アカデミックプログラム［B講演］ | 17. 生体機能関連化学・バイオテクノロジー：口頭B講演

2024年3月18日(月) 15:55 ~ 16:55 H936(9号館 [3階] 936)

## [H936-1vn] 17. 生体機能関連化学・バイオテクノロジー

座長：大嶋 孝志、李 秀栄

英語
15:55 ~ 16:15
[H936-1vn-01]
原子間力顕微鏡によるpH応答性リボン状タンパク質の構造とダイナミクスの解析

○Xin Li[1]、Thuc Toan PHAM[1]、菊池 幸祐[1]、伊達 弘貴[1]、鱒村 颯太[1]、上野 隆史[1] (1. 東京工業大学 生命理工学院)

日本語
16:15 ~ 16:35
[H936-1vn-02]
ペプチド阻害剤設計における動的構造の影響

○李 秀栄[1]、水口 賢司[1,2]、本多 優作[3]、高橋 大輔[3,4]、矢崎 亮[3]、大嶋 孝志[3] (1. 医薬基盤・健康・栄養研究所、2. 大阪大学蛋白質研究所、3. 九州大学大学院薬学研究院、4. 崇城大学薬学部)

日本語
16:35 ~ 16:55
[H936-1vn-03]
ヒト型抗体(T99wt)を抗体酵素に変換したときの立体構造変化の静的及び動的解析

○宇田 泰三[1,6]、加藤 龍一[2]、重田　育照[3]、廣田 俊[4,5]、一二三　恵美[6] (1. 九州先端研、2. 高エネルギー加速器研究機構、3. 筑波大学、4. 奈良先端科学技術大学院大学、5. CREST、6. 大分大学)

# Structure and dynamics of pH-responsive ribbon-like protein analyzed by atomic force microscopy

([1]*School of Life Science and Technology, Tokyo Institute of Technology*) ○Xin Li,[1] Thuc Toan Pham,[1] Kosuke Kikuchi,[1] Koki Date,[1] Souta Masumura,[1] Takafumi Ueno[1]

**Keywords**: Protein Assembly; Ribbon-like Protein; pH-responsiveness; Atomic Force Microscope; Force Curve

Proteins form hierarchical structures equipped with unique functions through self-assembly. Type 51 Refractile body (R-bodies) is a ribbon-like protein assembly and punctures cell membranes by its pH-responsive extension.[1,2] R-bodies possess 400-nanometers-wide coil-like morphologies at neutral pH and turn into 20-micrometers-long spiral-like morphologies at acidic pH (Figure 1a, b). This transformation is reversible and happens in less than one second. These features inspire us to use R-bodies as mechanochemical actuators, however, the structural and mechanical properties of R-bodies remain largely unclear.

In this study, we aimed to characterize R-bodies using an atomic force microscope (AFM) to quantify their structural and mechanical properties. We directly observed the spiral morphology in solution on mica and measured their size and stiffness. We also established the methodology to prepare fragmented R-body sheets (Figure 1c), which allowed us to investigate the precise thickness and curling of sheets by AFM. Furthermore, the synthesized R-body mutants, exhibited different properties compared to the wild-type, providing the underlying structural and mechanical insights of R-bodies.

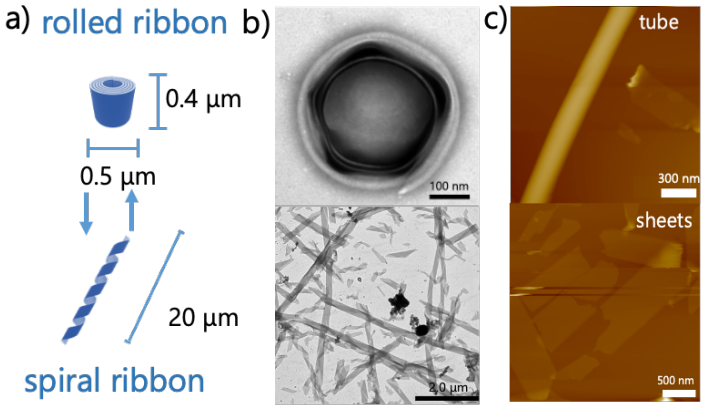


Figure 1. R-body coil and spiral morphologies. a) Schematic map, b) TEM and c) AFM.

1) Pond, F. R, *Microbiol. Rev.* **1989**, 53, 25–67.
2) Polka, J. K., *ACS Synth. Biol.* **2016**, 5, 303–311.

# Impact of dynamical structure on peptide inhibitor design

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**Keywords**: *Protein-Protein Interaction, Peptide Inhibitor, Unnatural Amino Acids, Molecular Dynamics Simulation*

Protein-protein interactions (PPIs) are essential for many biological functions and are therefore important targets for drug design. Medium-sized peptides have the potential to inhibit specific PPIs, which is difficult with small molecules. A major problem is how to suppress and stabilize the inherent conformational flexibility of peptides. This problem can be solved by introducing unnatural amino acids into the peptide.[1] Not only do unnatural amino acids stabilize the conformation of the peptide, but they also have additional benefits, such as improving peptidase resistance. While this approach is promising, it remains elusive how and to what extent the peptide dynamics are controlled by the inserted unnatural amino acids.

