Visualization and Optical Trapping of an Individual Submicrometer-Sized Assembly in Aqueous Solution: Aminated Polyethylene Glycol (PEG-A) Complexed with Palmitic Acid and DNA in Poly(ethylene glycol) (PEG) Solution

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Abstract: The present study reports the visualization and optical trapping of an individual submicrometer-sized assembly from synthetic polymers using a laser. The assembly was made of PA (palmitic acid) and PEG-A (amino pendant-containing poly(ethylene glycol)) labeled with FITC (fluorescein-5-isothiocyanate type I) at the ratio of PEG-A:FITC = 10:1. The individual submicrometer-sized assemblies were detected at a concentration 50 times lower than the critical aggregation concentration of the PEG-A/PA assembly expected by surface tension measurement. In addition, submicrometer-sized particles were prepared from individual DNA molecules, T4 DNA (166 kbp), in a poly(ethylene glycol) solution. These synthetic assemblies were successfully trapped and transported using a laser, as well as DNA particles. Finally, laser manipulation is shown to be an effective method to judge whether the pair interaction of submicrometer-sized objects is attractive or repulsive.

Manipulation at the molecular level of individual chemical and biological substances in solution is currently a challenging target in physics, chemistry, and biology. In particular, direct observation and manipulation of individual objects is helpful in determining the physical and chemical characteristics of the object. Even when the size of the macromolecules is much smaller than the wavelength of visible light, they can be visualized by staining with a suitable fluorescent dye.[1] Thus far, direct observations of the dynamics and the conformation of biopolymer in solution using fluorescent microscopy have yielded several interesting findings. For instance, conformational change in individual polymers such as DNA molecules has been studied in both the presence and absence of shear flow, and the dynamics of individual DNA in an aqueous solution have been measured in real time.[2−4] In addition, the interaction of DNA and proteins such as RNA polymerase was elucidated, where RNA polymerase bound to a fluorescent dye was visualized via the motion of sliding along a DNA chain.[5] Moreover, various mobilities of individual DNA molecules, depending on the molecular weight in agarose gel, have been studied extensively, demonstrating that DNA macromolecules were alternately contracted and lengthened as they moved and formed a U-shape for extended periods.[6,7] Furthermore, the elasticity and tension of DNA and various proteins were measured by trapping micrometer-sized beads that were attached to the molecules using a laser.[8−11]

In contrast to the recent increase in the number of optical microscopic studies on biopolymers, few reports have examined the visualization of individual synthetic polymers in a solution. This may be due to the difficulty in visualizing individual synthetic polymers because of the smaller contour length compared to DNA and other biological macromolecules. Studies on the hydrodynamic property of synthetic polymers have been carried out primarily by light scattering and spectral analysis. These methods afford information on the physicochemical characteristics of the ensemble of the polymer chains. Scanning probe microscopy has been extensively applied to the measurement of the conformation of individual polymer chains.[12] Although this tool is very useful for the direct observation of

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(1) Morikawa, K.; Yanagida, M. *J. Biochem.* **1981**, *89*, 693−700.
(2) Matsumoto, S.; Morikawa, K.; Yanagida, M. *J. Mol. Biol.* **1981**, *152*, 501−516.
(3) Matsumoto, M.; Sakaguchi, T.; Kimura, T.; Doi, M.; Minagawa, K.; Matsuzawa, Y.; Yoshikawa, K. *J. Polym. Sci. B: Polym. Phys.* **1992**, *30*, 779−783.
(4) LeDuc, P.; Harber, C.; Bao, G.; Wirtz, D. *Nature* **1999**, *399*, 564−566.
(5) Kabata, H.; Kurosawa, O.; Arai, I.; Washizu, M.; Margarson, S. A.; Glass, R. E.; Shimamoto, N. *Science* **1993**, *262*, 1561−1563.
(6) Smith, S. B.; Aldridge, P. K.; Callis, J. B. *Science* **1989**, *243*, 203−206.
(7) Schwartz, D. C.; Koval, M. *Nature* **1989**, *338*, 520−522.
(8) Arai, Y.; Yasuda, R.; Akashi, K-i.; Harada, Y.; Miyata, H.; Kinosita, K., Jr.; Itoh, H. *Nature* **1999**, *399*, 446−448.
(9) Perkins, T. T.; Quake, S. R.; Smith, D. E.; Chu, S. *Science* **1994**, *264*, 822−826.
(10) Smith, S. B.; Finzi, L.; Bustamante, C. *Science* **1992**, *258*, 1122−1126.
(11) Veigel, C.; Bartoo, M. L.; White, D. C.; Sparrow, J. C.; Molloy, J. E. *Biophys J.* **1998**, *75*, 1424−1438.

