Protein encapsulation into thermo-responsive biodegradable nanospheres

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ABSTRACT

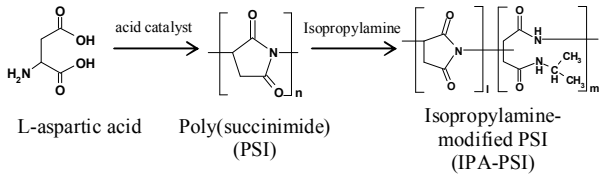
Polysuccinimide (PSI), a polycondensation product of L-aspartic acid by an acid catalyst, reacts with isopropylamine and results in a novel thermo-responsive polymer with biodegradability and biocompatibility. Although the isopropylamine-modified polysuccinimide (IPA-PSI) has the same side chain structure as that of ordinary thermo-responsive polymer, poly(N-isopropyl acrylamide) (PNIPAAm), IPA-PSI with 45 % of IPA substitution shows irreversible thermo-induced phase transition. This feature is available for protein encapsulation in the nanospheres using temperature change. The dominant factor for the encapsulation is mainly electrostatic interaction between the negative charge of polymer and the positive charge of protein surface. In fact, more than 90 % of hemoglobin can be easily encapsulated in IPA-PSI nanospheres, and which is released from the polymer without any denaturation.

INTRODUCTION

In recent years, thermo-responsive polymers possessing LCST have been investigated mainly in biomedical field such as DDS and separation technology [1]. So far, however, the thermo-responsive polymers synthesized are not biodegradable. Therefore, we tried to synthesize a new biodegradable thermo-responsive polymer derived from polysuccinimide (PSI).

PSI shows remarkable hydrolysis and biodegradable properties, and we can easily introduce a variety of side chains by aminolysis with amine derivatives. Owing to peptide bond in main chain, alkylamine-modified PSI (AA-PSI) has a possibility to be a biodegradable polymer [2]. Here, we selected isopropylamine, as an alkylamine because isopropyl groups gave the same side chain structure as ordinary thermo-responsive polymer, poly(N-isopropyl acrylamide) (Poly(NIPAAm)) which shows reversible liquid-solid phase transition [3].

We synthesized isopropylamine-modified PSI (IPA-PSI, Poly[α, β($_{DL}$-asparate isopropyl amide)-co-succinimide]) with a desired substitution degree as shown in **Scheme 1** and investigated their thermo-responsive colloidal properties of aqueous polymer solution. In this presentation, we mainly show the specific thermal property of IPA-PSI and the potential for protein encapsulation.



Scheme 1. Synthesis of PSI and IPA-PSI

EXPERIMENTAL METHODS

Synthesis of IPA-PSI

Polysuccinimide (PSI) was synthesized by acid-catalyzed polycondensation of L-aspartic acid in the presence of phosphoric acid under the reduced pressure. After reaction, the product was dissolved in DMF. Then the product solution was precipitated in the excess amount of water and successively washed with water until the pH of the suspension became neutral. The precipitate was filtered and then dried in vacuo at 323 K.

Synthesized PSI (4.85 g) was dissolved in dehydrated DMF (20 g) and was allowed to react with isopropylamine at 333 K for 4 hours under nitrogen atmosphere. After the reaction, the resultant mixture was precipitated in the excess amount of acetonitrile twice. The precipitate was filtered and then dried in vacuo at 323 K. The chemical structures of PSI and

IPA-PSI were confirmed by ^{1}H-NMR using DMSO-d_6 as a solvent. Then, the molecular weights of PSI and AA-PSI were determined by GPC measurement using DMF as a solvent.

Thermo-responsive properties of IPA-PSI

Aqueous IPA-PSI solution was heated stepwise in water bath. After heating up to 70°C, the solution was cooled down. The heating and cooling rate of samples was 1.0 K min^{-1} in both processes. We observed mean particle diameters of IPA-PSI aggregation by dynamic light scattering (DLS) during the solution was still turbid after cooling. In addition, the IPA-PSI aggregation collected by centrifugation and dried was observed by scanning electron microscopy (SEM).

To determine the LCST of IPA-PSI, the turbidity of aqueous IPA-PSI solution at 600 nm of wavelength was observed at different temperatures using a spectrophotometer equipped with peltier type thermostatic cell holder. LCST was defined as the temperature where the turbidity of the solution began to rise at a heating process.

Assuming that imide rings affect the formation of IPA-PSI nanospheres, aqueous NaOH solution was added slowly by dropwise into turbid IPA-PSI solution below pH 8 to cleave residual imide rings in IPA-PSI without any degradation of the main chains.

Encapsulation of hemoglobin into IPA-PSI nanospheres

Aqueous hemoglobin solution (pH 5.5) containing IPA-PSI was heated to 40°C in water bath to form polymer nanospheres encapsulating hemoglobin. The resultant suspension was centrifuged and hemoglobin concentration of the supernatant was measured to quantify the encapsulation efficiency. The aggregation obtained was re-dispersed in pure water, and aqueous NaOH solution was added in order to release the encapsulated hemoglobin from the IPA-PSI. The circular dichroism (CD) spectrum of the released hemoglobin was subsequently measured.

