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Clarification of the inner microenvironments in poly(*N*-isopropylacrylamide) hydrogels in macrogel and microgel forms using a fluorescent probe technique

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ABSTRACT

Temperature dependence of cross-linked poly(*N*-isopropylacrylamide) (PNIPAAm) gels in particle microgel and macrogel forms with different densities of crosslinking was studied by fluorescence measurements of 8-anilino-1-naphthalene sulfonic acid (ANS) doped into the hydrogels. The experiments for each sample were carried out for some weeks, by making sure that equilibrium was established at each temperature. The fluorescence intensity ratio of ANS at 460 and 540 nm was found to be useful as a measure of the polarity of the environment around ANS molecules in a gel. The results indicate that the environment of PNIPAAm chains in macrogel and microgel forms of different crosslinking densities are the same. Fluorescence depolarization of ANS first clarified that, after volume phase transition, the collapsed state of PNIPAAm gels is unlike a polymer film, but has free spaces where ANS can rotate freely because of the water molecules escaping from the PNIPAAm chains. The information of these free spaces produced by desorption of water molecules would be useful in the processing and utilization of PNIPAAm particle gels.

1. Introduction

The hydrogel of chemically crosslinked poly(*N*-isopropylacrylamide) (PNIPAAm) is a typical gel that undergoes volume phase transition (VPT). The VPT phenomenon was first discovered by Tanaka in 1978 [1]. In particular, the VPT process of PNIPAAm gels taking place during heating has been studied and utilized by many researchers since 1985 [2]. PNIPAAm itself is a temperature-responsive polymer, showing coil-globule transition [3]. These transitions are known to arise from the hydrophobic interactions between PNIPAAm segments and water. Below the VPT temperature (T_{vpt}), the amide group binds with water molecules through hydrogen bonding and the PNIPAAm chains are in an expanded hydrophilic state; the hydrogels are swollen. However, as the temperature is raised above T_{vpt}, water molecules cannot be attached by hydrogen bonding to the PNIPAAm chains, which come to interact more with each other by the inter- and intramolecular hydrophobic forces of side-chain groups of PNIPAAm; the polymer chains then go through an abrupt conformational rearrangement resulting in a collapsed hydrophobic state [4].

On the other hand, Pelton and Chibante [5] developed the preparation of crosslinked PNIPAAm particle microgels of submicrometer diameter; i.e., nearly the same as the wavelength of visible light. Because particle microgels have greater surface areas than the more usual macrogels, and the microgels in liquid solvent behave as solutions with high fluidity, PNIPAAm particle microgels are considered to be important materials responsive to temperature. Moreover, a variety of core–shell microgels composed of a cross-linked PNIPAAm and particles such as polystyrene and metal nanoparticles are prepared, characterized, and used as efficient and smart materials for nanoreacter, chemical and biological sensors, drug delivery and so on [6–13]. For example, PNIPAAm microgels containing metal nanoparticles are reported to show more efficient catalytic ability than the inherent metal nanoparticles [14–16]. The accumulation of information on the microenvironment of microgels has become more important in order to make the most of the VPT properties of PNIPAAm microgels.

The aim of the present paper is to examine whether the internal environment such as polarity of PNIPAAm macrogels and particle microgels are the same or not. This question also means addressing whether the surface and the inside of PNIPAAm hydrogels have the same polarity and mobility or not. Fluorescence quenching [17] and metal-enhanced fluorescence [18,19] have successfully been employed to observe stimuli-responsive swelling and shrinking behavior of

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microgels consisting of PNIPAAm. However, we considered that the addition of molecules whose fluorescence is strongly dependent on polarity and mobility was more efficient for our purpose, i.e., monitoring environmental changes. Thus, we applied a fluorescence probe method to give answers to the questions described above. So far, we have reviewed the efficiency of this method in some references [20,21] and applied fluorescence probe technique to some gel systems [22–25].

Concerning the application of the fluorescence probe to PNIPAAm particle gels, doping of the microgel solutions with pyrene was carried out [26–28]. Each paper succeeded in showing the results suitable for purpose, but because pyrene is so hydrophobic that it does not dissolve readily in water, it moves to the more hydrophobic area, such as the aggregated region after VPT. Thus, Matsumura and Iwai [29] reported important work on PNIPAAm gels by the introduction of phenylphenanthrene groups to the PNIPAAm chains. Probe molecules attached to polymer chain sometimes influence the microenvironments and the VPT process due to their bulkiness and/or polarity. Thus, we decided to apply another type fluorescence molecule to these PNIPAAm gel systems to examine the microenvironment in detail. Moreover, we were particular about whether the system was in equilibrium at each temperature, because the formation of the helix structure and three-dimensional network observed for isotactic polystyrene/decalin gels took more than two weeks at each temperature to attain equilibrium [30].

The probe used here was 8-anilino-1-naphthalene sulfonic acid (ANS), which was first applied to biochemical research by Weber and Laurence in 1954 [31]. It has the interesting property of fluorescing weakly in water and strongly in non-polar organic solvents or when bound to proteins. In fact, the peak intensity of ANS fluorescence increases 200 times with decreasing polarity of the solvent, while the peak wavelength is blue-shifted by 90 nm [32,33]. These drastic changes make ANS one of the most well-known fluorescent probes for detecting hydrophobic environments. In the present paper, we applied ANS to the PNIPAAm gel system, and obtained information about the microenvironments in the PNIPAAm hydrogels when the crosslinking densities of particle gels and macrogels were changed.

