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研究課題名（和文）タンパク質の保護活性と標的能を有した糖導入分解性双性イオン高分子ナノ粒子の設計

研究課題名（英文）Design of degradable glycopolymeric nanoparticles for the safe and targeted delivery of proteins

研究代表者

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研究者番号：７０８４８０４３

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）：本研究では、RAFT重合により糖付きゼウィッターイオン性ミセルを調製した。このミセルは、さまざまなストレス条件下で驚くべきタンパク質安定化能を示し、同時に本来の性質も保持している。重要な点として、タンパク質はミセルからほぼ完全な活性で安全に回収可能である。本研究は、スキャフォールド様ミセルシステムの初めての例であり、低温保存なしであらゆる条件下で保管・輸送できる商品化可能なタンパク質製品の開発の可能性を示した。これにより、タンパク質安定化や保存、デリバリーのための革新的な戦略の開発に貢献し、タンパク質ベースの治療薬の進歩や重要なバイオ医薬品の保存が可能となる。

研究成果の学術的意義や社会的意義
This research revolutionizes protein storage and delivery, enabling off-the-shelf products without low-temperature storage. It improves purification, processing, and production of therapeutic proteins, while offering versatile hydrogel-based protection for long-term storage.

研究成果の概要（英文）：Our research showcases successful glycopolymeric zwitterionic micelles via RAFT polymerization. These micelles effectively stabilize proteins under stress while preserving their native properties. Recovered proteins retain almost full activity. The study introduces a novel scaffold-like micellar system, enabling easily transportable protein products without low-temperature storage. Additionally, the PEG/PVA-coupled PLLSA hydrogels encapsulate proteins at therapeutic concentrations, maintaining their structure and function under extreme temperature fluctuations. The synergy between PLLSA and PEG/PVA enhances protein stabilization against freezing as well as thermal stress. These findings revolutionize long-term storage of therapeutic proteins, including antibodies and vaccines, with versatile hydrogel designs for customized protein protection and delivery under severe stress. Overall, this research advances protein-based therapeutics and preserves critical biomedical products.

研究分野：Polymeric Biomaterials

キーワード：RAFT Polymerization　Zwitterionic Polymers　Glycopolymers　Proteins　Denaturation　Ultracentrifugation　Hydrophobicity　Micelle

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

Therapeutic protein drugs are essential for treating severe medical conditions that cannot be addressed by other medications. However, protein instability poses a significant challenge in biopharmaceutics, and current methods lack the required efficiency for clinical use. Protein instability can lead to aggregation, denaturation, and reduced efficacy during transport and delivery, causing complications and limiting effective dosing concentration. Additionally, protein aggregation is associated with neurological disorders like Alzheimer's and Parkinson's diseases.

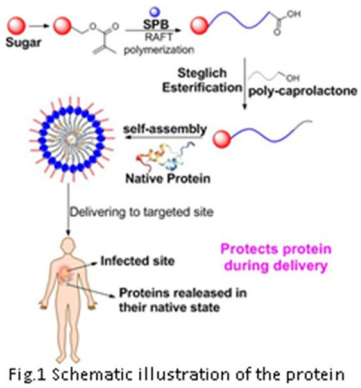

Fig.1 Schematic illustration of the protein protection micelles research

Previous research developed a poly-sulfobetaine (poly-SPB) polymer that exhibited promising efficiency in protecting proteins under severe stress. Enhancing the polymer's hydrophobicity significantly improved its protective capabilities. Nevertheless, the efficiency of the model remained inadequate for in vivo protein delivery. To address these limitations, this proposal aims to create a novel, biodegradable micelle system comprising sugars (for targeted cell delivery), poly-SPB, and a hydrophobic and degradable component like caprolactone. This system is expected to effectively deliver proteins in their native state, while ensuring biocompatibility, biodegradability, and protection against denaturation and aggregation (Fig. 1).

Drawing on previous work on polymer development and the potential of poly-sulfobetaine in suppressing protein aggregation, this research seeks to overcome previous limitations by incorporating sugars and a degradable unit like caprolactone into the micelle system. The objective is to develop an improved protein delivery system.This research holds significance due to the increasing demand for protein biopharmaceutics and the market success of protein therapeutics. Overcoming challenges such as protein aggregation, low stability, and limited delivery efficiency is crucial to unlock the full potential of these biotherapeutics. Moreover, the need for research and development in protein biopharmaceutics is highlighted by the challenges faced by Japan's bio-pharmaceutical industry in keeping pace with global advancements.

In summary, this research proposal aims to contribute to the development of a targeted protein delivery nanoparticle system capable of protecting proteins from aggregation and denaturation while safely delivering them to the desired site for treating various disorders.

２．研究の目的

The purpose of this study is to develop self-assembled micelles composed of poly-sulfobetaine (poly-SPB), trehalose, and polycaprolactone (PCL) for the protection and removal of proteins under various stress conditions. The researchers aim to address the challenge of protein denaturation and aggregation in protein biopharmaceutics by creating a protein stabilizer that can be easily separated from the protein solution. The goal is to preserve the native structure and activity of proteins while enabling their efficient isolation. The developed micelles hold potential applications in protein-based vaccines, drugs, and other protein products, offering improved stability and facilitating safe transportation and storage. Additionally, the study focuses on the design of a straightforward removal process that avoids protein denaturation.

