A method for an approximate determination of a polymer-rich-domain concentration in phase-separated poly(N-isopropylacrylamide) aqueous solution by means of confocal Raman microspectroscopy combined with optical tweezers

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HIGHLIGHTS

- Our method can be used for the estimation of polymer concentration.
- We obtained Raman signals from polymer-rich domain formed by optical tweezers.
- We estimated the polymer concentration to be ca. 30 mM (corresponding to 31 wt%).
- Optical tweezers can control the water content in polymer-rich domain.

GRAPHICAL ABSTRACT
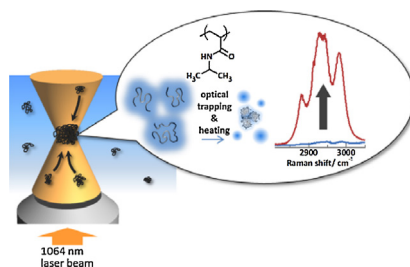
ARTICLE INFO

ABSTRACT

The paper demonstrates that a confocal Raman microspectroscope combined with optical tweezers is a promising technique to estimate polymer concentration in polymer-rich domain in phase-separated-aqueous polymer solution. The sample polymer is poly-(N-isopropylacrylamide) (PNIPAM) that is well-known as a representative thermo-responsive polymer. Optical tweezers can selectively trap the polymer-rich domain at the focal point in non-contact and non-intrusive modes. Such situation allows us to determine polymer concentration in the domain, which has been unclear due to a lack of appropriate analytical technique. It is applicable for a variety of other thermo-responsive polymers.

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1. Introduction

Thermo-responsive polymers have attracted much attention in the field of organic functional material science over the past 40 years since the first discovery of the phenomenon; thermo-responsive phase separation in solution [1–3]. Poly-(N-

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isopropylacrylamide) (PNIPAM) is a representative water-soluble thermo-responsive polymer. PNIPAM can homogeneously dissolve in water at room temperature, taking a hydrated random-coiled structure. Upon raising temperature above a lower critical solution temperature (LCST = 32 °C), the polymer exhibits coil-to-globule structural transition accompanied by dehydration of the polymer chains. The dehydrated globules aggregate with each other due to hydrophobic interactions, and consequently forming polymer-rich domains in solution (phase separation). It is reversible process. Since the phenomenon involves a wealth of physicochemical science and is applicable to biomedical engineering such as drug delivery [4], PNIPAM and related polymers have deeply been investigated by means of DSC [5,6], NMR [7,8], IR [9–11], and Raman spectroscopy [12]. It should be noted that kinetics and dynamics of such phase separation process are being recently revealed by our group using laser T-jump spectrometry[13,14] and single molecule tracking technique [15]. In spite of the past numerous studies, several important problems still remain unclear. For instance, quantitative analysis of polymer concentration (water content) in polymer-rich domains is a challenging task for deeper understanding of the phenomena. Raman microspectroscopy would be a powerful candidate for evaluating it. In this case, it is necessary to selectively obtain precise Raman spectra only of a polymer-rich domain that is micron-sized and continuously fluctuated by the Brownian motion in solution. It should be noted that in 2003 Maeda et al. reported Raman spectra of such a polymer-rich domain for a much dense aqueous PNIPAM solution (weight-average molecular weight M_w = 19000, 10–60 wt% concentration, corresponding to 6–80 mM concentration) [12]. In such solution with high concentration, polymer-rich domains naturally grew up to several tens of micrometers and adsorbed on a glass substrate to be immobilized. In such situation, it is no longer difficult to selectively access to a polymer-rich domain under an optical microscope. However, such dense solution causes entanglement of the polymer chains with each other and is far from normal dilute solution [15]. Determination of concentration of polymer-rich domain in more dilute solution is desirable and would provide new insights into the LCST behavior. For such dilute solutions (<10 wt%), we focus on a method of fixing a polymer-rich domain and to selectively measure vibrational spectra of it. Here, confocal Raman microspectroscopy combined with optical trapping technique is a powerful tool for such purpose as noted below.

