

Title	ドラッグデリバリー基材としての刺激応答性両性電解質高分子の合成
Author(s)	趙, 丹丹
Citation	
Issue Date	2020-03
Type	Thesis or Dissertation
Text version	ETD
URL	http://hdl.handle.net/10119/16665
Rights	
Description	Supervisor: 松村 和明, 先端科学技術研究科, 博士

氏 名	ZHAO Dandan		
学 位 の 種 類	博士(マテリアルサイエンス)		
学 位 記 番 号	博材第 488 号		
学 位 授 与 年 月 日	令和 2 年 3 月 25 日		
論 文 題 目	Synthesis of thermo- and pH-responsive ampholytic polymeric systems as delivery vehicles		
論 文 審 査 委 員	主査 松 村 和 明	北陸先端科学技術大学院大学	准教授
	松 見 紀 佳	同	教授
	金 子 達 雄	同	教授
	都 英 次 郎	同	准教授
	遊 佐 真 一	兵庫県立大学	准教授

論文の内容の要旨

Stimuli-responsive polymers can be used as drug carriers, smart surfaces, and for protein separation due to their unique properties that they can sense changes in the surrounding environment i.e. temperature, pH, light, glucose and so on. However, due to the complexity of the living system, single stimuli-responsive polymers can no longer fulfill the demands of the growing industry. Herein, multi-stimuli-responsive polymer materials have been designed and developed. Compared with conventional single-stimuli-based polymers, multi-stimuli-responsive polymer materials would be more intriguing since more functions and finer modulations can be achieved through more parameters.

Polyampholyte are polymers, which contains both positive charges and negative charges. The protein like structures allows these kinds of polymers to become highly biocompatible. The tunable properties of polyampholyte include mechanical properties, anti-biofouling characteristics, pH responsive or salt responsiveness. These functionalities allow these kinds of polymers to be used in multiple biomedical applications, like cryopreservation and drug delivery. Most of the stimuli responsive copolymer systems reported until date are made up of nonionic blocks. Copolymers with two ampholytic blocks have not been reported yet, and this can exhibit interesting characteristics. This is because the hydrogen bonds or electrostatic interaction between the charged groups will enable the polymer system to exhibit complex properties. However, this would be more challenging than the systems, which contain simpler structures.

In our previous works, we reported that poly-L-lysine (PLL)-based polyampholyte, COOH-PLL, showed an LCST type of phase separation, and could be controlled easily by molecular design. We used this polymer as a cryopreservation agent. On the other hand, we reported poly-sulfobetaine (PSPB) exhibited suppression of protein aggregation behavior. Additionally, PSPB have been widely used as thermoresponsive blocks to fabricate stimuli responsive polymers. Therefore, in this research, I synthesized multi-stimuli-responsive polymers by using COOH-PLL and PSPB, where both the individual blocks were ampholytes and in a subsequent study, I transformed this system into

self-assembled micelles. I intended to use this system as delivery vehicles and protein protecting agents.

In the first project, graft copolymers consisting of two different ampholytic blocks were synthesized via reversible addition fragmentation chain transfer polymerization (RAFT). These polymers showed dual properties of thermo- and pH-responsiveness in an aqueous solution. Ultraviolet-visible spectroscopy and dynamic light scattering were employed to study the phase behavior by varying temperatures and pH values. Unlike the phase transition behavior of other graft copolymers containing non-ionic blocks, the phase transition temperature of these polymers was easily tuned by changing the polymer concentration. Owing to the biocompatible and stimuli-responsive nature of the polymers, this system was shown to effectively release proteins (lysozyme) while simultaneously protecting them against denaturation. The positively charged lysozyme was shown to bind with the negatively charged polymer at the physiological pH (pH 7.4). However, it was subsequently released at pH 3, at which the polymer exhibits a positive charge. Protein aggregation studies using a residual enzymatic activity assay, circular dichroism, and a Thioflavin T assay revealed that the secondary structure of the lysozyme was retained even after harsh thermal treatment. The addition of these polymers helped the lysozyme retain its enzymatic activity and suppressed its fibrillation. Both polymers showed excellent protein protection properties, with the negatively charged polymer exhibiting slightly superior protein protection properties to those of the neutral polymer. The presence of the polyampholyte structure enables these polymers to act as protein release agents, while simultaneously protecting the proteins from severe stress.