2. Materials and methods

2.1. Materials

Macrogels (M60, M100 and M500) and particle gels (P100 and P500) of cross-linked PNIPAAm gels were polymerized while changing the molar ratio of *N*-isopropylacrylamide (NIPAAm) to the cross-linking agent, tetra(ethylene glycol) diacrylate (TEGDA), in deionized (Milli-Q) water; i.e., M60 is the macrogel whose molar ratio of NIPAAm to TEGDA is 60:1. These gels were prepared by radical polymerization at room temperature using ammonium persulfate (APS) as a polymerization initiator and N,N,N′,N′-tetramethylethylenediamine (TMEDA) as an accelerator of producing radicals from APS: the molar ratio of TMEDA to APS was 1.6:1 for macrogels and 3:1 for particle gels, The molar fraction of APS was 1/140 or 1/400 of the total molar amounts of vinyl groups of NIPAAm and TMEDA for the synthesis of particle gels and macrogels, respectively. When particle PNIPAAm gels were synthesized, tween20, a surfactant, was added to the reactant at 1 g/mol of NIPAAm. In this case, the polymerization process takes place only in the micelle state formed by tween20, so the prepared gels are limited to a small size.

The reaction was performed by dissolving 5 or 10 g of NIPAAm in Milli-Q water (~40 mL/g NIPAAm) together with the other reagents. The reactant mixture was purged with nitrogen gas to remove oxygen, and stirred overnight. After the completion of the reaction, the reactant mixture was repeatedly dialyzed with distilled water. In the case of the microgels, the water containing particle gels was filtered using glass funnels (Sibata Scientific Technology, pore size 4–5.5 μm) after the dialysis. All the gels were freeze-dried and freeze-crushed to a powder.

2.2. Characterization of materials

Infrared absorption spectra were measured on a Perkin Elmer SPECTRUM1000 FT-IR spectrophotometer with resolution of 1 cm^{-1}. For the FT-IR measurements, gel powders were mixed with KBr and pressed to a disk form. The synthesized gels were confirmed to be of PNIPAAm by these FT-IR measurements. Moreover, it was ensured that tween20 was perfectly removed from P100 and P500.

Thermal behavior of PNIPAAm hydrogels of different concentrations was monitored by DSC measurements with a Perkin-Elmer DSC8000. Each sample was packed into large-volume capsule. The processes of volume phase transition of all the gels in water were observed by slowly increasing temperature and confirming equilibrium by visual observation. Since the objective values are also important, we measured the onset temperature at DSC with heating rate of 1 °C/min, which was a little fast to determine the equilibrium temperatures: 33.8 °C (M100), 33.7 °C (M500), 33.3 °C (P100), and 33.2 °C (P500). It is clear that T_{vpt} of a PNIPAAm gel is almost independent of the form (macrogel or particle gel) and density of crosslinking.

Scanning electron microscopy (SEM) images were obtained using a JEOL JSM-6300 after drying the PNIPAAm gel, previously swollen with Milli-Q water, on a glass plate under vacuum. Dried gel samples were stuck onto a double-sided carbon tape and coated with platinum. Fig. 1 shows an SEM image of P100 where only particle gels exist, with no macrogel networks.

We measured the particle sizes of P100 and P500 in water by using a dynamic light-scattering method, using an FPAR-1000 particle size analyzer (Otsuka Electronics Co. Ltd), and found the particle diameters of P100 and P500 to be 80 nm and 35 nm, respectively. Each half maximum of light-scattering intensities was 40–210 nm for P100 and 20–70 nm for P500.

2.3. Measurements of fluorescence and fluorescence depolarization

Fluorescence spectra, fluorescence excitation spectra, and fluorescence polarization spectra were measured on a Hitachi F-7000 spectrofluorometer. Fluorescence measurements for the gels were carried out in a 1 cm wide quartz cell with an optical path length of 1 mm for their aerated states. Each gel sample was prepared in a cell by dissolving PNIPAAm gel powder in buffer solution (pH 6.86) containing ANS at 1.10×10^{-4} mol/L, which was kept strictly the same for all measurements. We checked the fluorescence of ANS in PNIPAAm hydrogels with different concentrations, and determined to adjust the concentration of all PNIPAAm gels to 3.0 wt%. A cell was set so that the side of the cell with a width of 1 cm was irradiated by the excitation light; i.e., the cell side with a width of 1 mm was placed facing the emission detector. The cell holder was set to contact the circulating device, and the temperature of the cell was controlled with an EYELA

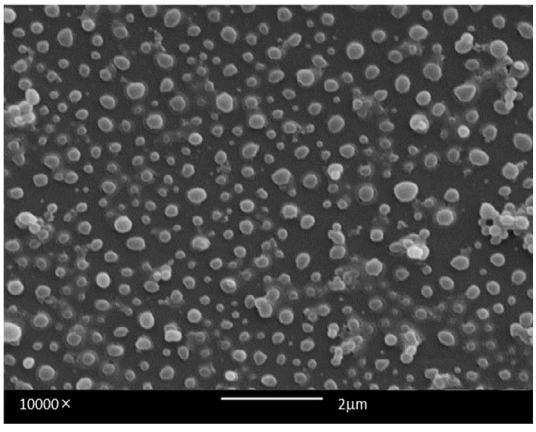

Fig. 1. SEM image of P100. The scale bar indicates a distance of 2 μm.