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A fluorescence study on the local environment of hydrogels: Double-network hydrogels having extraordinarily high mechanical strength and its constituent single-network hydrogels

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ABSTRACT

We studied the local environment of a novel double-network (DN) hydrogel and its constituent hydrogels, poly-(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) and poly-acrylamide (PAAm) gel, by fluorescence spectroscopy using a fluorescent probe. The steady-state spectra and lifetimes of the probe fluorescence in the three hydrogels were similar to those in bulk water, indicating that almost all the probe molecules reside in water pools that have local polarity similar to bulk water. The fluorescence anisotropy decay in PAMPS gel exhibits a bulk-like single exponential decay, indicating that the probe exists in sufficiently large water pools where the probe freely rotates, while it shows bi-exponential decays in PAAm and DN gels, suggesting that a part of the probe reside in confined water pools. This observation indicates that PAAm is entangled with PAMPS gel in DN gel realizing high mechanical strength, and PAAm plays the dominant role in forming the local water environment in DN gel.

1. Introduction

Hydrogels, which consist of hydrophilic polymers and water, have been attracting increasing attention in the fields such as tissue engineering [1], drug delivery systems [2], and gel chromatography. Increasing the mechanical strength of hydrogels is one of the most essential issues in producing better artificial human soft tissues. Conventional hydrogels are, in general, so fragile that they are unable to serve as substitutes for human soft tissues. Recently, a novel, double-network (DN) hydrogel was developed by combining highly cross-linked rigid polymers (first network) with flexible polymers (second network) [3]. The mechanical strength of this DN gel is as strong as that of articular cartilage.

The mechanical properties of DN gels are dependent on the properties of the constituent polymers, the molar ratios of individual monomers, and the cross-linking density. The optimal DN gel was synthesized by combining the anionic polyelectrolyte, poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS), as the first network, and the linear neutral polymer, polyacrylamide (PAAm), as the second network, with the molar ratio of PAMPS:PAAm = 1:20 (see Fig. 1 for the individual monomer structures). In spite of the high water content (~90 wt.%), the DN gel achieves surprisingly high mechanical strength compared with its constituent single-network (SN) hydrogels. For example, the fracture energy of the DN gel ranges from 100 to 1000 J/m^2, although the values for the PAMPS (~98 wt.%) and the PAAm (~88 wt.%) gels are ~1 and ~100 J/m^2, respectively. Early studies [4–8] suggested that the two constituent polymers in the DN gel associate favorably with each other, and that this association of the polymer chains may be responsible for the superior mechanical properties of the DN gel. The PAMPS gel is rigid but brittle, while the PAAm gel is soft and ductile. Thus, in DN gel, it was considered that the rigid and brittle PAMPS network can serve as a 'shock absorber' which fractures at a relatively low stress, and the soft and ductile PAAm network can act as a 'glue' that holds the overall polymer networks together. This is a model proposed to explain the molecular origin of the enhanced toughness of the DN gel [3,4,6,9,10]. In this model, however, the property of the internal water is not mentioned although the water is the predominant constituent of the hydrogels.

Because the content of water in hydrogels is as high as 90 wt.%, understanding of its property is important. In fact, different states or different types of water in hydrogels have been discussed [11]. One of the most evident facts is that there are different types of water with distinctively different thermodynamic properties observed by differential scanning calorimetry [12]. A number of dynamical studies have also been performed concerning the self-diffusion of water and transportation of solute molecules through the polymer matrix [13–16]. As for the rotational dynamics of fluorescent probe molecules in hydrogels, Eimer and coworkers

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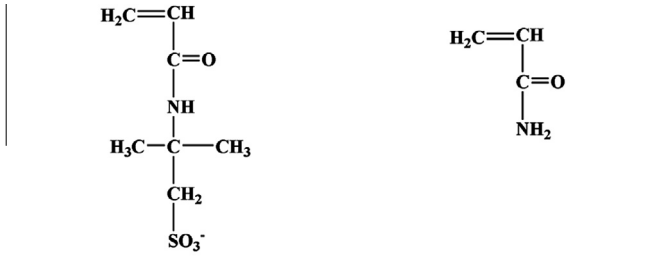

Fig. 1. Molecular structures of monomer units of PAMPS and PAAm polymers.

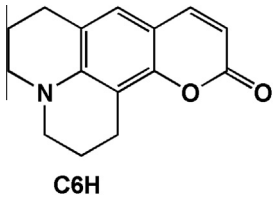

Fig. 2. Molecular structure of coumarin 6H, C6H.

examined it in PAAm gels by changing the amount of cross-linking agent and solvent for the gel (water–glycerol and water–methanol mixture) [17,18]. The aim of their work was to see how the difference of the local environment affects the local dynamics of the probe molecule. Datta and coworkers also studied the local environment of PAAm gels using the probe. They found that the rotational diffusion of the probe in the hydrogel was fast and the time constant is shorter than their instrumental response time (60 ps) [19].

