits. The amyloid core showed dynamic behavior changing from the globular shape to linear over time. Unlike amyloid beta peptide, the alpha-synuclein oligomer dissociates with time. Overall, our studies demonstrated the precise encapsulation of amylogenic peptides into a restricted space which usually difficult to study in solution.

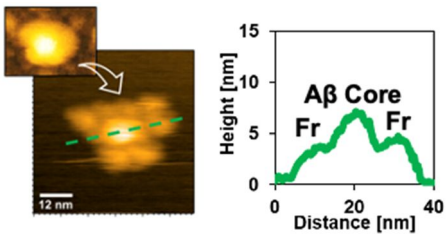

Figure 2: AFM results showing the presence Amyloid core into the disassembled ferritin cage.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　0件）

１．著者名 Basudev Maity, Yuki Hishikawa, Diannan Lu, Takafumi Ueno	４．巻 172
２．論文標題 Recent progresses in the accumulation of metal ions into the apo-ferritin cage: Experimental and theoretical perspectives	５．発行年 2020年
３．雑誌名 Polyhedron	６．最初と最後の頁 104-111
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.poly.2019.03.048	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Basudev Maity,　Zhipeng Li,　Kento Niwase,　Christian Ganser, Tadaomi Furuta, Takayuki Uchihashi, Diannan Lu, Takafumi Ueno	４．巻 22
２．論文標題 Single-molecule level dynamic observation of disassembly of the apo-ferritin cage in solution	５．発行年 2020年
３．雑誌名 Physical Chemistry Chemical Physics	６．最初と最後の頁 18562-18572
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D0CP02069A	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Basudev Maity
２．発表標題 Disassembly reaction of the ferritin cage observed by high-speed AFM
３．学会等名 100th Annual Meeting of the Chemical Society of Japan 2020（国際学会）
４．発表年 2020年

１．発表者名 Basudev Maity
２．発表標題 High-speed AFM observation of ferritin cage disassembly in solution
３．学会等名 The first international symposium on Molecular Engine.（国際学会）
４．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関