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内　容　の　要　旨

The detection of synthetic polymers in a crowding solution has attracted keen interest in biomedical applications. It has been considered that the first event after an artificial material is inserted into a living body is the interaction with proteins. If the protein and the synthetic polymer form an association, the diffusion of synthetic polymers will change. One of the commonly used techniques for polymer diffusion is dynamic light scattering (DLS), which is a family of photon correlation spectroscopy (PCS). DLS has often been employed to measure the particle size of colloidal submicron-size particles. When submicron-size particles are dispersed in solution and their sedimentation is negligible, the fluctuation of scattered light is the result of the Brownian motion of particles. If the scattering contrast of a particle is enough high, the constituents can be organic, inorganic, or any mixture of them. Therefore, DLS has become a powerful tool for monitoring the diffusion dynamics in a colloidal suspension. This versatility, however, makes it difficult to measure a target in a crowding circumstance. DLS may detect signals from all scatterers. Moreover, it is difficult to observe the scattered light of small particles when large particles coexist because the scattering intensity is much emphasized for a large particle.

If we could label the target with a fluorescent chromophore, fluorescence correlation spectroscopy (FCS) enables us to monitor the diffusion even in a crowding solution, since FCS detects only the fluctuation of the fluorescent light. This thesis shows the application of FCS for monitoring a single-polymer diffusion in a crowding solution. The aqueous solution of poly(*N*-isopropylacrylamide) (PNiPAm) is selected as the test case, where phase separation occurs by heat. In the vicinity of the phase separation, the polymers gather toward a macroscopic phase change. DLS cannot monitor the single-polymer diffusion in this process

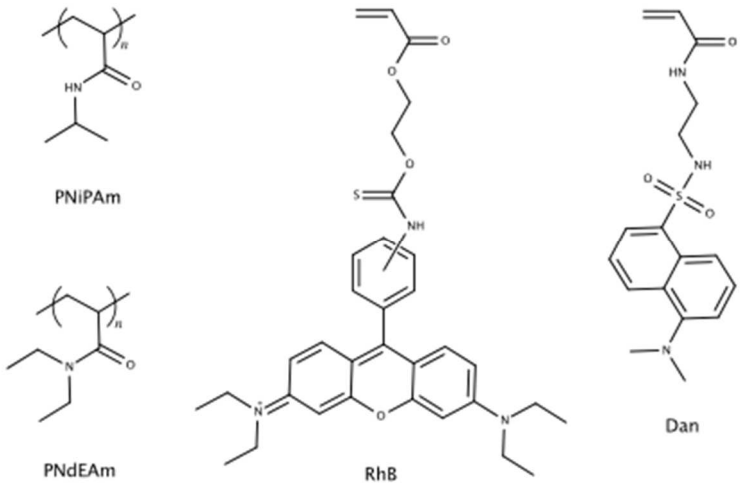

Figure.1 Chemical structures of PNiPAm, PNdEAm, RhB, and DAN.

because of the large contrast between the aggregate and the unimer.

Fluorescence-labeled PNiPAm and poly(*N,N*-diethylacrylamide) (PNdEAm) with various meso diad (*m*) contents were synthesized according to the literacture. Chemical structures of PNiPAm and PNdEAm homopolymers are shown in Figure 1. The molecular weight and the tacticity of polymers are controlled by reversible addition-fragmentation chain transfer (RAFT) polymerization with a Lewis acid catalyst. *N*-[2-[[[5-(Dimethylamino)-1-naphthalenyl]sulfonyl]amino]ethyl]-2-propenamide (DAN) was labeled to monitor the change in the microenvironment around the polymer chain in water. Acryloxyethyl thiocarbamoyl rhodamine B (RhB) was employed for FCS measurement. The molar ratio of DAN or RhB to the monomer in preparation is 1×10^{-3}. The polymer products were recovered by freeze-drying after dialysis to water. The number-averaged molecular weight (M_n) and polydispersity (M_w/M_n) of the prepared samples were determined by size exclusion chromatography

(SEC; Intelligent HPLC system). The eluent was *N,N*-dimethylformamide (LiBr 10 mM) at 60 °C with a flow rate of 0.35 mL min^{-1}. ^{1}H NMR spectra were recorded on a JEOL (Tokyo, Japan) JNM- LAMBDA spectrometer (500 MHz). The tacticity of the samples was represented by the *m* content, which was determined from the methylene proton peaks of the polymer measured in DMSO-d_6 at 145 °C.

