Synthesis of thermo- and pH-responsive ampholytic polymeric systems as delivery vehicles

Abstract

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