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the detailed morphology of polymer chains and their assemblies at high resolution, scanning probe microscopy does not easily afford direct information on the conformation of polymer chains exhibiting free thermal motion in bulk solution. Recently, in addition to DNA molecules,[13,14] synthetic polymer assemblies have been visualized using fluorescence microscopy, and bulk manipulation using a laser has also been successfully performed.[15,16] A single DNA molecule could be trapped by a laser without a micrometer-sized bead only when its conformation takes on a compact state upon the addition of a suitable condensing agent.[14] In addition, focusing a laser beam into a polymer solution induces polymer assemblies and such assemblies can be trapped in solution.[15,17] However, the methodology on the direct observation and manipulation of an individual synthetic polymer assembly has not been well developed yet.

The present study reports successful experimental results of direct observation and laser manipulation of individual submicrometer-sized assemblies from synthetic polymers. The synthetic polymer assembly used in this study consisted of PEG-A (amino pendant-containing poly(ethylene glycol)) labeled with FITC (fluorescein-5-isothiocyanate type I) and PA (palmitic acid). The assembly was trapped and successively transported by a YAG laser. In addition, laser manipulation is shown to be an effective method for diagnosing whether the pair interaction of submicrometer-sized objects is attractive or repulsive.

Experimental Methods

FITC-Labeled PEG-A. A polymer assembly system made of PEG-A[18] (M_n = 3900) and PA (Wako Pure Chemical Industries, Ltd.) was selected as the subject material in the present study. The average number of amino groups in PEG-A was 8.28 per polymer chain. PEG-A was fluorescently labeled with FITC (Molecular Probes) as follows: FITC (1.93 mg) in methanol (350 μL) was added dropwise with stirring to PEG-A (198 mg) dissolved in 1 mL of water (mole ratio of FITC/PEG-A = 0.1). After the mixture was stirred for 24 h, the solution was gel-filtered twice using a Sephadex G-50. The polymeric product was then collected and freeze-dried to form a syrup (159 mg). The elution profile, monitored by UV detection, showed that almost all the FITC molecules were covalently bound to the amino residues. The molar ratio of FITC to NH_2 was quite small (1 to 80). Thus, the FITC is expected to have a negligibly small effect on the polymer assembly formation.

Polymer Complex of PEG-A/PA. PEG-A labeled with FITC was then dissolved in water together with 10 mM PA at a molar ratio of NH_2/COOH = 2. The concentration of the stock solution of PEG-A/PA was [NH_2] = 20 mM. The PEG-A/PA complex solution was diluted in a 20 mM Tris-HCl buffer (pH 8.5) for each experimental condition. A 20 mM PEG-A solution containing no PA was also stocked as a control.

Microscope System. A fluorescence image of the assembly was obtained using a Carl Zeiss microscope, Axiovert 135 TV, equipped with a 100× oil-immersion objective lens (NA: 1.3) and with a highly sensitive Hamamatsu SIT TV camera, as shown in Figure 1. A Nd:YAG laser (1064 nm, ca. 180 mW) was used for optical trapping. The laser beam was expanded to 7.5 mm in diameter and was introduced into the microscope through mirrors and a dichroic mirror. The beam was focused on the focal plane of the object under fluorescence microscopic observation. Individual assemblies could be observed with the blurring effect, even when the size of the assembly was less than the wavelength of the observation light.

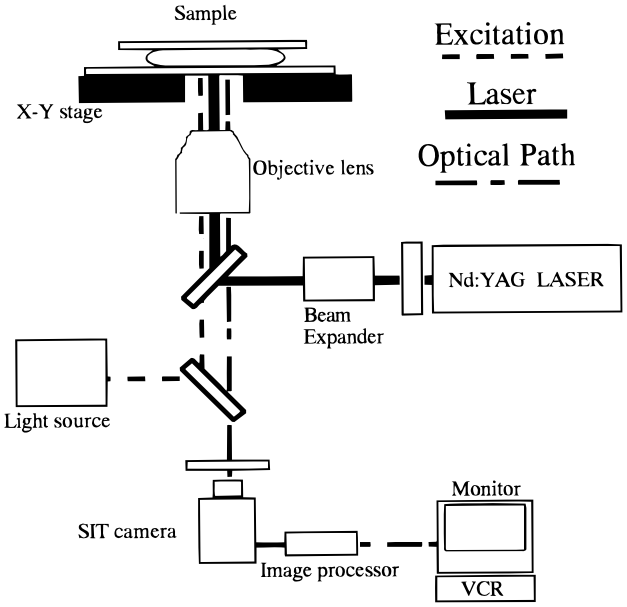

Figure 1. Microscope system used for the optical trapping.

