Optical Trapping of Amino Acids in Aqueous Solutions†

Yasuyuki Tsuboi,* Tatsuya Shoji, and Noboru Kitamura*

Department of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

Received: July 29, 2009; Revised Manuscript Received: November 27, 2009

In this paper, we demonstrated that the photon forces that are generated by a near-infrared (1064 nm) focused laser beam can manipulate (optically trap) small amino acid molecules in aqueous solutions. Under observation with an optical microscope, we observed the gradual growth of a particle-like assembly of objects at the focal point during laser irradiation into an aqueous arginine solution. The observation was a molecular assembly of arginine by means of confocal Raman microspectroscopy. Such molecular assemblies were also observed for other amino acids (glycine, proline, serine, and alanine), showing that the optical manipulation technique can be extensively applied to the micromanipulation of amino acids. From experimental observations and numeral calculations, we consider that the origin of the present manipulation phenomenon is ascribed to optical trapping, not of individual molecules, but of molecular clusters of amino acids.

Introduction

In 1970, Ashkin first reported that a tightly focused laser beam caught a small latex particle (diameter 0.6−2.7 μm) in an aqueous solution.[1] Ashkin and his group have subsequently developed this particular technique of "optical trapping".[2] Optical trapping of a particle is based on photon forces that fall into the following two categories, depending on a mutual relationship between the size of an object (d) and the wavelength of the incident light (λ). When $d \gg \lambda$, the photon force can be explained within the framework of the Mie's scattering theory. The photon force is generated by changes in the momentum of the light upon refraction of incident light by a microparticle. Since a laser beam can be used to manipulate latex particles, living cells, bacteria and so on,[2−6] the optical trapping technique has been widely employed in various research fields. For instance, it was recently reported that photon force can guide neuronal growth cones.[7] On the other hand, when $d \ll \lambda$, Rayleigh's theory can be used to explain the optical trapping of an object. The force responsible for optical trapping is called the "dipole gradient force", which can be expressed by the following equations:

$$U = -\alpha|\mathbf{E}|^2/2 \tag{1}$$

$$\alpha = 4\pi\varepsilon_2 r^3 \frac{(n_1/n_2)^2 - 1}{(n_1/n_2)^2 + 2} \tag{2}$$

where U is the potential energy of trapping due to the dipole gradient force and $\mathbf{E}$ is the electric field vector of the incident light. α is the "polarizability" of a particle to be trapped, r is the radius of the particle, and ε_2 is the dielectric constant of the surrounding medium. n_1 and n_2 are the refractive indices of the particle and the surrounding medium, respectively. Thus, the equations indicate that the condition for stable trapping of a particle is $U \gg k_bT$, where k_b and T are the Boltzmann constant and the temperature of the system, respectively. It is noteworthy that theoretical studies on optical trapping of nanoparticles are actively in progress.[8]

Using the dipole gradient force, we can, in principle, optically trap a small molecule (with a relationship in which $n_1 > n_2$) in a homogeneous solution.[9,10] In such a small regime of a particle size, even a metallic nanopartcile ($d < 50$ nm) can be optically trapped as Svoboda and Block demonstrated.[11] Yoshikawa et al. also reported optical trapping of small metallic nanoparticles.[12,13] The photon force can exert an influence on the molecule, i.e., molecular trapping and manipulation. Molecular manipulation possibly provides a significant impact on chemistry as well as bioscience. Indeed, such optical trapping of a single small molecule was first reported by Chieu and Zare. They demonstrated biased diffusion and optical trapping of a single DNA molecule labeled with a dye.[14] Another group also reported that translational diffusion of molecules was suppressed at the focal point of the incident light due to a shallow optical potential.[15]

On the other hand, the targets of molecular trapping have hitherto been limited to a few large macromolecular systems: natural polymers such as DNA and artificial polymers such as poly(*N*-isopropylacrylamide), poly (vinyl methyl ether) and so on.[16−18] Because the trapping potential energy U is proportional to the volume (r^3) of the trapped particle (eq 2), the target molecule needs to be large enough to satisfy the trapping conditions ($U \gg k_bT$). Note that such optical trapping of a small nanoparticle is promoted by using pulsed laser light according to a recent report by De et al.[19]

