

## Crystallization in Unsaturated Glycine/D<sub>2</sub>O Solution Achieved by Irradiating a Focused Continuous Wave Near Infrared Laser

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Received June 22, 2010; Revised Manuscript Received September 12, 2010

**ABSTRACT:** The crystallization of glycine in unsaturated solution is made possible by laser trapping of its molecular clusters due to photon pressure of a focused continuous wave near-infrared laser beam. Always one single crystal is spatiotemporally formed at a focal spot, and then it undergoes dissolution, eventually leading to repetitive crystallization and dissolution. The polymorph characterization of the crystal formed in unsaturated solution confirmed the  $\gamma$ -form, which is not obtainable by conventional crystallization methods. The preparation probability of the  $\gamma$ -form compared to the  $\alpha$ -form is much higher than that in the supersaturated solution.

Crystallization in solution is a crucial process in separation and purification for a wide range of substances in scientific and industrial laboratories, especially in pharmaceutical fields.<sup>1</sup> Generally, it has been achieved by various methods of solvent evaporation, solution cooling, crystal seeding, and so on.<sup>2,3</sup> Over the past decade, laser-induced crystallization has attracted increasing attention. It is expected to crystallize membrane proteins which are not obtainable by conventional methods, give a crystal with better quality, prepare a crystal faster and more efficiently, and control the polymorph if available. Garetz, Myerson, et al. demonstrated crystallization of several organic compounds, and polymorph control of several amino acids by applying a nanosecond laser pulse into their highly supersaturated solutions.<sup>4–6</sup> In 2002, one of the present authors (H.M.) and collaborators succeeded for the first time in protein crystallization by irradiating femtosecond laser pulses into its solution.<sup>7</sup> Recently we have demonstrated the spatiotemporal crystallization,<sup>8</sup> crystal growth,<sup>9</sup> and polymorph control<sup>10</sup> of glycine by irradiation with an intense continuous wave (CW) near-infrared (NIR) laser beam. For all laser crystallization methods, the supersaturation is always necessary, but if crystallization in unsaturated solutions is made possible, new crystallization and crystal growth phenomena will be explored. In this paper, we present the first demonstration of crystallization of glycine in unsaturated D<sub>2</sub>O solution by utilizing CW NIR laser irradiation. One single crystal was always formed spatiotemporally, and then it dissolved after growing to a few tens micrometer size, leading to repetitive crystallization and dissolution. Furthermore, it was shown that laser control of the glycine crystal polymorph was more successful compared to the supersaturation condition, which strongly supports crystallization in unsaturated solution.

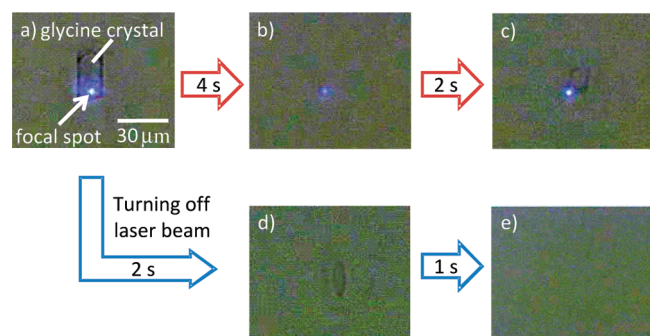
D<sub>2</sub>O was employed as a solvent to suppress laser-induced heating, since H<sub>2</sub>O has a higher absorption coefficient due to the overtones of the OH vibration at 1064 nm of the trapping laser. Glycine solutions of saturation 50 and 68% were prepared by dissolving 0.11 and 0.15 g of glycine in 1.0 g of D<sub>2</sub>O, respectively, and the solutions were kept at 60 °C during vigorous shaking for 3 h, followed by slow cooling down to room temperature. These concentrations are a half concentration of saturated and supersaturated solutions, respectively, used in previous work.<sup>10</sup> A 15  $\mu$ L portion of the solution was dropped into a hand-made sample bottle with a hydrophilic bottom surface, giving a thin

solution film. The bottle was completely sealed to avoid solvent evaporation, and it was set on the stage of an inverted microscope. The experiment setup for laser trapping was almost the same as in our previous reports.<sup>8–10</sup> A linearly polarized CW Nd<sup>3+</sup>:YVO<sub>4</sub> laser beam ( $\lambda$  = 1064 nm, Spectra Physics, J20-BL-106C) was introduced into an inverted microscope and focused at the air/solution interface of the thin glycine solution film through an objective lens (60 $\times$  magnification, NA 0.90). The laser power throughout the objective lens was varied from 0.8 to 1.4 W. Only one single crystal was always prepared in our experiment, which was repeated for 10 samples under each condition. Crossed Nicols images of the formed crystal were collected with an EMCCD video camera (Flovel, ADT-40C). The surface height of the solution before/after laser irradiation was measured with a laser confocal displacement meter (Keyence, LT-9030).<sup>11</sup> The polymorph of formed crystals was identified by Fourier transform infrared (FTIR) measurement (HORIBA, FT-720) as described in our previous report.<sup>10</sup>