Preparation of the Compacted DNA. PEG (average molecular weight 6000, from Nihon Oils and Fats Co Ltd.) was dissolved in a Na-phosphate buffer (pH 7.2) and mixed with T4 phage DNA (Nippon Gene), DAPI (4′,6-diamidino-2-phenylindole, Wako Pure Chemical Industries, Ltd) as a fluorescent dye, and 2-ME (2-mercaptoethanol) as an antioxidant. The final concentrations were as follows: 20 mM Na-phosphate buffer, 0.6 μM DNA in nucleotide, 0.6 μM DAPI, 2%-(v/v) 2-ME, 60 mg/mL PEG, and 50 mM $MgCl_2$.[14] In an aqueous solution without condensation agents, DNA stained with the fluorescent dye is observed as an elongated coil. Upon addition of PEG and $MgCl_2$, the DNA undergoes domestic conformational transition, transforming into a tightly compacted state.[19] The DNA chain is expected to take on a hexagonal closed packed structure.[20,21] The hydrodynamic radius of the compacted DNA was found to be approximately 60 nm based on the measurement of the Brownian motion by fluorescence microscopy.

Electron Microscopic Measurement. A sample for electron microscopy examination was prepared at [NH_2] = 0.2 mM and mounted on carbon-coated copper grids (No. 200). The sample was then negatively stained with 1% uranyl acetate and was observed by using a JEOL 1200 EX transmission electron microscope (Tokyo, Japan) at 100 kV.

Surface Tension Measurement. The PEG-A/PA mixture was placed in a capillary. A sample (5 mL in volume) was dripped and the number of droplets was counted. Measurement using distilled water was also performed. The surface tension was estimated from the surface tension of distilled water (72.28 dyn/cm at 23 °C) determined from the ratio of the number of droplets of distilled water to that of the sample.

Results

To check the existence of polymer assembly produced from PEG-A/PA, the mixture of PEG-A labeled with FITC and PA was investigated via fluorescence microscopy. Figure 2b shows

(12) Kumaki, J.; Nishikawa, Y.; Hashimoto, T. *J. Am. Chem. Soc.* **1996**, *118*, 3321−3322.
(13) Chiu, D. T.; Zare, R. N. *J. Am. Chem. Soc.* **1996**, *118*, 6512−6513.
(14) Katsura, S.; Hirano, K.; Matsuzawa, Y.; Yoshikawa, K.; Mizuno, A. *Nucleic Acid Res.* **1998**, *26*, 4943−4945.
(15) Borowicz, P.; Hatta, J.; Sasaki, K.; Masuhara, H. *J. Phys. Chem. B* **1998**, *102*, 1896−1901.
(16) Gensch, T.; Hofkens, J.; van Stam, J.; Faes, H.; Creutz, S.; Tsuda, K.; Jerome, R.; Masuhara, H.; De Schryver, F. C. *J. Phys. Chem. B* **1998**, *102*, 8440−8451.
(17) Sasaki, K.; Shi, A.-Y.; Kopelman, R.; Masuhara, H. *Chem. Lett.* **1996**, 141−142.
(18) Koyama, Y.; Umehara, M.; Mizuno, A.; Itaba, M.; Yasukouchi, T.; Natsume, K.; Suginaka, A.; Watanabe, K. *Bioconjugate Chem.* **1996**, *7*, 298−301.
(19) Minagawa, K.; Matsuzawa, Y.; Yoshikawa, K.; Doi, M.; Khokhlov, A. R. *Biopolymers* **1994**, *34*, 555−558.
(20) Maniatis, T.; Venable, J. H., Jr.; Lerman, L. S. *J. Mol. Biol.* **1974**, 84, 37−64.
(21) Noguchi, H.; Saito, S.; Kidoaki, S.; Yoshikawa, K. *Chem. Phys. Lett.* **1996**, 261, 527−533.

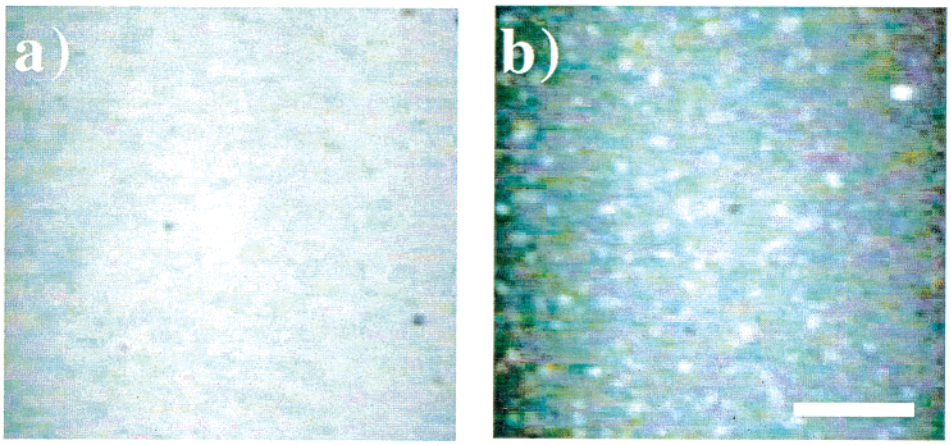

Figure 2. Fluorescence images of (a) FITC labeled PEG-A solution at $[NH_2] = 0.2$ mM and (b) PEG-A/PA assembly at $[NH_2] = 0.2$ mM. The scale bar indicates 10 μm.