The optical trapping technique would be the only viable methodology of accessing and '3D-manipulate' a molecule in solution in noncontact-mode. The experimental observation of slow diffusion of small molecules within an optical potential would suggest the possibility of trapping small molecules and, thus, it would be fruitful to explore the trapping and manipulation of small molecules in solution. In particular, the manipulation of bioactive functional molecules has great potential in bio/medical science. In the present study, we demonstrate optical trapping of several amino acids in aqueous (D_2O) solutions by focusing a near-infrared CW laser beam (λ = 1064 nm). The

† Part of the "Barbara J. Garrison Festschrift".

* To whom correspondence should be addressed. E-mail: twoboys@sci.hokudai.ac.jp (Y.T.); kitamura@sci.hokudai.ac.jp (N.K.).

10.1021/jp9072334
Published on Web 12/23/2009

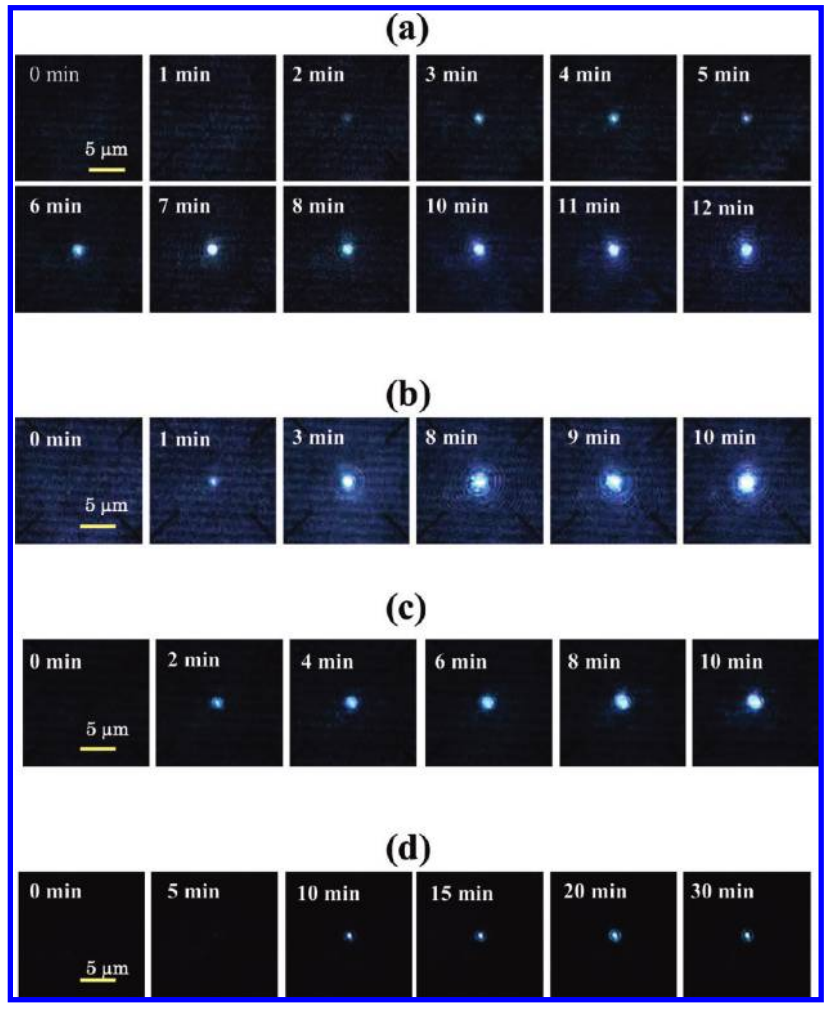

Figure 1. Optical micrographs (backward light scattering images) of the optical trapping of L-arginine in heavy water solutions. The laser irradiation time is shown in each image (0 min corresponds to the time just as the laser is switched on). Laser power (P_{eff}) and concentration of sample solution: (a) 0.6 W for a 1.0 M solution, (b) 1.3 W for a 1.0 M solution, (c) 1.3 W for a 0.1 M solution, and (d) 2.0 W for a 0.01 M solution.