RESULTS AND DISCUSSION

IPA-PSI with 30-50 % substitution was dissolved into water and the IPA-PSI solution indicates became turbid at a heating process. However, the solution was also turbid even after cooling below the LCST as seen in **Figure 1**.

The IPA-PSI shows an irreversible thermo-responsibility, while ordinary thermo-responsive polymer such as poly(NIPAAm), which has the same side chain as IPA-PSI, shows reversible liquid-solid phase transition by temperature change. This indicates that phase transition of poly(NIPAAm) cannot produce the stable polymer particles even below LCST. The specific thermo-responsibility of IPA-PSI would give us significant information for stabilization of polymer nanospheres, and which has a great potential for the simple preparation of polymer nanospheres using temperature change.

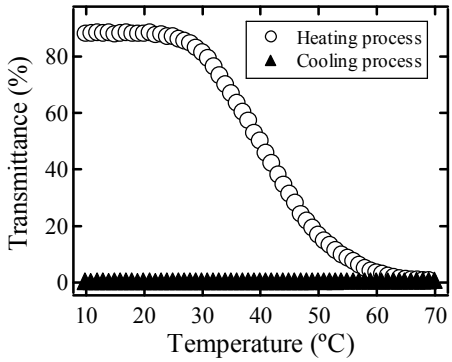


Figure 1. Temperature dependence of optical transmittance for 1wt% aqueous IPA-PSI solution.

The irreversible phase transition of IPA-PSI with hemoglobin facilitates the formation of IPA-PSI nanospheres containing hemoglobin. The supernatant after centrifugation was almost transparent colorless solution, indicating that almost all hemoglobin was encapsulated in the nanosphere aggregation. **Figure 2** shows the effect of protein concentration on the encapsulation efficiency of hemoglobin. From this result, it is clear that more than 90% of

hemoglobin was incorporated into the IPA-PSI nanospheres and that there was the encapsulating capacity of hemoglobin.

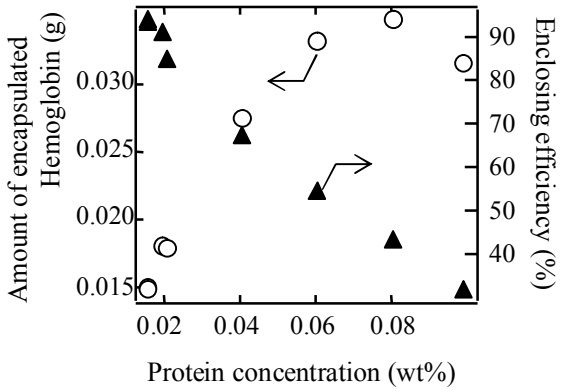


Figure 2. Effect of protein concentration on the enclosing efficiency into IPA-PSI nanospehres.

The zeta potential data of the IPA-PSI nanospheres encapsulating hemoglobin show the dependence of feed protein concentration, as seen in **Figure 3**. The negative surface charge of the hemoglobin-encapsulated nanospheres is based on the carboxyl groups of IPA-PSI, and which is decreased with increasing the amount of the encapsulated hemoglobin. Therefore we concluded the hemoglobin encapsulation was occurred by the electrostatic interaction between proteins and polymers.

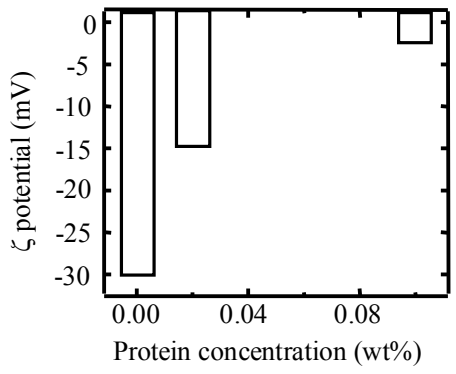


Figure 3. Effect of protein concentration on zeta potential of IPA-PSI nanospheres.

In addition, we added aqueous NaOH solution to the colloidal solution in order to dissolve polymer nanospheres and release hemoglobin. As a result, we recovered transparent red solution, indicating that hemoglobin was completely released. From CD spectra of the released hemoglobin solution, we found that hemoglobin was not denatured by this treatment as shown in **Figure 4**.

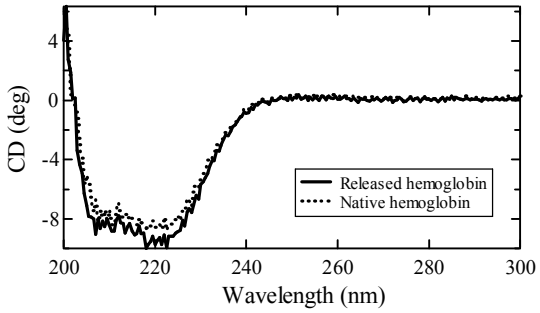


Figure 4. CD spectra of native and released hemoglobin.

CONCLUSION

We succeeded in the synthesis of a new biodegradable thermo-responsive polymer derived from PSI. The side chain structure and the substitution degree strongly affected the thermo-responsibility of polymers. The irreversibility of phase transition was dominated by the amount of imide ring remained in main chain. Moreover, we developed the polymeric nanosphere encapsulating protein by thermo-induced phase transition without using organic solvent. This method is suitable for preparation of biodegradable polymer nanospheres encapsulating protein.

REFERENCES

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