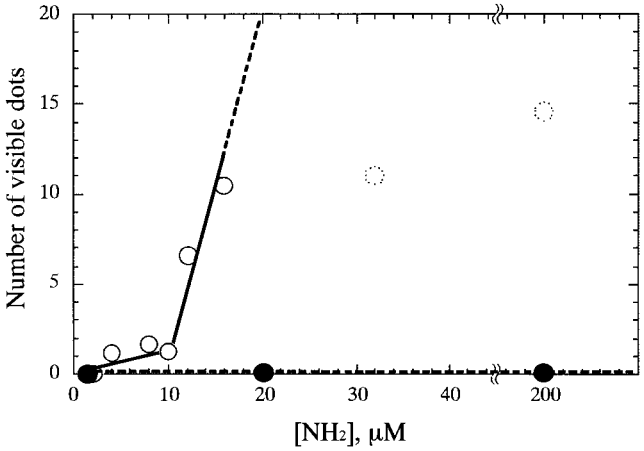

Figure 3. Dependence of the number of visible objects derived from PEG-A labeled with FITC (closed circle) or PEG-A/PA (open circle) with respect to the polymer concentration in the solution, excluding those attached on the glass surface by fluorescence microscopy. Before observation, each sample was diluted from a stock solution and equilibrated to room temperature (24 °C) for 10 min. Observable objects in a 600 μm^2 area having a thickness of 10 μm were counted.

a fluorescence image of the polymer assembly of the PEG-A/PA at $[NH_2] = 0.2$ mM. White dots indicate the fluorescence images of the PEG-A/PA assembly. These bright dots exhibited significant Brownian motion in the solution. In contrast, no optical dots were observed in the FITC-labeled PEG-A solution at $[NH_2] = 0.2$ mM, and only fluorescent background was seen (Figure 2a). We also confirmed that there were no bright dots in a 20 mM Tris buffer (pH 8.5). The fluorescent dots were thus attributed to the polymer assemblies from PEG-A labeled with FITC and PA.

Figure 3 shows the relationship between the number of visible fluorescent spots in the solution and the concentration of PEG-A/PA, as represented by the concentration of amino residue. No bright dots were detected at less than $[NH_2] = 2$ μM. Only a few luminous dots became visible with increased amino residue. The number of visible dots increased abruptly at $[NH_2] = 12$ μM. Around this concentration, most of the dots exhibited significant Brownian motion. Above $[NH_2] = 20$ μM, the visible objects attached to the cover slips increased, whereas the number of objects in the bulk solution remained almost constant (see the broken circle in Figure 3). The FITC-labeled PEG-A solution was also observed (Figure 3, closed circle) up to $[NH_2] = 0.2$ mM, but no bright dots were detected.

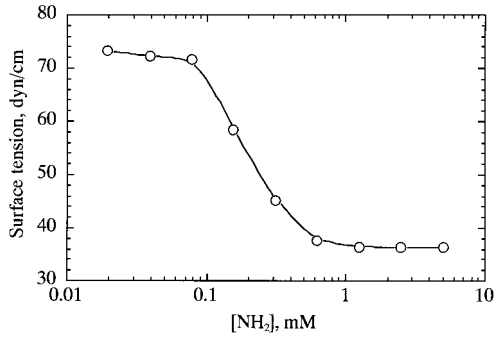

Figure 4. Surface tension of PEG-A/PA solution depending on the concentration of the polymer (at a molar ratio of NH_2/COOH = 2).

The critical assembly concentration of the PEG-A/PA was also evaluated using the surface tension method (Figure 4). The surface tension began to diminish at $[NH_2] = 80$ μM and became almost constant at $[NH_2] = 600$ μM. According to the conventional theory for cmc (critical micelle concentration),[22] the critical assembly concentration would be 600 μM, indicating that the macromolecular system starts to assemble at a much lower concentration than that expected by the surface tension measurement for cmc.