Here, we analyzed the conformational properties of peptide inhibitors targeting SARS-CoV-2 spike protein using both public[2] and in-house data. To this end, features representing the dynamical conformation of the peptide were obtained from molecular dynamics simulations. Comparison with available experimental data showed that these features (distance between peptide ends, residue fluctuations, and side chain orientation) correlated with the activity of the designed peptides, indicating that nonnatural amino acids indeed help stabilize the conformation. It appears that better correlation with experimental data can be obtained by accounting for changes in conformational dynamics upon binding to the target. These results emphasize the importance of considering dynamic conformation when designing peptide inhibitors. Further details will be discussed in the presentation.

1) T. Tsuji, K. Hashiguchi, M. Yoshida, T. Ikeda, Y. Koga, Y. Honda, T. Tanaka, S. Re, K. Mizuguchi, D. Takahashi1, R. Yazaki, and T. Ohshima. *Nat. Synth.* **2022**, 1: 304-312.
2) P. Karoyan, V. Vieillard, L. Gómez-Morales, E. Odile, A. Guihot, C-E. Luyt, A. Denis, P. Grondin, and O. Lequin. *Commun. Biol.* **2021**, 4: 197.

# ヒト型抗体(T99wt)を抗体酵素に変換したときの立体構造変化の静的及び動的解析

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Static and dynamic analysis of structural changes between T99wt antibody and the catalytic antibody T99-Pro95(-) converted from T99wt. (*[1]Nanotech. Lab., ISIT, [2]High Energy Accel. Res. Org., [3]Center Com. Sci., Tsukuba Univ.,[4]Div. Mat. Sci., NAIST, ,[5]JST-CREST,[6]Inst. Res. Mgmt., Oita Univ.)* ○Taizo Uda,[1,6] Ryuichi Kato,[2] Yasuteru Shigeta,[3] Shun Hirota,[4,5] Emi Hifumi[6]

Pro95 in the CDR-3 of the antibody light chain is highly conserved. When the Pro is deleted, the antibody acquires antigen-degrading activity. The T99-Pro95(-) mutant, which deletes Pro95 in the human-type antibody light chain T99wt, was converted to a catalytic antibody that degraded Amyloid-beta. In this study, static (X-ray crystallography) and molecular dynamics simulations analyses (MD) of the structural changes associated with this conversion were carried out. From the analysis, it was clarified that the distances between Asp1(O)-His93(H) in the structure of T99-Pro95(-) were shortened, while it was not observed in T99wt.
*Keywords: Catalytic antibody, Steric conformation, X-ray analysis, Molecular dynamics*

【目的】抗体の軽鎖 CDR-3 に存在する Pro95 は高度に保存されている。この Pro95 を欠失させると抗体軽鎖は抗原分解機能を持つように変換される[1]。僅か一残基の変異がこうした性質を獲得することは、免疫学的、生化学的および構造学的観点から興味深い。本研究では、ヒト型抗体軽鎖 T99 野生型(T99wt)と、これを Pro95(-)変異型（抗体酵素）に変換したときの X-線結晶構造解析（静的解析）と分子動力学シミュレーション解析(MD)を行い、両者の解析から抗体酵素の構造と機能の関連性を検討した。
【実験手法】サンプルの結晶化は 0.1 M Sodium cacodylate, 36% PEG2000MME、Beam line は BL-5A/PF KEK や BL32XU/SPring-8 で実行した。MD 計算には Amber22 を用いNPT アンサンブルのもと 300ns 実行し、解析には最後の 50ns のデータを使用した。
【結果と考察】ヒト型抗体軽鎖 T99wt および抗体酵素に変換された T99-Pro95(-)の結晶構造は、それぞれ、2.6 および 2.0 Å の解像度で解析できた。その結果、触媒三つ組残基 (Asp1, Ser27a, His93)の中で Asp1(O)-His93(H)間の距離が Pro95 を欠失させると 5.72Å短縮され、Asp の $CO^-$ と His の $N\text{-}H^+$ とが強い相互作用を持つようなり、活性サイトが形成すると考えられた (Fig. 1)。一方、MD を用いて T99wt および T99- Pro95(-)の動的解析を行った結果では、His93 残基と Asp1 残基の立体構造は大きく揺らぎ、Pro95(-)の場合のみ上述した触媒三ツ組残基がかなり近接する時間帯の存在する事が分かった。この最近接構造（動的）と X-線結晶構造（静的）解析の結果とが類似していた。また、MD で観られた大きな揺らぎは、抗原を Induced fit し、抗体酵素との親和性を高めると推測されるばかりでなく、抗体酵素の特徴である multi-cleaving sites 説を裏付ける事にも繋がると考えられた。

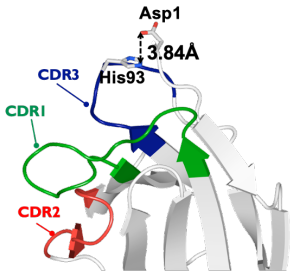

**Fig.1 Structure of T99-P95(-) (X-ray crystallography)**

1) Hifumi et al., *Science Adv.,* **6**(13), eaay6441(2020).