Figure 1 shows a typical example of crystallization behavior for the 50% solution at 1.1 W of laser power. Just after focusing the laser beam at the air/solution interface, only reflection of the trapping laser from the interface was observed. At 415 s after starting laser irradiation, the crystallization was observed at the focal spot, where the generated crystal grew up to about 30  $\mu$ m at 450 s while being trapped (Figure 1a). Interestingly, after a subsequent 4 s, the crystal suddenly underwent dissolution and became unidentified by the EMCCD camera (Figure 1b). Further laser irradiation led to crystal regrowth to a few tens of micrometers (Figure 1c), followed by the repetitive behavior of dissolution (b) and crystal growth (c) randomly. All samples showed such crystallization/dissolution behavior as seen in Figure 1, while the average time required for crystallization was 405 s, which is four times longer than that in supersaturated solutions under the same irradiation conditions.<sup>10</sup>

We consider the crystallization behavior in the unsaturated solution in view of laser trapping due to the photon pressure of the focused beam. The laser power used in this work is estimated to be 0.4 GW/cm<sup>2</sup> at the focal point, too low to overcome Brownian motion of a single glycine molecule in D<sub>2</sub>O. The minimum size of the object to be trapped is calculated to be about 17 nm,<sup>11,12</sup> therefore, we considered that not glycine molecules but their liquid-like clusters in the solution should be gathered to the focal spot and reorganized by photon pressure, leading to the crystallization. The large liquid-like clusters have been reported by Myerson et al.<sup>13</sup> for the supersaturated solution but are expected

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**Figure 1.** Crossed Nicols images of the repetitive manner of (a) crystallization, (b) dissolution, and (c) crystal regrowth induced by laser irradiation, and those of the disappearance of the crystal at (d) 2 and (e) 3 s after turning off the laser beam.

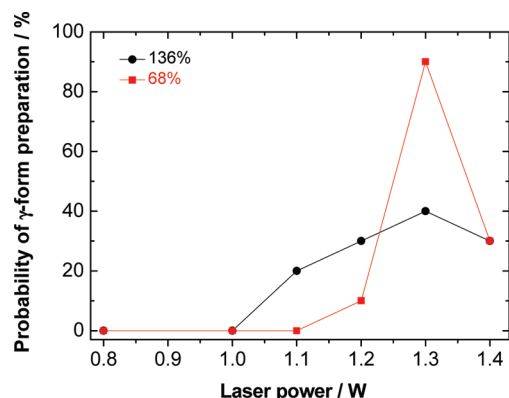
to be less for the dilute solution. Thus, we considered that the liquid-like clusters in the dilute solution are formed locally and transiently due to concentration fluctuation, and when photon pressure works on the clusters at the focal point, they may be trapped. Consequently, the cluster concentration inside the focal volume increases, inducing further laser trapping of the clusters, and eventually, the crystallization is triggered. Recently, Tsuboi et al. reported a notable result that the particle-like assembly of glycine was observed by focusing a laser beam into a solution less concentrated than the solution used in this study.<sup>12</sup> We suggest that this phenomenon can be explained as described above.

It should be noted that the present laser trapping crystallization is observed only by focusing at the air/solution interface, where the clusters possibly become larger due to the limited molecular diffusion and enhanced alignment, with the trapping force nonlinearly increasing with time. In contrast, no crystallization took place upon irradiation at the glass/solution interface or in the solution. Instead, we found that focusing the laser beam at the glass/solution interface induces the formation of a large dense liquid droplet, as reported previously for the supersaturated solution.<sup>11</sup> This focal position dependence is indeed one of the significant factors for the crystallization.

The size of the generated crystal became much larger than that of the focal spot of about 1  $\mu\text{m}$ . After the focal spot and its surroundings were fully occupied by the crystal, the dissolution started, since photon pressure could not gather the clusters anymore. When the dissolution proceeded and the crystal size was reduced to less than the focal spot, laser trapping of the clusters was again induced, giving a smaller but comparable size crystal (Figure 1c), and then followed by the repetitive behavior of dissolution and crystal growth.