Next, we evaluated the actual size of the polymer assembly of PEG-A/PA. The image size of the assemblies observed by fluorescence microscopy is always larger than their actual size, due to the blurring effect from the resolution limit of the optical wavelength and the high sensitivity of the SIT camera. On the basis of the measurement of the Brownian motion for the individual assemblies using fluorescence microscopy, the hydrodynamic radius of the assembly can be estimated using the Stokes−Einstein relation, which is applicable to spherical particles.[23] On the basis of the measurement of the mean square displacement of the center of mass for the individual polymer assembly, the hydrodynamic radius of the polymer assembly at $[NH_2] = 20$ μM is evaluated to be 38 ± 11 nm. In addition, the morphology of the polymer assembly was observed using electron microscopy. Figure 5 shows an example of an electron micrograph at $[NH_2] = 0.2$ mM. Several kinds of specific morphology are observed for this condition. One typical morphology is composed of small and elliptic assemblies that are approximately 30 nm in length along the long axis (Figure

(22) Martin J. *Surfactant Science Series*; Dekker: New York, 1987; Vol. 23, pp 109−183.

(23) Doi, M.; Edward, S. F. *The Theory of Polymer Dynamics*; Clarendon, Oxford, 1986.

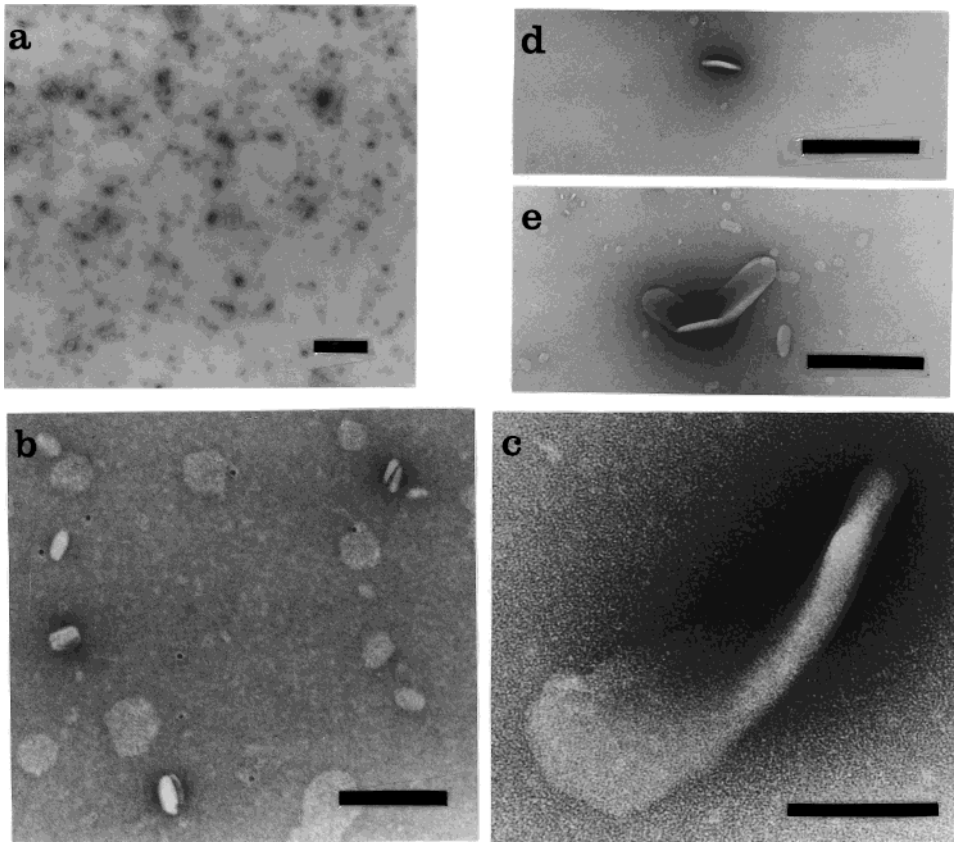

Figure 5. Transmission electron micrographs of PEG-A/PA assembly at $[NH_2] = 0.2$ mM: (a) morphology of PEG-A/PA; (b) Small assembly; (c, d) rod- or ribbon-like structure; and (e) ribbon-like structure. Scale bars indicate 100 and 500 nm for parts b, c and parts a, d, e, respectively.

5a,b). Another typical morphology is a rod- or ribbon-like structure having a length of approximately 200 nm (Figure 5c,d). Elongated morphology is also found (Figure 5e). These structures could be observed neither with PEG-A alone nor with Tris buffer in the absence of polymers.

Trapping of the polymer assembly at $[NH_2] = 20$ μM was performed using a Nd:YAG laser (1064 nm, ca. 180 mW).[14] When the laser was switched on, the complex fluctuating around the point at which the laser focused was pulled into the laser focus site. It has been reported that H_2O absorbs an incident 1064 nm laser beam, which might lead to temperature elevation of the sample solution. In our optical system, the laser incident is focused in the optical system so that the trapped assembly is fixed at the same location.[14] Figure 6 shows the transportation of the assembly by the laser. The trapped assembly is indicated by the white arrow, and the other assembly is attached on the glass surface (Figure 6a,a′). In moving the stage from left to right, the attached assembly on the glass moved in the same direction, and only the trapped assembly remained at the focused point (Figure 6b,b′). Similarly, upon moving the stage downward, the attached assembly was transported (Figure 6c,c′ and Figure 6d,d′).