'photon-force-induced' molecular assembling of amino acids was studied by video microscopy and Raman microspectroscopy.

Experimental Section

Commercially available L-arginine (Tokyo Kasei, 99.0%), D,L-proline (Wako, 98.0%), glycine (Wako, 99. 0%), and D,L-serine (Wako, 98%) were used as-supplied. Heavy water (Wako, D_2O, 99.9%) was used as a solvent. Since heavy water is transparent at 1064 nm, any thermal effects are negated, and we can discuss experimental results solely in terms of photon force effects.[20] The details of the experimental methods and apparatus were similar to those reported previously.[9,10,21] Briefly, a CW Nd^{3+} YAG laser beam (1064 nm) was introduced into a confocal microscope and focused into a sample solution. Simultaneously, Ar laser beam (488 nm) was introduced into the microscope coaxially for the excitation of Raman Scattering at the focal spot. The spatial resolution of Raman spectroscopy is less than 500 nm in the $x-y$ plane and 2 μm in the z direction. Laser power, P_{eff}, represents the effective value as measured after passing through the objective lens. The sample solutions (0.01−1.0 mol/L) were centrifuged at 13 900g for 5 min and then filtered through 0.20 μm cellulose acetate syringe filters to eliminate impurities. A 50 μL droplet of the supernatant liquid was poured into an inert paraffin oil in a well (diameter 7 mm, depth 10 mm) and the well was sealed with a cover glass, as reported in the previous study.[22]

Results and Discussion

Microparticle Formation of L-Arginine in Aqueous Solutions. Aqueous solutions of glycine, D,L-proline, D,L-serine, and L-arginine were irradiated with a focused 1064 nm laser beam under an optical microscope. For all of these solutions, we observed photon-force-induced molecular assembling of the amino acids above certain threshold values of amino acid concentrations and laser powers (P_{eff}). These values varied from system to system. In the present study, we mainly dealt with arginine because arginine has high solubility in water, moreover it is a large amino acid that is favorable for optical trapping due to large polarizability. As a representative example, the results for aqueous L-arginine solutions (1.0, 0.1, and 0.01 M) are displayed in Figure 1 as the temporal evolutions of the microscope images upon irradiation. These correspond to backward-light (488 nm)-scattering-images taken under dark-field-observation. After irradiating the laser beam into the 1.0 M solution (Figure 1a, $P_{eff} = 0.6$ W, $\Delta t = 0-1.0$ min), we did not confirm molecular assembling of the amino acid. Upon prolonged irradiation, we confirmed particle formation at the focal point and, at $\Delta t = 2.0$ min, the particle size increased, as shown by the temporal change in the image. At $\Delta t = 10$ min, the size of the particle seemed to reach an equilibrium value. Under more intense laser irradiation (Figure 1b, $P_{eff} = 1.3$ W), the growth rate and the equilibrium size of the microparticle increased. For a more dilute solution (0.1 M, Figure 1c or 0.01 M, Figure 1d), it took a longer irradiation time (Δt) to confirm