The present study is an attempt to understand the local water environments in the hydrogels, in particular that in the DN gel. We performed a steady-state and time-resolved fluorescence study on the DN gel and its constituent SN gels, the PAMPS and the PAAm gel, using a polarity-sensitive probe, coumarin 6H, C6H (Fig. 2). Fluorescence spectroscopy is widely used to study local environments in a variety of systems such as polymer solution [20], copolymers [21–24], micelles [25–27], and lipids [28]. The steady-state spectrum and lifetime of the fluorescence of polarity-sensitive probe provide information of the local polarity around the probe molecules. Furthermore, time-resolved fluorescence anisotropy measurements allow us to examine the local viscosity through the rotational dynamics of the probe. To the best of our knowledge, the present paper reports the first fluorescence study of the DN gel.

2. Experimental section

2.1. Sample preparation

The procedures for preparing the hydrogels are described in the literature [29]. Briefly, the hydrogels were synthesized by the radical polymerization with irradiation of UV light. The PAMPS gel was synthesized from a 1 M aqueous solution of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) (Tokyo Chemical Industry) with 4 mol% of the cross-linking agent, *N*,*N*′-methylenebisacrylamide (MBAA) (Wako), and 0.1 mol% of the UV initiator, 2-oxoglutaric acid (Wako). The PAAm gel was synthesized from a 2 M monomer solution of acrylamide (AAm) with 4 mol% of the cross-linking agent in the presence of the 0.1 mol% initiator. The DN gel was synthesized by a two-step sequential synthesis. The first network, PAMPS, was synthesized by UV irradiation from a 1 M AMPS solution with 4 mol% of MBAA and 0.1 mol% of the UV initiator. This first network hydrogel was then immersed in a 2 M AAm solution containing 0.1 mol% of the initiator, and then this sample was again irradiated by UV light. In this case, the AAm solution was prepared without MBAA. The optimized conditions reported in the previous paper [29], i.e., the cross-linking density for the first network and initial molar ratio of AMPS and AAm (AMPS:AAm = 1:20), was used for synthesizing the toughest DN gel. The water content ratios of the resultant PAMPS, PAAm, and DN gel are ~98, ~88, and ~90 wt.%, respectively.

C6H (Sigma Aldrich) was used as received. All hydrogel samples were prepared with ~1 mm thickness, and they were immersed in saturated C6H solutions (2 μM) for one day before the measurements. The hydrogel samples were held between two glass plates separated by a 1 mm silicone spacer to prevent water evaporation during the measurements.

2.2. Fluorescence measurements

Picosecond time-resolved fluorescence spectra were measured using a streak camera (C4334, Hamamatsu). The second harmonic (400 nm) of the output from a Ti:sapphire regenerative amplifier system (Spitfire, Spectra-Physics, 1 kHz) was used for excitation. The time resolutions of the fluorescence lifetime measurement and the anisotropy measurement were determined by the sweep range adopted, and they were ~400 and ~20 ps, respectively. The lifetime measurements were carried out using the magic angle polarization condition. In the anisotropy measurement, the excitation light was set to vertical with a Glan Thompson Prism, and the parallel and perpendicular fluorescence were collected by rotating a film polarizer placed in front of the spectrometer. The polarization dependence of the detection sensitivity was corrected by tail-matching of the parallel and perpendicular fluorescence intensity at sufficiently long delay time that no further transient spectral changes were observed (i.e., 2000 ps). The steady-state fluorescence spectra were measured with a fluorescence spectrometer (Fluorolog, Horiba Jobin Yvon). All the measurements were performed at room temperature.

3. Results and discussion

3.1. Steady-state emission and fluorescence lifetime: local polarity

Fig. 3 shows the steady-state fluorescence spectra of C6H in bulk water and in the hydrogels, which were obtained with excitation at 400 nm. As seen in this figure, the fluorescence spectra of C6H in the hydrogels are almost the same as that in bulk water although a

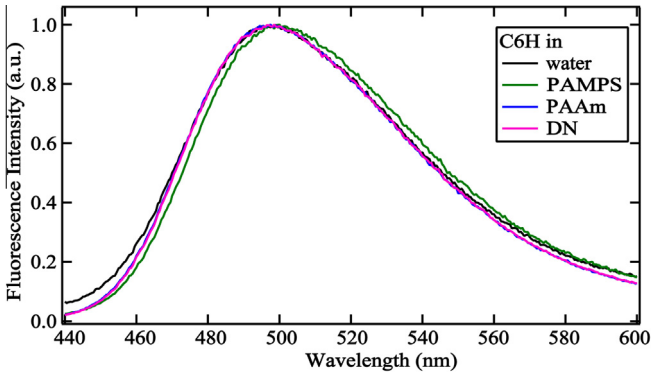

Fig. 3. Normalized fluorescence spectra of C6H in water (black), and in the PAMPS (green), PAAm (blue), and DN (pink) gels. The excitation wavelength is 400 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of article.)