We attempted to bring the targeted assemblies into contact with each other and collapse them by laser manipulation. Figure 7 shows the trapping and collision of the PEG-A/PA assemblies (Figure 7a−c), as well as those for the compacted DNAs (Figure 7d−f). The PEG-A/PA assembly or compacted DNA was led into the focused point of the laser and then moved to another PEG-A/PA assembly or compacted DNA that was trapped, whereupon contact occurred, as shown in Figure 7b and Figure 7e, respectively. Upon turning off the laser power, the two systems exhibited markedly different behavior. The PEG-A/PA assemblies separated by thermal agitation (Figure 7c), but the stuck DNAs remained as a single object (Figure 7f), indicating the repulsive and attractive interactions between the particles, respectively.

It is known that the temperature of the trapped object is raised due to the absorption of the incident 1064 nm laser beam by H_2O.[24,25] However, we have noted that, during the laser trapping, the polymer assembly and the compacted DNA do not exhibit any change in the fluorescence image, such as a collapsion and decollapsion. Thus, we think that this photothermal effect has a negligible influence on the experimental results in the present study.

Discussion

In the present study, we have shown that submicrometer-sized particles can be directly observed via fluorescence microscopy. Currently, the formation of small assemblies, nanometer to micrometerin size, from polymers and surfactants is the central issue in colloid science. One useful and simple method to understand the critical assembly concentration of polymers is measurement of surface tension, which seems to show a critical concentration. The surface tension decreases, due to an increase in the concentration of surfactant, and then reaches a plateau. Such a change has been attributed to the accumulation of the surfactant on the surface of the water. However, precise insight on the initial process of the assembling phenomena has been difficult to examine in detail using macroscopic methods such as surface tension measurement. The solubility of PA is 0.00072 g/100 g in water at 20 °C (about 30

(24) Ishikawa, M.; Misawa, H.; Kitamura, N.; Fujisawa, R.; Masuhara H. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 59−65

(25) Hofkens, J.; Hotta, J.; Sasaki, K.; Masuhara, H.; Iwai, K. *Langmuir* **1997**, *13*, 414−419.