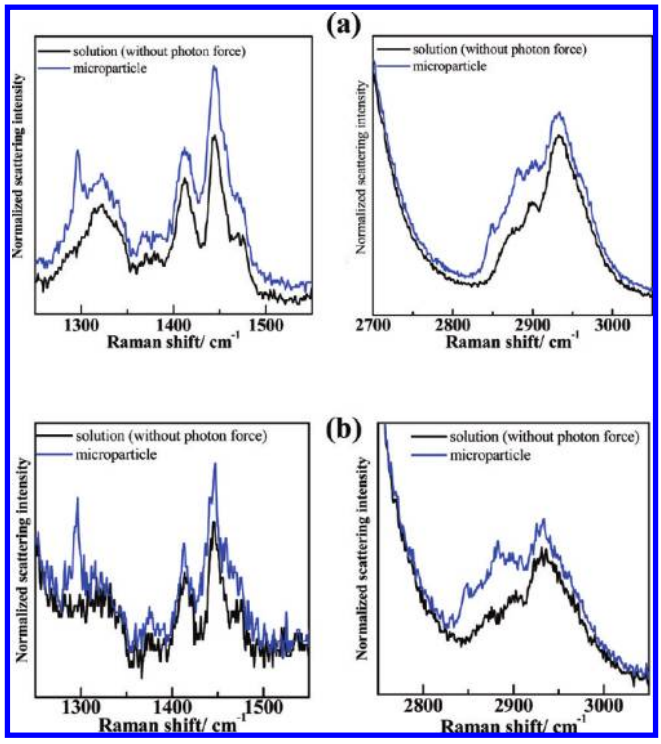

Figure 2. Raman spectra of sample solutions (before 1064 nm irradiation, black line) and microparticles (blue line) formed by photon force. (a) $P_{eff} = 1.3$ W for 1.0 M aqueous L-arginine solution. (b) $P_{eff} = 1.3$ W for 0.1 M aqueous L-arginine solution.

particle formation, and the equilibrium particle size was smaller than that in the 1.0 M solution, though the precise sizes of the particles was hardly determined owing to light-scattering in the images.

The phenomena observed in the present study are analogous to those that occur in the photon-force-induced molecular assembling of artificial polymers and a protein (lysozyme).[9,10,22] Note that the backward-scattering intensity increases with increasing P_{eff}, suggesting that the density of the particle-like-object increases with increasing laser irradiation power. The particle formation process depended on P_{eff}, and was observed at $P_{th} \geq 0.3$ W for 1.0 M to ~10 mM solutions. For sample concentrations below 1 mM, stable optical trapping was not observed, even under intense laser irradiation ($P_{eff} \sim 1$ W).

Raman Microspectroscopy of an L-Arginine Solution. We performed in situ confocal Raman microspectroscopy to chemically identify the particles produced under laser irradiation. The results for 0.1 and 0.01 M L-arginine solutions are shown in Figure 2, together with a reference spectrum of an aqueous homogeneous L-arginine solution. The intensity of the spectra was normalized to that of the O−D stretching band of D_2O (2650 cm^{-1}), which was used as a reference spectrum. The Raman scattering peaks at 1320 and 1444 cm^{-1} (black lines in Figure 2, panels a and b) are assigned to the CH_2-wagging and CH_2-scissoring bands of L-arginine, respectively.[23−30] The broadband observed at 2200−2800 cm^{-1} is safely assigned to the O−D stretching bands of D_2O. The relatively sharp peaks observed between 2800−3000 cm^{-1} were assigned to the $-CH_2-$ and $-CH-$ vibrational bands of the amino acids.[31]

The Raman spectrum of the particle produced in the 1.0 M solution (Figure 2a, blue line) agreed well with that of the reference arginine solution (black line). We can clearly recognize a one-to-one correspondence of the peaks between the two spectra. Note that the scattering intensity (signal intensity) of the particle is stronger than that of the solution. Also, the Raman spectrum of the particle formed in the 0.1 M solution (Figure 2b, blue line) agreed with that of the relevant reference sample solution (black line), and the scattering intensity of the particle increased relative to that of the reference solution. From these results, we conclude that the particles formed by the laser irradiation are arginine assemblies trapped by the photon force phenomenon. The increase in the signal intensity from the particle compared with that from the reference solution indicates an increase in the concentration of arginine at the focal point, induced by photon force.

If we consider the characteristics of the Raman spectra of the particles, the relative ratios between several peak intensities that were observed for the particles were slightly different from those observed for the reference spectrum. For example, the Raman bands observed for the particle at 2880 and 2900 cm^{-1} ($-CH_2-$ and $-CH-$ stretching bands, respectively) are slightly stronger than those of the reference spectrum. Moreover, a new peak appeared at 1300 cm^{-1} in the Raman spectra of the particles. The observed phenomena were reproducible. One may ascribe these spectral changes to a sign of thermal decomposition of arginine by a photothermal effect. However, the temperature rise at the focal point is estimated to be 5 K at most when $P_{eff} = 1.0$ W based on a literature.[32] Hence such a thermal effect should be negligible. An origin of these new Raman signals may be ascribed to a formation of arginine clusters at focal point and is discussed in a latter section of optical trapping mechanism.