In the second project, cholesterol was introduced into the polymer system, which was synthesized in first project. After introducing hydrophobic cholesterol, these polymers can form micelles in an aqueous solution. The critical micelle concentration of the polymers was very low because of the introduction of a highly hydrophobic moiety like cholesterol. Atomic force microscopy (AFM) and Transmission electron microscopy (TEM) was employed to determine the morphology of the micelles. Meanwhile, small-angle X-ray scattering (SAXS) was also employed to study the reason of phase separation from a microscopic perspective. From the SAXS result, we found the LCST type phase separation of COOH-PLL occurred by the liquid-liquid phase transition and the UCST type phase separation of PSPB occurred by the coil-globule transition. In the future, I will use these micelles as hydrophobic drug delivery vehicles to study their ability to load the drugs and then subsequently release on changing the external environment.

To the best of my knowledge, this is the first study where a polymeric system has been developed that contains two different polyampholyte segments in a graft copolymer, thus imparting new and interesting characteristics to the graft copolymer. The use of COOL-PLL segment enables the phase transition temperature of the polymers to be easily tuned by changing the concentration or molar mass of the polymer. This phenomenon gives the possibility of micro-adjustment of the phase transition temperature and can allow these polymers to be employed for various applications by slightly altering the structure of the polymer. Meanwhile, the development of these novel dual thermos-responsive and pH-responsive polymers has further enhanced the understanding of the behavior of multiple stimuli-responsive polymers,

which will enable them to be used for various applications in the future.

Keywords: thermos-responsive polymer, pH-responsive polymer, micelle, protein protect, delivery vehicle

論文審査の結果の要旨

刺激応答性高分子は、温度や pH、光、電場、磁場など様々な外部刺激によりその構造や物性を変化させることが出来る材料として種々の分野で注目を浴びている。その中で、生体材料としては生体の温度や pH に応じて薬物放出機能を持つドラッグデリバリーシステム材料として大きな注目を浴びている。本論文では、下限臨界溶液温度 (LCST) を持つ、高温で構造変化し相分離するポリマーと、上限臨界溶液温度(UCST)を持つ、低温で構造変化し相分離するポリマーのグラフト共重合体を合成することで、多重温度応答性を持つ高分子を重合し、その物性を調べた。また、使用した LCST 型ポリマー部位は、pH にも応答して相分離挙動を示すことから、温度だけでなく pH 応答性の多重刺激応答性ポリマーとしての性質を示すことを確認した。一方、UCST 型のポリマー部位として選んだスルホベタインポリマー(PSPB)は、タンパク質の凝集を抑制する保護作用が報告されている。これらの機能をすべて併せ持ったグラフトポリマーを合成し、温度もしくは pH 依存的に目的タンパク質の活性を保持したまま、複合化/放出可能なキャリアーとしての分子設計を行うと共に、その物性発現および制御メカニズムについて考察した。

まず LCST 型ポリマーのカルボキシル化ポリリジン中のアミノ基を利用してトリチオカルボナート分子を導入し高分子連鎖移動剤を合成した。次にそれを基点とした可逆的付加開裂連鎖移動 (RAFT) 重合により SPB をグラフト重合させた。合成したポリマーは 20 度以下の低温および 40 度以上の高温で可逆的に相分離を起こす熱応答性挙動を示すことを確認し、また、pH によりゼータ電位をプラスからマイナスにコントロール可能であることも確認した。中性 pH においてリゾチームを吸着させ、酸性条件下でリリース出来ること、および、90℃30 分という非常にシビアな条件下でもリゾチームの凝集を防ぎ、活性を維持できることも確認した。次に、コレステロール分子を上記グラフトポリマーに導入することで刺激応答性高分子ミセルの合成にも成功した。TEM および AFM 観察から温度によるミセルサイズの変化を確認した。また小角 X 線散乱により、LCST と UCST において異なるスケールでの構造変化が起こっていることを確認した。UCST においてはシェル部分の SPB 分子鎖が温度上昇により広がることによる 200nm オーダーの粒子サイズの増大が観測されたが、LCST 挙動では十数 nm スケールという高分子鎖の密度揺らぎに起因する構造変化が観察された。このような相分離挙動の機序の解明により、さらに高機能な刺激応答性分子の設計に繋がることが期待される。以上、本論文は、新たな多重刺激応答性高分子の分子設計とそのタンパク質のデリバリー応用への道筋をつけた研究成果であり、学術的に有用と考えられる。よって、博士 (マテリアルサイエンス) の学位論文として十分価値あるものと認めた。