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Thermo-sensitive Self-assembled Micelles Prepared by Cholic Acid and Poly(*N*-isopropylacrylamide)

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Synthetic self-assemblies have been intensively investigated in recent decades and their nanosized structure is very attractive due to their extensive applications such as in colloid science, electronics, environmental technology, biotechnology and biomedical engineering (White-sides *et al.*, 1991; Lehn, 1993; Freedman, 1991; Akiyoshi and Sunamoto, 1996; Davis, 1981). Self-assemblies of block copolymer micelles (Xu *et al.*, 1991) or self-aggregates of hydrophobically modified polymers (Gue-noun *et al.*, 1996) have been investigated with respect to theoretical approaches to their formation or their biotechnological and pharmaceutical applications. The formation of self-assemblies from polymeric amphiphiles resembles that of low-molecular-weight amphiphiles, in that polymeric amphiphiles form micelles consisting of an inner core of hydrophobic segments and an outer shell of hydrophilic segments (Gao and Eisenberg, 1993). The hydrophobic inner core acts as a microcontainer and contains hydrophobic molecules. The hydrophilic outer shell enhances dispersion, inhibits intermicellar aggregation and interactions with other hydrophobic components irrespective of high inner core hydrophobicity.

Some Polymers, which exhibit a lower critical solution temperature (LCST), have recently attracted much attention in the study of controlled drug delivery. Among the LCST polymers, poly(*N*-isopropylacrylamide) (PNIPAAm) is the most popular thermo-sensitive polymer due to its dramatic and reversible transition behaviors. This polymer is water-soluble and hydrophilic. Hydrophobically modified PNIPAAm shows thermo-responsive water-solubility and can form heterogeneous structures composed of hydrophilic microdomains of PNIPAAm chains and hydrophobic microdomains of incorporated hydrophobic segments in an aqueous solution (Chung *et al.*, 1997). Due to their heterogeneous structure, the synthetic self-assemblies act as host systems for many hydrophobic molecules (Anton *et al.*, 1993). In this study, cholic acid (CA) was selected as a hydrophobic segment. CA is biologically the most detergent-like molecule in the body.

N-isopropylacrylamide (NIPAAm, Tokyo Kasei, Tokyo, Japan) was purified by recrystallization in *n*-hexane and dried in vacua at room temperature. Amine-terminated PNIPAAm (ATPNIPAAm), prepared according to the method reported by Chen *et al.* (Chen and Hoffman, 1995), was synthesized by polymerization of NIPAAm (5.65 g, 50 mmol) in methanol (20 ml) at 60°C for 22 h using 2,2'-azoisobutyronitrile (82.0 mg, 0.5 mmol) and 2-aminoethanethiol hydrochloride (113.0 mg, 1.0 mmol) as an initiator and a chain transfer reagent, respectively. The number-average molecular weight of ATPNIPAAm obtained by GPC was 7,880 and the molecular weight distribution of the obtained polymer was 1.74.

CA/PNIPAAm conjugate was prepared through coupling reaction of CA with ATPNIPAAm using N,N'-dicyclohexyl carbodiimide (DCC) as a coupling agent. CA (0.2 mmol), DCC (0.4 mmol), and ATPNIPAAm (0.2 mmol) were separately dissolved in dimethylsulfoxide. The DCC solution was added to the CA solution, and stirred for 30 min to activate the carboxyl group of CA. Subsequently, the ATPNIPAAm solution was dropped into the activated CA solution. The reaction was carried out at room temperature for ten days (Li, 1991). The formation of CA/PNIPAAm micelles and the IN loading procedure were carried out by a diafiltration method (Yokoyama *et al.*, 1994; Cho *et al.*, 1997; Jeong *et al.*, 1998; Kwon *et al.*, 1995). Briefly, 20 mg of CA/PNIPAAm was dissolved in 10 ml of N,N'-dimethyl formamide (DMF). To form polymeric micelles, the solution was dialyzed using a molecular weight cut off (MWCO) 12,000 dialysis tube against distilled water for 24 h and then freeze-dried. To prepare IN-loaded micelles, 20 mg of CA/PNIPAAm was dissolved in 10 ml of DMF. Subsequently, 20 mg of IN was added. The solution was solubilized entirely at room temperature, and dialyzed using a MWCO 12,000 dialysis tube against distilled

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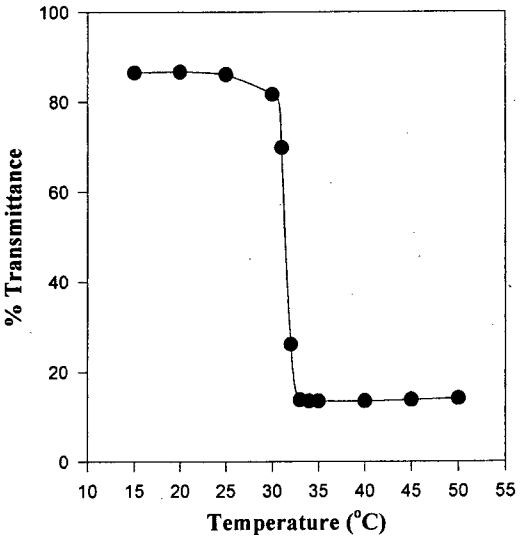

Fig. 1. Optical transmittance at 500 nm of CA/PNIPAAm micelle solution (concentration: 1 g/L) against temperature.