Optical Trapping of Other Amino Acids. We confirmed optical trapping of other amino acids (L-alanine, D,L-proline, glycine and D,L-serine) in aqueous solutions (0. One M) above certain threshold values, P_{th}. The P_{th} values were roughly estimated to be ≥0.3 W for L-proline, ≥1.3 W for L-alanine, and ≥1.3 W for glycine. The results clearly demonstrate that smaller molecules exhibit higher values for P_{th}. Figure 3 shows the light-scattering images of the optically trapped amino acids in aqueous solutions. From the figure, molecular assembling of an amino acid by photon force can be achieved by photoirradiation in several minutes. These results indicate that optical trapping (photon-force-induced molecular trapping) is generally applicable to amino acids in aqueous solutions.

Optical Trapping Mechanism. Factors governing molecular assembling of amino acids are worth discussing here. The energy condition necessary for optical trapping is $U \gg k_bT$, as described before, and, in many cases, $U \geq 10k_bT$ has been taken as a minimum criterion.[2,33] For example, we determined that the P_{th} value for assembling arginine in a 10 mM aqueous solution was 0.3 W. In this case, the radius of the trapped particle should be $r > 15$ nm to satisfy $U \geq 10k_BT$, as predicted from eqs 1 and 2.[34−36] This value (15 nm) is clearly larger than the radius of a single arginine molecule ($r \sim 1$ nm), implying that individual arginine molecules cannot be optically trapped by photon force under the present irradiation conditions. Hence, we should consider the following mechanism for the explanation of optical trapping of an amino acid.

Because an arginine molecule in an aqueous solution takes a zwitterionic structure with a large dipole moment, molecules will be likely to aggregate with each other due to Coulombic interactions and hydrogen bonding.[37,38] Hence, arginine would form clusters in a concentrated aqueous solution (10 mM). Such arginine clusters with $r > 15$ nm would be optically trapped and would assemble with each other at the focal point. The cluster trapping mechanism is consistent with the observation of the lowest concentration limit for trapping (10 mM for arginine), because such cluster formation is promoted by an increase in the concentration. Indeed, Kondow et al. reported

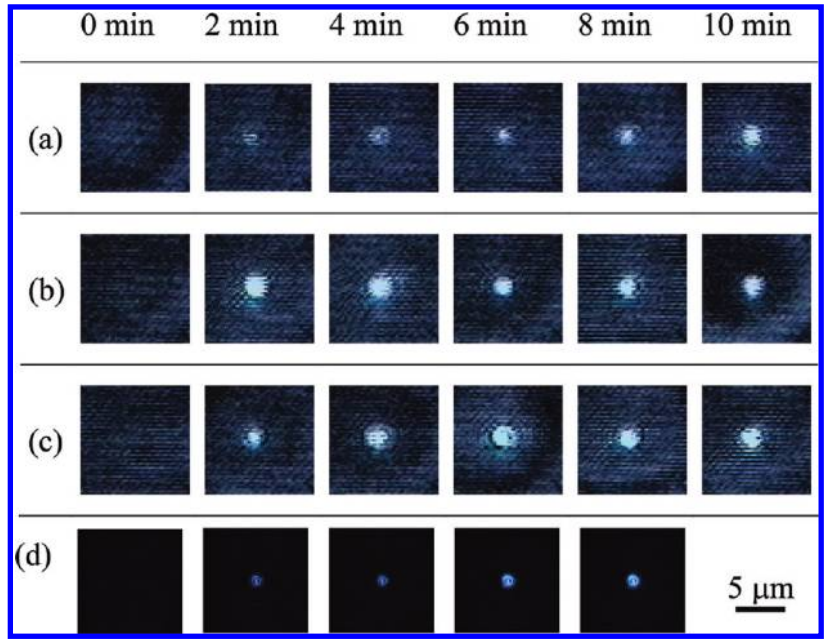

Figure 3. Optical micrographs (backward light scattering images) for optical trapping of four amino acids in heavy water solutions (0.1 M). (a) glycine, (b) D,L-proline, (c) D,L-serine, and (d) L-alanine. The laser irradiation time is shown in each image and $P_{eff} = 1.3$ W.