A confocal Raman microspectroscope combined with optical tweezers allows us to characterize polymer-rich domain in dilute solution with high accuracy in non-contact and non-intrusive mode [16]. Optical tweezers, which uses a tightly-focused laser beam to exert radiation force at a focal point [17], have been used for trapping and manipulating of microparticles such as living cells and polymer microspheres in bioscience [18]. This technique can also trap smaller nanoparticles such as polymer chains in aqueous solution [19]. Ishikawa et al. first reported optical trapping of PNIPAM in an aqueous solution [20]. By the combination of radiation force and a photothermal effect, the technique can selectively trap a micro-assembly of polymer-rich domain at a focal point. In 2005, we have analyzed the microparticle of PNIPAM by means of confocal Raman microspectroscopy (800–3500 cm^{-1})

combined with optical tweezers [21]. We confirmed that the micro-assembly formed by the method is composed of a globular-state of PNIPAM.

In the present work, we reveal the concentration of polymer-rich domain formed in dilute PNIPAM solution (3.6, 7.5, and 18 wt% corresponding to 2.5, 5.4, 15 mM, respectively). This method can be expanded to determine concentration in polymer-rich domain for other thermo-responsive polymer chains.

2. Materials and methods

An atactic-like PNIPAM was synthesized by RAFT (reversible addition-fragmentation chain-transfer) polymerization in the same manner as reported previously [22]. Weight-average (M_w) and number-average molecular weight (M_n) of the synthesized polymer chains were determined using size-exclusion-chromatography to be 15,000 and 10,000, respectively. This polymer was dissolved in distilled and deionized water (Advantec, AQUARIUS GSR-200) to be concentration of 2.5–270 mM (3.6–80 wt%).

Optical set-up of a confocal Raman microspectroscope (slit width 0.09 mm, resolution 6 cm^{-1}) (Tokyo Instruments, Inc., Nanofinder®) combined with optical tweezers has already been reported in our previous literatures [23,24]. A continuous-wave (cw) Ar$^+$ laser (λ = 488 nm, Coherent, Inc., Innova 70) was used for Raman excitation light source, while cw Nd^{3+}: YAG laser (1064 nm, Spectron Laser Systems Ltd., SL-902T) was used both for water-heating and optical trapping. These laser beams were co-axially focused into aqueous PNIPAM solutions with an oil-immersion objective lens (×100, N.A. = 1.30, CFI Plan Fluor) of an inverted microscope (Nikon Co., Ltd., Eclipse TE300). The confocal arrangement with a pinhole (100 μm) enables us to obtain spatially-resolved optical spectra only for a focal position. Raman signals was diffracted with 1200 grooves/mm of a grating and detected with a cooled CCD (Andor Tech.). Laser powers (after passing through the objective lens) were measured using a power meter (Ophir Optronics, NOVA). Ar$^+$ laser power was 5.0 mW at the sample position and the exposure time for each Raman measurement was set at 1800 s for one accumulation in this study. For polymer-rich domains formed by optical tweezers, the exposure started after 10 min of YAG laser irradiation. We have already confirmed that the Raman spectra were almost constant over 10 min of YAG laser irradiation. Namely, the polymer-rich domain reached to an equilibrium state after 10 min of YAG laser irradiation. To produce a standard curve of Raman scattering intensity versus PNIPAM concentration in aqueous solution, Raman microspectroscopy for PNIPAM solutions at each concentration was also carried out without optical tweezers.

3. Results and discussion

As a representative example, Fig. 1 shows optical micrographs of a PNIPAM aqueous solution (2.5 mM) under irradiation of YAG laser (0.19 W). Starting YAG laser irradiation, a microparticle was observed at the focal point (irradiation time t = 1.0 min). With irradiation time, the size of the microparticle gradually increased and it became constant (diameter 20 μm at t = 30 min). The
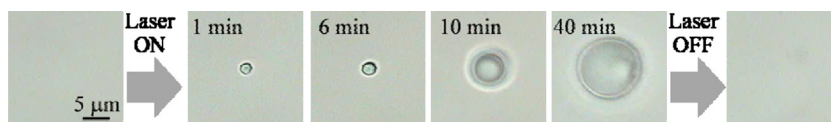
Fig. 1. Optical micrographs of a single polymer-rich domain formed by optical tweezers. A sample is an aqueous PNIPAM solution (2.5 mM corresponding to 3.6 wt% concentration). YAG laser power is 0.19 W. Times in the micrographs express irradiation time of YAG laser.