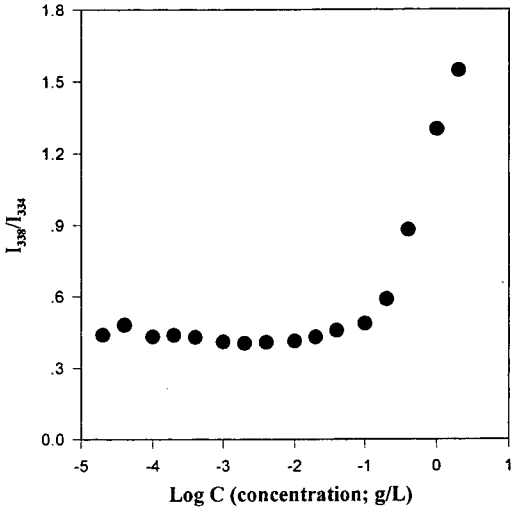

Fig. 2. Plot of the intensity ratio (I_{338}/I_{334}) from pyrene excitation spectra vs. log c of the CA/PNIPAAm in distilled water. λ_{em}=360nm, [pyrene]=6.0×10^{-7} M.

water. The solution was then freeze-dried. The prepared micelles were kept refrigerated at 4°C until use.

The CA/PNIPAAm micelle solution showed thermo-sensitive characteristics at a specific temperature range with a phase transition in optical transmittance change as shown in Fig. 1. It was measured at 500 nm with UV-VIS spectrophotometer (Shimadzu, UV-1201, Japan), and we can confirm the transition around 31.5°C, the LCST of the polymer.

The micellar structure formation of CA/PNIPAAm was determined through use of the fluorescence probe technique using pyrene by spectrofluorophotometer (Shimadzu, RF-5301PC, Japan) (Wilhelm *et al.*, 1991). To obtain sample solutions, a known amount of pyrene in acetone was added to each of a series of 20 ml vials and the acetone was evaporated. The final concentration of pyrene was 6.0×10^{-7} M. 10 ml of various concentrations of CA/PNIPAAm solutions were added to each vial and then heated for three hours at 65°C to equilibrate the pyrene and the micelles, and subsequently left to cool overnight at room temperature. The excitation spectra of pyrene were examined in the various concentrations of CA/PNIPAAm. Pyrene was preferentially partitioned into hydrophobic inner cores with a change of photophysical properties of molecules (Wilhelm et al., 1991). The intensity ratio I_{338}/I_{334} takes on the value characteristic of pyrene in water at low concentrations, and the value of pyrene entirely in the hydrophobic domain. Fig. 2 shows a plot of I_{338}/I_{334} vs. log c. This result represents a ratio that was almost flat at lower concentrations, and rapidly increases at higher concentrations. The critical micelle concentration (CMC) was taken from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points of low concentrations. The estimated CMC value was 8.9×10^{-2} g/L.

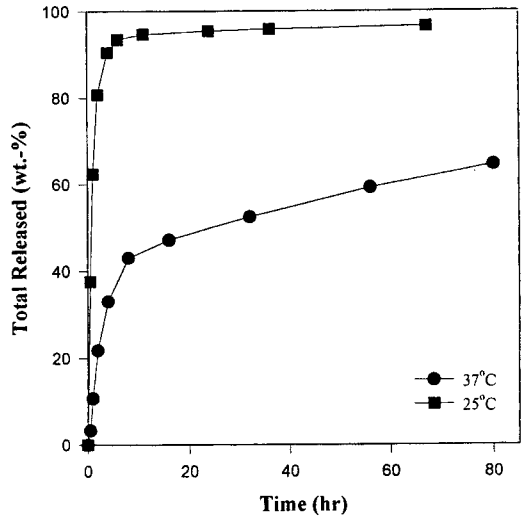

Fig. 3. Release of IN from CA/PNIPAAm micelles in PBS (0.1 M, pH 7.4) at 25 and 37°C (n=3).

The release experiment in vitro was carried out as followed: 5 mg of IN-loaded CA/PNIPAAm micelles and 1 ml phosphate buffered saline (PBS, 0.1 M and pH 7.4) were put into a dialysis tube (MWCO 12,000). The dialysis tube was then introduced into the vial with 10 ml PBS (0.1 M, pH 7.4), and the media was stirred at 100 rpm at the desired temperature. At specific time intervals, the entire medium was removed and replaced with fresh PBS. The released amount of IN from CA/PNIPAAm micelles was measured with UV-VIS spectro-

photometer (Shimadzu, UV-1201, Japan) at 312 nm. Fig. 3 shows the release kinetics of IN from the micelles of CA/PNIPAAm as a function of temperature. The release of IN was dependent on temperature, and slowly released with as increase in temperature. It is thought that the structure of PNIPAAm in the micelle was changed from an expanded form to a compact form when the temperature is raised above a critical point, around 31.5°C.

It can be expected that drug release from thermo-sensitive CA/PNIPAAm micelles may be applied to a site-specific drug delivery system by modulating the temperature of the target site.

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