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Abstract of Thesis

Name (Yuxi Lin)	
Title	Study on the mechanism of protein aggregation based on new macroscopic views (新しい巨視的な観点に基づく蛋白質凝集の機構に関する研究)

Abstract of Thesis

In the last decade, protein aggregation has become increasingly a hot topic in diverse research fields due to its linkage to a number of severe human pathologies and potential applications to biological and bioinspired materials.[1, 2] Up to now, most of studies have been focused on how environmental factors, including temperature, solvent composition, liposome surfacc, additives, etc., regulate protein aggregation based on microscopic viewpoints such as structural properties of proteins. In contrast, understanding of the mechanism underlying protein aggregation from macroscopic viewpoints including macroscopic properties of solubility and supersaturation of protein solution is largely unknown. In this thesis, I performed systematic examination on the aggregation of lysozyme and cytochrome *c* and provided new macroscopic insights into protein aggregation using the phase diagram.

The aggregation of lysozyme at various water/alcohol (2,2,2-trifluoroethanol, 1,1,1,3,3,3-hexafluoro-2-propanol, and ethanol) mixtures was first investigated in the absence and presence of ultrasonication.[3] Lysozyme showed the marked variety of its aggregation behaviors depending on the type and concentration of alcohol. Without alcohol and at low alcohol concentrations, lysozyme remained soluble due to high solubility. At moderate and high concentrations of alcohol, lysozyme adapting to partially and extensively helical structures formed amyloid fibrils or amorphous aggregates. Further evidences proved that both amyloid fibrillation and amorphous aggregation occurred at the alcohol concentrations where lysozyme concentration exceeded over its equilibrium solubility. Sonication revealed the phase transition of apparently soluble lysozyme to amyloid fibrils, indicating supersaturation-limited amyloid fibrillation. These results also revealed that sonication is a powerful method to disrupt the high metastability of supersaturation which keeps kinetically solubility. Based on these results, I constructed phase diagrams of lysozyme aggregation in alcohol/water mixtures with and without sonication, and proposed a "protein misfolding funnel" for illustrating alcohol-induced lysozyme aggregation.

I next examined the aggregation behaviors of three distinct conformations of cytochrome *c*, holo cytochrome *c*, apo cytochrome *c*, and silver-bound apo cytochrome *c*, at various concentrations of

2,2,2-trifluoroethanol and 1,1,1,3,3,3-hexafluoro-2-propanol and constructed understandable phase diagrams of cytochrome *c* aggregation.[4] The phase diagrams showed that the aggregation propensity of holo cytochrome *c* was low due to its intrinsically high solubility. Removal of heme groups generated unfolded apo cytochrome *c*, which decreased the solubility and promoted the propensity for amorphous aggregation. Silver-bound apo cytochrome *c* increased the aggregation capacity to protofibrils due to subtle changes in structures of apo cytochrome *c* by strong binding of silver. However, mature amyloid fibrils were not detected for any of the cytochrome *c* variants or its aggregation-prone fragment. These results implicated inherently low amyloidogenicity of cytochrome *c*, which is attributed to the low metastability on supersaturation.

Although lysozyme and cytochrome *c* are obviously different proteins with distinct amino acid sequences, function, and structures, aggregation behaviors of both proteins share a general mechanism: the concentration of proteins which exceeds over their solubility limit is a thermodynamic prerequisite for protein aggregation and the metastability on supersaturation is a critical kinetic determinant for the formation of mature amyloid fibrils. I expect that the macroscopic mechanism of protein aggregation proposed here will be common to aggregation of other proteins and peptides, and will contribute to the deeper understanding of protein aggregation from a new perspective.

Reference

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

アルツハイマー病やパーキンソン病といった神経変性疾患および白内障の発症にはアミロイド線維や不定形凝集体という蛋白質の異常凝集物が関与する。その形状・構造的な特徴および構造に基づくミクロ的な凝集反応の機構が明らかになりつつある一方、蛋白質の異常凝集形成の機構の巨視的な理解は乏しかった。本論文では蛋白質の溶解度および過飽和現象に着目し、様々な生物物理学的な手法を用いて、蛋白質の凝集形成の巨視的な特徴を研究した。

アルコール混合液中のニワトリ卵白リゾチームの凝集研究では、アルコール濃度依存的な凝集反応を網羅的に調べ、凝集の相図の作成に初めて成功した。アルコールはリゾチームの溶解度を低下させ、凝集体形成を引き起こした。さらに、核形成依存的なアミロイド線維の形成は、過飽和の準安定性によって速度論的に制限されるが、超音波のような強力な撹拌によって過飽和の準安定性が解消されることを発見した。次に、構造的に異なる三種類のシトクロム c およびそのフラグメントの凝集形成を系統的に調べ、凝集の相図を作成した。超音波処理とアルコール添加に関わらず、シトクロム c およびフラグメントは、低い過飽和の準安定性のために不定形凝集体あるいはプロトフィラメントを形成することを明らかにした。一連の蛋白質の凝集形成の巨視的な研究は、既存のミクロ的な観点では未知であった蛋白質凝集の一般的機構の解明につながる重要な成果である。

以上、本論文はアミロイド線維を中心とする蛋白質異常凝集の理解を深めるものであり、関連分野の進展に大きく貢献する優れた成果である。よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。