PREPARATION OF HYDROGEN BONDING POLYMER STRUCTURES USING ULTRA HIGH PRESSURE TECHNOLOGY AS DRUG CARRIER

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Introduction

Polymeric assembly is a powerful tool for producing functional materials that combine properties and many respond to external conditions (1, 2). Self-assembly of amphiphilic polymers to create nano-micelle (3) or nano-gels (4) has been eagerly studied. They are formed by complex combination of weak interactions, such as hydrophobic interaction, electrostatic interaction and hydrogen bond. To fabricate self-assemble molecules or to break them by controlling these intermolecular forces, alternating concentration and/or temperature is mainly adopted. Here we have focused on pressure, which is one of the fundamental physical parameters as well as concentration and temperature, could also be used for controlling the intermolecular forces to maintain the self-assembled molecules. Pressure processing technology ranging from 1 to 100,000 atmosphere (atm) has been utilized in several fields, such as earth science, material science, food processing, chemistry and biology. In chemistry and biology, the behavior of molecules, such as proteins, artificial peptides and synthetic polymers, has been studied under high pressure condition. It was reported that pressure affected the aggregation properties of elastin, elastin-like peptide (5), poly(N-isopropylamide) (PNIPAM) (6) and poly(N-vinylisobutyamide) (PNVIBA) (7), which exhibit lower critical solution temperatures (LCSTs) derived from their hydrogen bonding and hydrophobic properties.

The strength of pressure at over 6,000 atm is thought as ultra high pressure (UHP). Under UHP condition, the fact that the hydrogen bond between inter/intra molecules is emphasized than electrostatic and hydrophobic interactions is known well (8, 9). Previously, we found that nano-, micro-particles and hydrogels of poly(vinyl alcohol)(PVA) mediated by hydrogen bonding interaction were formed by pressurization (10). Moreover, for drug delivery system, we also reported the formation of hetero-assembling of PVA and DNA using UHP technology and gene delivery into mammalian cells in vitro (11).

In this study, we have demonstrated the preparation of novel assembly via hydrogen bond by UHP treatment using various hydrogen bonding polymers, such as poly(ethylene glycol) (PEG), poly(vinyl pyrrolidone) and dextran. It is well known that aqueous two-phase separation of PEG and dextran having high molecular weights is formed because of their molecular repulsion between them. We hypothesized that the inter/intra molecular hydrogen bonding interaction of PEG and dextran could be induced by UHP processing, in which the hydrogen bonding interaction is strengthen (Figure 1). The assembly of hydrogen bondable polymers was investigated by pressurization under various conditions. The assembly of those and DNA, in which the hydrogen bonding interactions between bases are formed, was also examined by UHP treatment as one of biomedical applications.

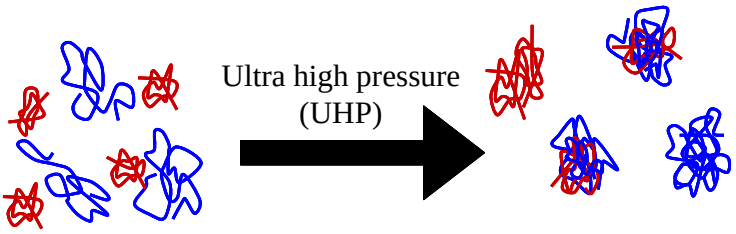

Figure 1. Illustration of polymeric assembly induced by ultra high pressure.

Experimental Part

Poly(ethylene glycol)(PEG), dextran and poly(vinyl pyrrolidone) (PVP) having various molecular weights were used (Table 1). Their chemical structures are shown in Figure 2. They were dissolved in water at concentration of 10 w/v%, respectively and then autoclaved at 121 ˚C for 10 minutes. The PEG solution was mixed with dextran solution and PVP solution at the ratio of 1 to 1, respectively. Their mix solutions were treated under ultra high pressure condition at 10000atm, 25 ˚C for10 min using high pressure machine (Dr.Chef; Kobe steal. Co. Ltd.). The obtained solution was observed by visual observation and the size of the obtained solution was measured using by dynamic light scattering (DLS) measurement. Also, the changing of their sizes during heat treatment was examined by DLS measurement in order to confirm whether the driven force of their assemblies is the hydrogen bonding interaction.

Table 1. Various polymers used.

Polymers	Mw
PEG6	6,000
PEG8	8,000
Dextran60	60,000-90,000
Dextran500	500,000
PVP40	40,000
PVP360	360,000

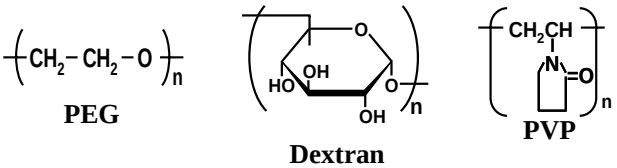

Figure 2 Chemical structures of polymers used

Results

When 10 w/v% solutions of PEG6, PEG8 and PEG35 were pressurized at 10,000 atm, 25 ˚C for10 min, respectively, the solutions were still translucent. Also, in the case of dextran60, dextran100, dextran500, PVP40 and PVP360, there was no change in visual observation of them as they were treated by UHP processing (Figure 3). When the DLS measurement of PEG6 solution and dextran60 solution with/without UHP treatment was carried out at 25 ˚C, the average diameters of PEG and dextran were approximately 4 and 8 nm, respectively, irrespective of UHP treatment. Also, no change in sizes of PEG6 and dextran60 was detected by DLS measurement at 50 ˚C (Table 2). These results indicate that the assemblies of themselves were not induced by UHP treatment. On the other hand, the two-phase separation of mixture solution of PEG6 (5 w/v%) and dextran60 (5 w/v%) was obtained by UHP treatment although the alteration of solution was not observed with mixing of them (Figure 4). The light scattering was observed in lower phase, suggesting that apparent molecular weight was increased by the formation of the assembly of PEG6 and dextran60. In order to confirm the formation of the assembly by UHP treatment, DLS measurement of the mixture solution was performed before and after UHP treatment. The average diameter of their molecules in the mixture solution was increased by UHP