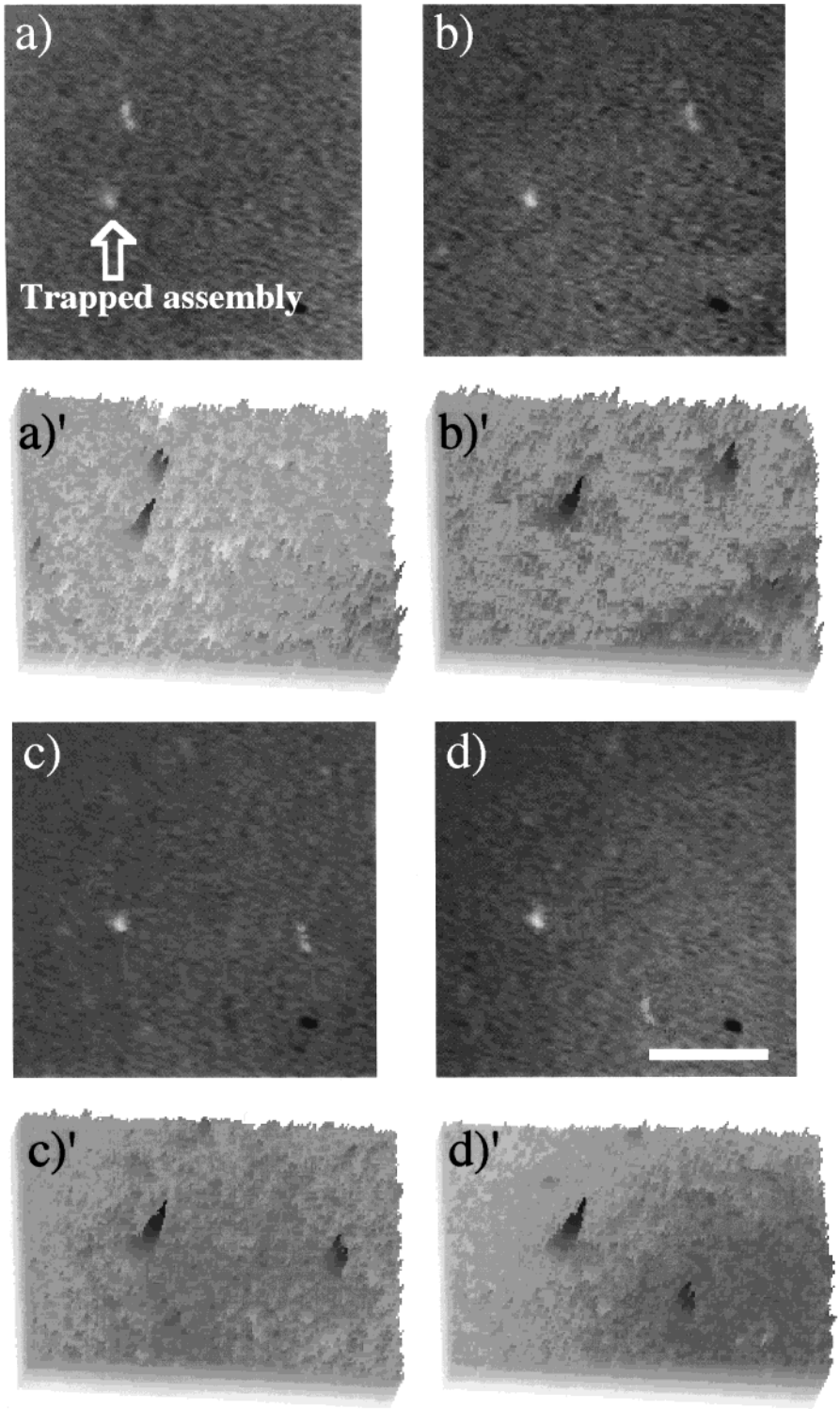

Figure 6. Fluorescence images of the PEG-A/PA complex that was trapped and transported by the Nd:YAG laser. The object adsorbed on the glass surface indicates the actual movement of the stage; thus the trapped polymer assembly (indicated by an arrow) is effectively transported in reference to the adsorbed objects. (a′−d′) Quasi-three-dimensional surface plot for each photograph a−d, where the vertical axis indicates the intensity of the fluorescence. The scale bar indicates 10 μm.

μM), which is close to the starting point of a change in the value of surface tension. The observable assembly by fluorescence microscopy is found to exist at a concentration 50 times lower than that expected from the measurement of surface tension (see Figure 4). This discrepancy is at least partly due to the difference in the experimental parameter for the surface tension measurement, having originated from the effect of the surface of the monitor, and is from the bulk aqueous phase. The present data basically suggest that, in solution, PA interacts preferentially with PEG-A rather than be dissolved, even when PA is present in the solution at a lower concentration than its solubility limit. In addition, this indicates that even prior to cmc, stable micellar assemblies are generated.

As shown in Figure 5, the outline of the polymer assembly in the solution is ellipsoidal. On the other hand, the trapped assembly is observed as a compacted round object. It is expected that a nonspherical particle prefers the orientation along the incident direction of the laser due to the optical pressure.[26] The