FCS measurement was carried out by a homemade apparatus with a confocal optical setup with a cross-correlation arrangement. The avalanche photodiode detectors (H10682-110, Hamamatsu photonics) were used with Flex02- 12D/C digital correlator (correlator.com). The emission signal was collected after splitting the signal using a dichroic mirror (#86-334, Edmund), and filtering with a long-pass filter (#49- 027, Edmund). The fluorescence species were excited by a YAG laser at λ = 532 nm. FCS measurement was performed stepwise at each temperature with an equilibration time of at least 30 min^{-1}. It should be equivalent with a scanning rate of less than 0.1 ° C min^{-1}. For the FCS measurement, we chose RhB, because the emission wavelength and intensity are stable during the phase change of the aqueous solution of PNiPAm.

The FCS profiles were analyzed by fitting the following fitting function,

$$g(\tau) = 1 + \frac{1}{<N>}\left\{ f\left(\frac{1}{1+\tau/\tau_{D,1}}\right)\left(\frac{1}{1+(1/s)^2\tau/\tau_{D,1}}\right)^{1/2} + (1-f)\left(\frac{1}{1+\tau/\tau_{D,2}}\right)\left(\frac{1}{1+(1/s)^2\tau/\tau_{D,2}}\right)^{1/2} \right\} \quad (1)$$

where $\tau_{D,n}$ (n = 1, 2) represents the relaxation time of diffusants, s is the aspect ratio of the confocal volume, $<N>$ is the average number of the fluorescent dye molecules in the confocal volume element, and f is the fraction of the first component. The radial and axial dimensions of the confocal volume are represented by w_I and w_Z, respectively. For determining s ($=w_Z/w_I$), Rhodamine 6G (diffusion coefficient, D = 4.14 × 10^{10} m^2 s^{-1} at 25 °C) was used.

The fluorescent shift of the DAN probe reveals that the temperature range of dehydration for PNiPAm chains is much narrower than that for PNdEAm. The sharp dehydration of polymer chains may give rise to the characteristic thermoresponsive behavior of PNiPAm in water. For meso-rich PNiPAms, the dehydration point (T_{dh}), which is defined as the temperature where the single chains start assembling in the solution, locates far from the cloud point (T_c). That is, the dehydration of the chain occurs antecedently before the system undergoes a macroscopic liquid-liquid phase separation (LLPS). For PNdEAm, however, the dissociation between T_{dh} and T_c is not found. For the aqueous solution of PNiPAm with 52 % of the *m* content