The research objectives are as follows:

- Develop a degradable in vitro model for protein delivery applicable in the preclinical phase of drug development.
- Protect proteins from denaturation and aggregation during transport and delivery.

- Enhance compatibility and targeting capabilities of the delivery system through sugar incorporation.
- Evaluate protein protection efficiency and degradation properties of protein-encapsulated micelles in vitro.
- Demonstrate the delivery of protein-encapsulated polymer system to target cells and the release of proteins in their native state.

３．研究の方法

The research method employed in this study is summarized as follows:
The following report summarizes the research method employed and the work that has already been completed in the development of self-assembled micelles for protein protection and separation.

Synthesis of triblock copolymer:

The copolymer PSPB-b-PTrH was successfully synthesized by introducing methacrylate groups to sugars (Trehalose) followed by RAFT polymerization with the SPB monomer.
Both random and block copolymers were synthesized to investigate their respective effects.
Furthermore, the block copolymer PCL-b-PSPB-PTrH was synthesized using the following method: Poly-SPB Macro-CTA was first synthesized by RAFT polymerization, followed by anionic ring opening polymerization of ε-caprolactone using the hydroxyl group at the terminal of Macro-CTA as the initiator.

Protein stability studies: Lactate dehydrogenase (LDH) and recombinant human insulin were employed as model proteins.
The proteins were subjected to various stress conditions to assess the effectiveness of the micelles in protecting them.

Isolation of pure proteins: Following exposure to stress conditions, pure proteins in their native state with complete activity were successfully isolated from the protein-micelle mixture.
The work conducted so far has demonstrated the successful synthesis of the copolymers PSPB-b-PTrH and PCL-b-PSPB-PTrH, as well as their effectiveness in protecting proteins under different stress conditions. The isolation of pure proteins from the protein-micelle mixture further validates the potential of this system for protein protection and subsequent separation (Fig. 2).

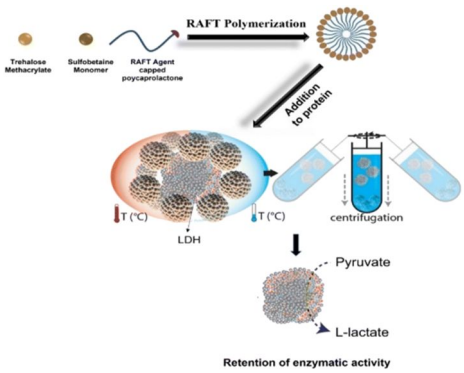

Fig.2 Schematic illustration representing the preparation of the self-assembled micelles and subsequent protection of proteins (from thermal and cold stress) and removal using ultracentrifugation.

The findings from this research contribute to the development of self-assembled micelles as a promising approach for protein stabilization and removal, holding significant implications for protein-based therapeutics, vaccine stability, and transportation/storage of proteins. The report highlights the progress made in addressing the challenge of developing removable protein stabilizers and emphasizes the importance of further research in this field.

４．研究成果

In summary, our research has successfully demonstrated the preparation of glycopolymeric zwitterionic micelles composed of poly-SPB and trehalose via RAFT polymerization. These micelles exhibit remarkable protein stabilization capabilities under various stress conditions while preserving the native properties of the proteins. Importantly, the proteins can be safely recovered from the micelles with nearly full activity. This study represents the first example of a scaffold-like micellar system for protein stabilization, offering potential for the development of off-the-shelf protein-based products that can be stored and transported under any condition without the need for low-temperature storage.

Furthermore, we have developed a zwitterionic-based polymeric system using polyampholyte-based hydrogels containing PEG/PVA coupled with PLLSA (poly-L-Lysine). These hydrogels possess excellent swelling capability and can encapsulate proteins in therapeutically sufficient concentrations. The encapsulated proteins maintain their native structure and function even after exposure to heat, cold, and freeze-thaw cycles. The synergistic effect of PLLSA and PEG/PVA enhances protein stabilization, with PVA providing protection against freezing stress and PEG offering thermal stress protection.

This research has significant implications for the long-term storage and preservation of therapeutic proteins, including antibodies and protein-based vaccines. It provides a versatile toolbox for hydrogel design, allowing for the customization of protein protection and delivery systems under conditions of severe stress.

Overall, these findings contribute to the development of innovative strategies for protein stabilization, storage, and delivery, paving the way for the advancement of protein-based therapeutics and the preservation of critical biomedical products.

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２．論文標題 Polyampholyte‐Based Polymer Hydrogels for the Long‐Term Storage, Protection and Delivery of Therapeutic Proteins	５．発行年 2023年
３．雑誌名 Advanced Healthcare Materials	６．最初と最後の頁 2203253～2203253
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/adhm.202203253	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

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掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.biomac.1c01126	査読の有無 有
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〔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

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