treatment, indicating that the assembling of PEG6 and dextran60 by pressurization (Table 2). The diameter of the assembly was decreased by heat treatment. It suggests that the driving force of the assembly formed by UHP treatment is hydrogen bonding interaction. Similarly, the phase separation of mixture solution of PEG6 (5 w/v%) and PVP40 (5 w/v%) was formed by UHP treatment (Figure 4).

In order to investigate the assembling of PEG and dextran by UHP treatment in detail, PEG and dextran having higher molecular weights were used. The two-phase separation of PEG8 and dextran60 having light scattering in lower phase was induced by UHP treatment, while that of PEG8 and dextran500 was already obtained before UHP treatment. Also, the phase separation was already formed by mixing of PEG6 and dextran500 before UHP treatment. From these results, it was clear that the assembly tended to be formed using dextran having optimal molecular weight. It seems that the assembly could be inhibited due to the intense molecular repulsion between PEG and dextran having higher molecular weight.

When the mixture solution of DNA and PEG6 was pressurized at 10,000 atm for 10 min, the assembling of them was confirmed by agarose gel electrophoretic analysis. The assembly of DNA and dextran60 was also formed by UHP treatment. From these results, they would be utilized as a novel drug carrier.

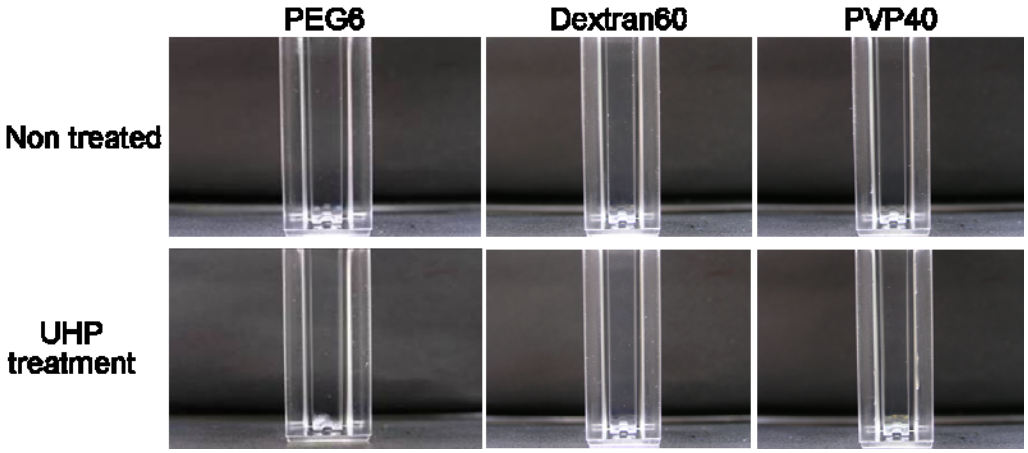

Figure 3. Photographs of polymer solutions of 10 w/v% before and after UHP treatment.

Table 2. DLS measurement of polymer solutions before and after UHP treatment.

Mixtures	Size/nm		
	Non UHP	UHP	UHP
	25℃ (nm)	25℃ (nm)	50℃ (nm)
PEG6	3.6±0.01	3.5±0.04	4.4±0.05
Dextran60	8.1±0.1	7.9±0.04	8.0±0.02
PEG6/Dextran60	112.0±16.0	140.7±7.0	109.5±3.5

* Analysis by cumulant method

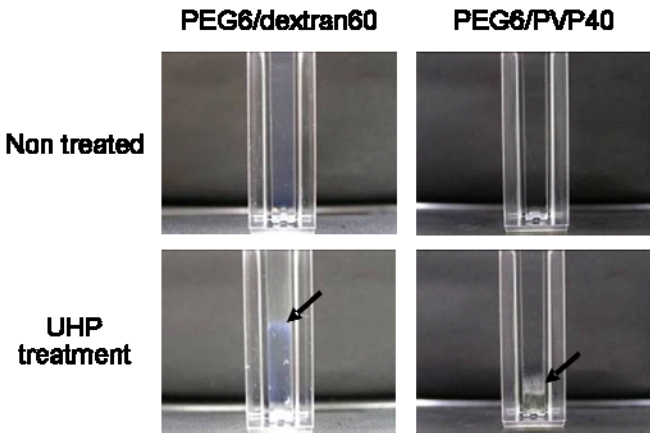

Figure 4. Photographs of mixture solutions of PEG6 and other polymers used before and after UHP treatment.

Discussion

This report described the successful formation of a novel polymeric assembly using UHP technology. DLS measurement confirm the successful assembling of PEG and dextran mediated by hydrogen bonding interaction. Also, PEG and dextran were also assembled with DNA, which has hydrophobic and hydrophilic moieties, by UHP treatment. It is expected that this methodology could be applied to build a structure by the manipulating molecular interactions to develop novel assembly.

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