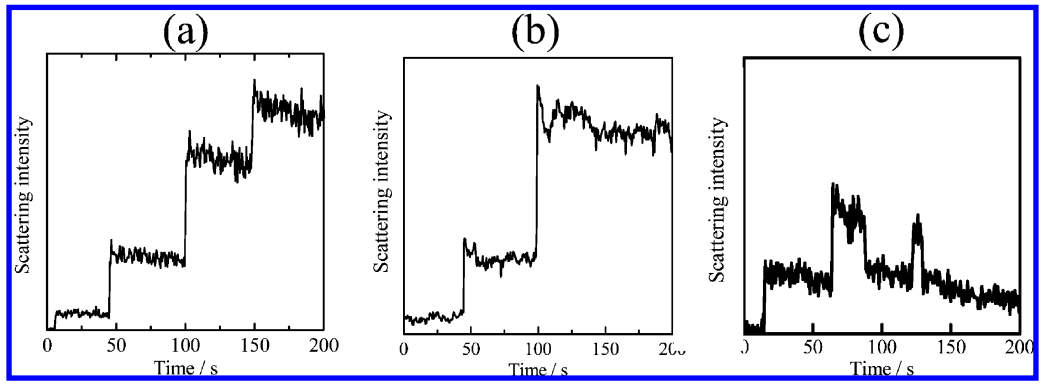

Figure 4. Representative example of temporal evolution of backward scattering intensity during optical trapping in 10 mM aqueous L-arginine solution. Laser power: (a and b) $P_{eff} = 0.6$ W and (c) $P_{eff} = 0.4$ W.

the existence of hydrated arginine clusters, $H^+(Arg)_m(H_2O)_n$, in an aqueous solution, as demonstrated by a liquid beam technique combined with mass spectroscopy.[37] At an arginine concentration of >10 mM, they clearly detected arginine clusters with $m = 1 \sim 14$. We assume that such arginine clusters, whose size satisfies the trapping conditions ($r > 15$ nm), is trapped one by one at the focused spot of the laser beam.

To examine this assumption, the temporal evolution of the backward-light-scattering intensity during optical trapping (corresponding to Figure 1) was measured. The results are shown in Figure 4: 10 mM arginine solution at $P_{eff} = 0.6$ W. As clearly seen in Figure 4, the scattering intensity did not increase gradually, but in a step-by-step manner with time. If each arginine molecule was optically trapped in a molecule-by-molecule manner, the scattering intensity would increase gradually with irradiation time. To be more specific, the sudden rise (step) of the scattering intensity in Figure 4 would be ascribed to one-by-one optical trapping of each arginine cluster during translational diffusion of the clusters across the focused spot. The sudden rise showed irregular steps (Figure 4, panels b and c), indicating a size distribution of the clusters. In addition, with decreasing P_{eff} to 0.4 W (decreasing optical potential), different behavior was observed as shown in Figure 4c. In this case, stable trapping time was shortened and sudden drop of scattering light were observed obviously. This means the escape of trapped clusters from the focal point due to a shallow optical potential. On the other hand, total intensity of scattering light increased with P_{eff}, corresponding deeper optical potential can trap more clusters. These behaviors are consistent with the cluster trapping mechanism.

Because such examples of cluster formation would also hold for other amino acids, the cluster trapping mechanism can explain the present observations. Indeed, it is well-known that amino acids frequently forms liquid-like-clusters in aqueous solution.[39] At the laser focused volume, the molecular density will be further increased by photon forces, and cluster structures is possibly be altered by intermolecular interactions at the trapping potential[40] where the optical binding force is enhanced[41] We consider such structural changes would be reflected in the Raman spectrum of the particle (Figure 2). Note that such signal increases in step-by-step manner has been observed for polymer bead trapping (diameter, 24−200 nm)[42] In contrast to polymer latex beads, optical properties, sizes, density, structure of liquid-like-clusters of amino acids still remain unclear. They are continuously fluctuated in water. The present study revealed that such *soft* clusters can be trapped to form molecular assembly.