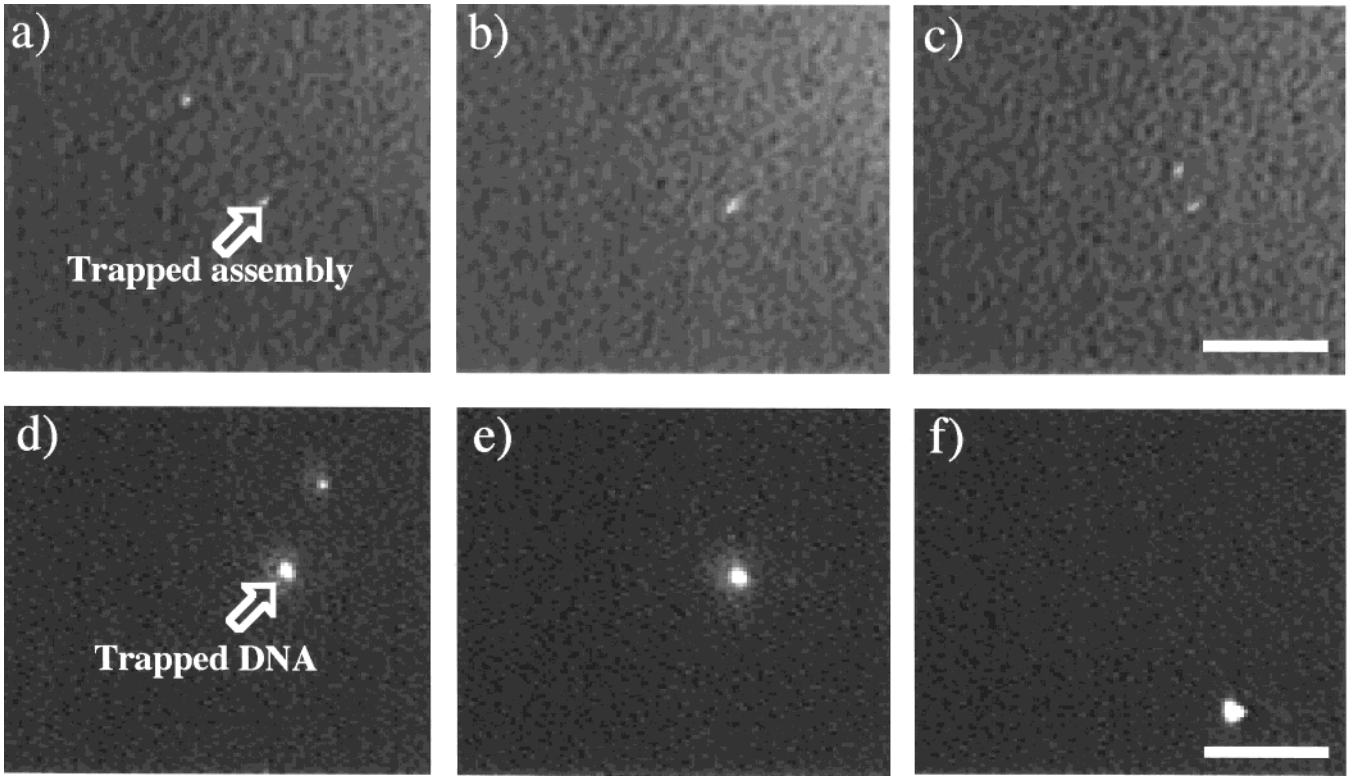

Figure 7. Photographs used to determine the stickiness of the objects: (a−c) The PEG-A/PA assembly (20 μM of amino residue) and (d−f) the compacted DNA using the condensing agent, PEG-$MgCl_2$.[14] (a, d) The objects were transported via movement of the stage to the focus point (indicated by an arrow). (b, e) After contact, the laser illumination was switched off. (c, f) The photos show that the polymer assemblies are not sticking to each other, whereas the compacted DNAs are revealed to be sticky. The scale bars indicate 10 μm.

change in the apparent shape from ellipsoidal to circular is thus attributed to this effect of optical pressure.

Interaction between submicrometer objects has been the subject of intense study. At present, the standard interpretation for this behavior is the D.L.V.O. theory.[27] This theory provides a picture of the manner in which interaction colloidal particles behave, i.e., the interaction between the charged particles is repulsive at long distances in Coulombic terms, but can be attractive in terms of van der Waals attraction. To clarify the stability of colloidal solutions, long-range repulsive interaction between objects is essential. However, the short-range attractive interaction is not a necessary condition for the stability of the solution, including the "metastable state". Thus, discrimination as to whether the "short-range" interaction is attractive or repulsive is important to obtain a correct picture of the physicochemical characteristics in the colloidal system. The present study affords a powerful new methodology by which to solve this problem. In our system, PEG-A/PA assemblies are found to be repulsive to each other. The results of the electrophoresis performed in our laboratory using fluorescence microscopy have revealed that these assemblies exhibited a slightly positive electrostatic charge (data are not given). Such a remaining positive charge in the PEG-A/PA system causes the repulsive interaction between the assemblies. On the other hand, it has become clear that the tightly packed DNA chain is almost neutral in the electronic charge. Thus, the neutral DNA particles have no effective Coulombic repulsion to each other. On the basis of these considerations, the difference in the manner of pair-interaction is attributed to the charge of Coulombic repulsive interaction.

In our optical system, it was difficult to observe an individual single synthetic polymer such as PEG-A labeled with FITC. Because one FITC is included in ten PEG-A molecules, there may be numerical PEG-A molecules in one assembly. In addition, considering the solubility of PA up to 10 mM, PA should form an assembly similar to a micelle with PEG-A and is soluble in the bulk solution. The structure of polymer assembly is expected to be as follows: the hydrocarbon chains of PA assemble with hydrophobic interaction and the carboxyl residues have favorable interaction with the positive charged PEG-A via electrostatic interaction, that is PEG-A forms an envelope around PA molecules.

The mixture of PEG-A and PA constructs a polymer assembly and the steady assembly can be manipulated by a laser under fluorescence microscopy. These results suggest that controlling the conformation of the polymer provides a powerful method to improve the effectiveness of optical trapping. Thus, laser manipulation is expected to serve as a useful tool in various applied fields, such as medical chemistry, molecular biology, and pharmaceutical chemistry.

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(26) Nishioka, M.; Tanizoe, T.; Katsura, S.; Mizuno, A. *J. Electrostatics* **1995**, *35*, 83−91.

(27) Irja, P. *Surfactant Science Series*; Dekker: NewYork, 1992; Vol. 42, pp 2−15.