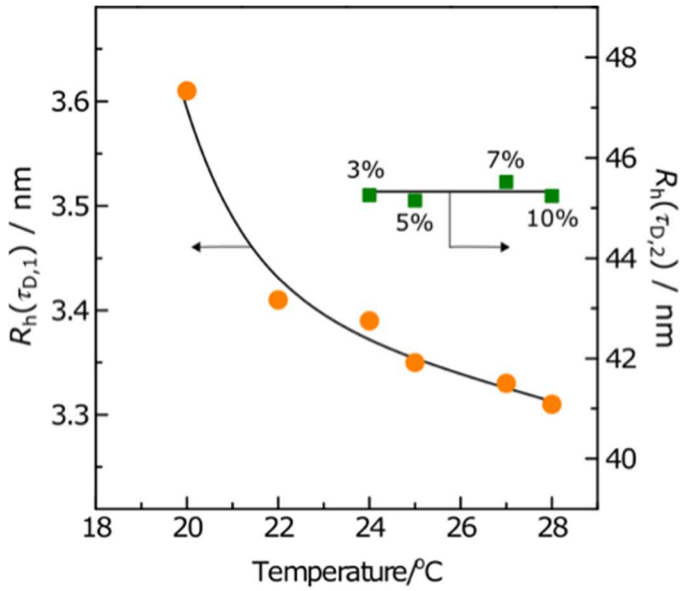

Fig.2 Temperature dependence of R_h values of *i*-52r

(*i*-m52), we carried out FCS measurement. The concentration of the polymer solution was adjusted to 0.1 wt% by mixing RhB-labeled *i*-52r and unlabeled *i*-52 to control the number of chromophores for FCS measurements. This makes it possible to monitor the single-polymer diffusion under a crowding condition.

The temperature dependence on R_h of diffusants is shown in Figure 2. Below 24 ° C, PNiPAm *i*-52r exists as a single chain, and then its R_h decreases with increasing temperature. When the temperature closes to T_{dh} (ca. 25 ° C), a droplet with *ca.* 45 nm is formed. This result suggests that a droplet having R_h ~ 45 nm is formed at around T_{dh} and increases its population until T_c. That is, nano-order droplets are stabilized in the intermediate state between T_{dh} and T_c. The existence of the nano-order droplets should be related to the acute dehydration of PNiPAm in the vicinity of the macroscopic LLPS. The sharp dehydration of PNiPAm chains may enable an acute condensation of polymers in droplets, causing a viscoelastic hindrance in the coalescence of droplets.

審査の結果の要旨

本論文は，生物学的相分離で観測される液滴成長阻害の要因を明らかにするために合成高分子水溶液系をモデルとして用い，高分子の脱水和挙動とサブミクロンサイズの液滴安定化の関連を明らかにしたものである．蛍光相関分光（FCS）計を構築し，合成高分子の単一拡散挙動を夾雑溶液化で検出できるようにしたことは，特筆に値する．

具体的には，立体制御を行なったpoly(N-isopropyl acrylamide) (PNiPAm)の水溶液中における脱水和挙動を蛍光プローブ法を用いて明らかにし，曇点近傍の夾雑環境において単一分子鎖の拡散挙動をFCS法いよって観測可能にした．PNiPAmの相分離挙動はその特異性から長年研究されており，立体制御の効果や曇点以下での高分子鎖の拡散挙動などその報告は多岐に渡る．しかしこれまで，サブマイクロスケールの凝集体が形成される曇点近傍の高分子鎖の拡散挙動は測定が困難であった。これに対し申請者は，蛍光標識されたもののみの拡散物質を調べることのできるFCS法に着目し，合成高分子溶液系に応用した．対照実験としてマクロスケールまで液滴成長が停止しない poly(*N,N*-dietylacrylamide) (PNdEAm)水溶液系との比較実験を行なっている．

得られた主な結果は以下のように要約できる．

(1) PNiPAm鎖の脱水和はPNdEAmに比べて非常に狭い温度範囲で起こり，脱水和が温度に対して急激に進行する．

(2) 立体規則性がイソタクチックに寄っていくと PNiPAmの脱水和温度とポリマー水溶液の相分離温度が解離することを明らかにした．

(3) FCSを用いて相分離温度近傍の夾雑環境下で，濃厚ドロプレットの成長が抑制される安定な中間状態が存在することを明らかにした．

これらのことからドロプレットの成長が抑制されるのは，高分子鎖の急激な脱水和が要因であることが示された．本研究は，細胞生物学で脚光を浴びる液液相分離とドロプレット安定化機構を，非生物由来の物質によって解明した重要なものであり，生物学的相分離のメカニズムに対して新たな知見を与えたと評価できる．また，申請者が自立して研究活動を行うのに必要な能力と学識とを有することを証したものである．