When we turned off the laser after the first crystal was formed, it immediately started dissolution (Figure 1d) and disappeared (Figure 1e) within several seconds. Consequently, we consider that the unsaturated condition was kept during the irradiation. The surface height before and after laser irradiation was measured using a laser confocal displacement meter, on which we confirmed that only 7% of the solvent was evaporated during laser irradiation for 400 s. In other words, the concentration of solution after laser irradiation was calculated to be approximately 59%, which is clearly less than the saturation. Therefore, laser trapping crystallization can be confirmed for unsaturated glycine solution in the present work.

Another experimental support for the laser trapping crystallization in unsaturated solution was obtained by the investigation of the crystal polymorph. The 68% solution was used because the formed crystal showed a slow dissolution rate compared to the 50% one. When similar crystallization behavior was observed as described above for the 50% one, the spigot was slightly opened for solvent evaporation in order for the first generated crystal to



**Figure 2.** Probability of the  $\gamma$ -form preparation of glycine in the 136% and 68%  $\text{D}_2\text{O}$  solutions as a function of laser power.

grow large. After 3 h, we obtained a crystal large enough to characterize its polymorph by FTIR spectra. The measurement revealed that two kinds of crystal polymorph of  $\alpha$  and  $\gamma$  were formed depending on laser power for the supersaturated solution.<sup>10</sup> Figure 2 shows the probability of  $\gamma$ -form preparation among 10 samples of 68 and 136% solutions<sup>10</sup> as a function of laser power. It is well-known that the  $\alpha$ -form is prepared by conventional crystallization methods, and our laser trapping crystallization, with the laser power less than only 1.0 W, also gives the  $\alpha$ -form.<sup>10</sup> This means that laser trapping with this power works only for gathering glycine clusters more efficiently but does not result in the specific orientation of glycine molecules leading to the  $\gamma$ -form.

When the power was increased to 1.1 and 1.3 W for the 136% solution, the probability of  $\gamma$ -form preparation in the supersaturated solution increased up to 20 and 40%, respectively, as shown in Figure 2. It is worth noting that the  $\gamma$ -form crystal was produced in the 68% solution with the highest probability of 90% at 1.3 W. We consider that in more dilute solution not only association of the clusters but also their formation itself is induced by photon pressure, so that the orientation of glycine molecules in the clusters is more controlled by the laser beam. As a result, the crystal polymorph can be determined more efficiently by the laser. The probability dramatically decreased to 30% at 1.4 W, eventually giving a bell-shaped curve, which can be ascribed to a heating effect. It is well-known that glycine molecules can absorb 1064 nm photons by overtone vibrational modes, as discussed previously.<sup>10</sup> A similar crystallization result for  $\gamma$ -form formation in the supersaturated solution was reported by Garetz et al. using linearly polarized nanosecond pulse irradiation.<sup>5</sup> Thus, the present high probability of  $\gamma$ -form preparation is experimental evidence which strongly supports that the crystallization occurs in unsaturated solution.

In summary, we have demonstrated for the first time the crystallization of glycine in unsaturated  $\text{D}_2\text{O}$  solution by applying photon pressure of a focused CW NIR laser beam. Surface height measurement and polymorph analysis revealed that the solution was still below unsaturation during laser irradiation. The crystallization behavior in the dilute solution was discussed on the basis of trapping of the liquid-like clusters and the alignment of glycine molecules at the surface. In addition, we presented novel behavior of crystallization and dissolution specifically observed in the diluted solution under photon pressure. The success of spatio-temporally induced crystallization and polymorph control in unsaturated solution may enable us to prepare a high quality of crystal, reduce the necessary amount of a solute molecule for crystallization, and elucidate the crystallization mechanism.

**Acknowledgment.** The present work is partly supported by a KAKENHI (S) grant (a Grant-in-Aid for Scientific Research)

(No. 18106002) from the Japan Society for the Promotion of Science (JSPS) to H.M., the MOE-ATU Project (National Chiao Tung University) of the Ministry of Education, Taiwan, to H.M., the National Science Council of Taiwan (No. 0970027441) to H.M., a KAKENHI grant on Priority Areas “Strong Photon-Molecule Coupling Fields” from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) (No. 21020022) to T.S., and a KAKENHI (C) grant (No. 20550136) to T.S.

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