Concluding Remarks

We demonstrated experimentally that photon forces trap amino acids in aqueous solutions. Amino acid molecules were assembled at the focused spot of the incident light in a few minutes. The growth rate of amino acid aggregates increased with increasing concentrations of the amino acid and the laser power (P_{eff}). The origin of the present optical trapping of amino acids was ascribed to the cluster trapping mechanism, and the technique can be extended to other small molecules or small oligomers. Such molecular trapping and manipulation based on photon force has a wealth of applications, such as selective drug delivery to a living cell, molecular separation, targeted synthesis of bioactive molecules (protein), and so on. Indeed, Sugiyama and Masuhara recently demonstrated the photon-force-induced crystallization of glycine in a supersaturated solution[43,44] In this case, the glycine clusters experimentally verified in the present study possibly play a role as a precursor for the crystal nucleus.

Acknowledgment. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan to the Priority Area "Strong Photon-Molecule Coupling Fields (470)" (No. 19049004), No. 20550002, and the Global COE Program (Project No. B01: Catalysis as the Basis for Innovation in Materials Science).

References and Notes

(1) Ashkin, A. *Phys. Rev. Lett.* **1970**, *24*, 156.
(2) Ashkin, A.; Dziedzic, J. M.; Bjorkholm, J. E.; Chu, S. *Opt. Lett.* **1986**, *11*, 288.
(3) Ashkin, A.; Dziedzic, J. M. *Science* **1987**, *235*, 1517.
(4) Ashkin, A.; Dziedzic, J. M.; Yamane, T. *Nature* **1987**, *330*, 769.
(5) Block, S M.; Goldstein, L. S. B.; Schnapp, B. J. *Nature* **1990**, *348*, 348.
(6) Sasaki, K.; Koshioka, M.; Misawa, H.; Kitamura, N.; Masuhara, H. *Appl. Phys. Lett.* **1992**, *60*, 807.
(7) Carnegie, D. J.; Stevenson, D. J.; Mazilu, M.; Gunn-Moore, F.; Dholakia, K. *Opt. Exp.* **2008**, *16*, 10507.
(8) Dienerowitz, M.; Mazilu, M.; Dholakia, K. *J. Nanophtonics* **2008**, *2*, 021875.
(9) Tsuboi, Y.; Nishino, M.; Sasaki, T.; Kitamura, N. *J. Phys. Chem. B* **2005**, *109*, 7033.
(10) Tsuboi, Y.; Nishino, M.; Matsuo, Y.; Ijiro, K.; Kitamura, N. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 1926.
(11) Svoboda, K.; Block, S. M. *Opt. Lett.* **1994**, *19*, 930.
(12) Yoshikawa, H.; Matsui, T.; Masuhara, H. *Phys. Rev. E* **2004**, *70*, 061406.
(13) Tanaka, Y.; Yoshikawa, H.; Itoh, T.; Ishikawa, M. *J. Phys. Chem. C* **2009**, *113*, 11856.
(14) Chiu, D. T.; Zare, R. N. *J. Am. Chem. Soc.* **1996**, *118*, 6512.
(15) Ichikawa, M.; Matsuzawa, Y.; Koyama, Y.; Yoshikawa, K. *Langmuir* **2003**, *19*, 5444.
(16) Hofkens, J.; Hotta, J.; Sasaki, K.; Masuhara, H.; Taniguchi, T.; Miyashita, T. *J. Am. Chem. Soc.* **1997**, *119*, 2741.
(17) Borowicz, P.; Hotta, J.; Sasaki, K.; Masuhara, H. *J. Phys. Chem. B* **1997**, *101*, 5900.
(18) Osborne, M. A.; Balasubramanian, S.; Furey, W. S.; Klenerman, D. *J. Phys. Chem. B* **1998**, *102*, 3160.
(19) De, A. K.; Roy, D.; Dutta, A.; Goswami, D. *Appl. Opt.* **2009**, *48*, G33.
(20) Juodkazis, S.; Mukai, N.; Wakaki, R.; Yamaguchi, A.; Shigeki Matsuo, S.; Misawa, H. *Nature* **2000**, *408*, 178.
(21) Tsuboi, Y.; Nishino, M.; Kitamura, N. *Polym. J* **2008**, *40*, 367.
(22) Tsuboi, Y.; Shoji, T.; Kitamura, N. *Jpn. J. Appl. Phys.* **2007**, *46*, L1234.
(23) Garfinkel, D. *J. Am. Chem. Soc.* **1958**, *80*, 3827.
(24) Lyng, F. M.; Faoláin, E. Ó.; Conroy, J.; Meade, A. D.; Knief, P.; Duffy, B.; Hunter, M. B.; Byrne, J. M.; Kelehan, P.; Byrne, H. J. *Exp. Mol. Pathol.* **2007**, *82*, 121.
(25) Haussühl, S.; Karapetyan, H. A.; Sukiasyan, R. P.; Petrosyan, A. M. *Cryst. Growth Des.* **2006**, *6*, 2041.
(26) Petrosyan, A. M. *Vibrat. Spectrosc.* **2006**, *41*, 97.
(27) Petrosyan, A. M.; Sukiasyan, R. P. *J. Mol. Struct.* **2008**, *874*, 51.
(28) Sun, Z. H.; Yu, W. T.; Cheng, X. F.; Wang, X. Q.; Zhang, G. H.; Yu, G.; Fan, H. L.; Xu, D. *Opt. Mater.* **2008**, *30*, 1001.
(29) Sun, Z. H.; Xu, D.; Wang, X. Q.; Liu, X. J.; Yu, G.; Zhang, G. H.; Zhu, L. Y.; Fan, H. L. *Cryst. Res. Technol.* **2007**, *42*, 812.
(30) Sanker, R.; Muralidharan, R.; Raghavan, C. M.; Jayavel, R. *Mater. Chem. Phys.* **2008**, *107*, 51.
(31) Howell, N. K.; Arteaga, G.; Nakai, S.; Chan, E. C. Y. L. *J. Agric. Food Chem.* **1999**, *47*, 924.
(32) Ito, S.; Sugiyama, T.; Toitani, N.; Katayama, G.; Miyasaka, H. *J. Phys. Chem. B* **2007**, *111*, 2365.
(33) Harada, Y.; Asakura, T. *Opt. Commun.* **1996**, *124*, 529.
(34) Keenan, G. L. *J. Biol. Chem.* **1929**, *83*, 137.
(35) Keenan, G. L. *J. Biol. Chem.* **1924**, *62*, 163.
(36) Lacourt, A.; Delande, N. *Microchim. Acta* **1964**, *52*, 547.
(37) Toyama, N.; Kohno, J.; Mafuné, F.; Kondow, T. *Chem. Phys. Lett.* **2006**, *419*, 369.
(38) Myung, S.; Fioroni, M.; Julian, R. R.; Koeniger, S. L.; Baik, M. H.; Clemmer, D. E. *J. Am. Chem. Soc.* **2008**, *128*, 10833.
(39) Chattopadhyay, S.; Erdemir, D.; Evans, J. M. B.; Ilavsky, J.; Amenitsch, H.; Segre, C. U.; Myerson, A. S. *Cryst. Growth Des.* **2005**, *5*, 523.
(40) Burns, M. M.; Fournier, J.-M.; Golovchenko, J. A. *Phys. Rev. Lett.* **1989**, *63*, 1233.
(41) Chaumet, P. C.; Nieto-Vesperinas, M. *Phys. Rev. B* **2001**, *64*, 035422.
(42) Hosokawa, C.; Yoshikawa, H.; Masuhara, H. *Phy. Rev. E* **2005**, *72*, 021408.
(43) Sugiyama, T.; Adachi, T; Masuhara, H. *Chem. Lett.* **2007**, *36*, 1480.
(44) Sugiyama, T.; Adachi, T.; Masuhara, H. *Chem. Lett.* **2009**, *38*, 482.

JP9072334