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RAPID COMMUNICATION

Single crystal formation of amino acid with high temporal controllability by combining femtosecond and continuous wave laser trapping

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Abstract We investigated laser trapping crystallization of glycine by using femtosecond (fs) laser as a trapping light source. Impulsively exerted fs laser pulses crystallized glycine more effectively than that induced by continuous wave (CW) laser trapping. Highly efficient crystallization and crystal growth behavior indicates fs laser irradiation increased the concentration not only at the focal spot, but also around the laser focus. Furthermore, we found that irradiation of fs pulses to CW laser-induced locally high supersaturation region enables immediate crystallization. Spatiotemporally controlled triggering of a single crystal formation with sub-second time resolution has achieved by integrating fs and CW laser trapping techniques.

1 Introduction

In solution crystallization, nucleation profoundly impacts the ability to determine crystal structure and size distribution. Therefore, elucidating a nucleation process with a high spatial and temporal resolution is crucial to investigate primary crystallization processes. However, controlling arbitrarily the position and time of the crystallization is generally considered to be rather difficult. From the viewpoint of spatiotemporal control of crystallization enabling its dynamics and mechanism studies, laser-induced crystallization of molecules has attracted considerable attention. Four laser-induced crystallization methods have been developed so far. The first method is based on the optical Kerr effect induced by nanosecond laser pulses to a supersaturated aqueous solution of urea [1], glycine [2], and potassium chloride in agarose gel [3]. Under the intense electric field exerted by nanosecond laser pulses, forced reorientation of solute molecules in the solution resulted in crystallization. Recent reports demonstrated that the study of the dynamics and mechanism of laser-induced crystallization is possible by monitoring a population change of crystals in highly supersaturated solutions [4]. However, repeated and widely irradiated nanosecond laser pulses randomly generate many crystals in time and space, explaining the difficulty in spatiotemporally controlled initiation of crystallization which is difficult when using this method.

The second method achieves crystallization by using cavitation bubble formation and the resulting mechanical force generation, which are caused by femtosecond (fs) laser-induced break-down of water in a supersaturated lysozyme protein solution [5]. This method is currently the only one which enables crystallization of proteins by laser irradiation. However, this method can increase nucleation probability but not growth speed. A few days or longer are necessary to confirm the crystallization. Crystals are randomly formed around the cavitation bubble, explaining the inability to determine where and when the crystallization is initiated. The third method achieves crystallization by focusing high-power near-infrared (NIR) continuous wave (CW) laser to the air/solution interface of a saturated and unsaturated glycine solutions [6–8]. CW laser trapping can form only one single-crystal at the focal point of the

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trapping laser of ~GW/cm^2. Crystallization can be accounted for the local concentration increase in the solute molecule at the focal spot. At the solution surface, local surface deformation is observed and the subsequent liquid–liquid phase separation forms a high-concentration dense liquid droplet [7]. The fourth method can induce crystallization by focusing the NIR CW laser of several tens of mW on a thin gold film in aqueous solution of glycine [9]. Surface of a microbubble formed by heating the gold thin film is used to generate the dense liquid droplet, and crystallization from the droplet is eventually induced. Although the latter two methods possess high spatial controllability with the resolution of a few μm, their temporal controllability of the crystallization is limited from several tens of seconds to tens of minutes. Namely, controlled triggering of crystallization with high temporal controllability is difficult. The ability to commence crystallization arbitrarily and immediately by laser irradiation allows us to investigate the dynamics of primary crystallization process with high temporal resolution.

Efficient laser trapping of small Rayleigh particles with an ultrashort laser pulses has received considerable attention. Tamai et al. demonstrated the feasibility of achieving laser trapping of 3.3 nm CdTe quantum dots by irradiation of picosecond laser pulses with two orders of magnitude lower than that used in CW laser trapping [10]. By using fs laser, Jiang et al. [11] observed the stable trapping of 60-nm gold nanoparticle and unconventional trap split due to the nonlinear optical effect. Laser trapping of polystyrene nanoparticle by fs laser pulses revealed an efficient accumulation of the particles and additional unconventional polarization-dependent off-axis scattering of the particles [12]. Above studies involving the efficient trapping of small particles by ultrashort laser pulses imply that a new laser crystallization phenomenon may occur, and novel spatiotemporally controlled triggering of crystallization is expected by femtosecond laser trapping. This study demonstrates that fs laser trapping crystallization of the simplest amino acid, glycine, in aqueous solution is more efficient than that by CW laser trapping. A spatiotemporally controlled single crystal formation method with μm and sub-second controllability is developed by integrating fs and CW laser trapping. We conclude that fs pulse trapping more significantly enhances the accumulation of glycine clusters increases their local concentration at and around the focal spot, as well as accelerates molecular reorganization, nucleation, and crystal growth than those induced by CW laser.

2 Materials and experimental setup

Glycine (Wako Pure Chemical, >99.0 %) and deuterated water (D_2O, Sigma-Aldrich, >99 %) were used without further purification. Glycine solution with a supersaturation (SS) of 0.9–1.0 was prepared by heating at 60 °C for 24 h, followed by slow cooling to room temperature. A sample solution of 15 μL was sealed in a homemade glass chamber to prevent rapid evaporation during measurement. A trapping laser was focused to the air/solution interface of the solution through a high N.A. objective lens (0.95, 60×) of an inverted optical microscope (IX-71, Olympus). A diode-pumped Ti:Sapphire laser (Tsunami, SpectraPhysics, 800 nm) was operated both in fs pulse (~120 fs, 80 MHz) and CW modes (CW_{800}). A CW Nd:YVO_4 laser (1064 nm, J20-BL10-106Q, Spectra Physics) was used as another CW trapping light source (CW_{1064}). Fs pulse and CW lasers were spatially overlapped and focused on the solution surface by compensating for chromatic aberration with telescopes. Laser power mentioned in this study was determined through an objective lens. As is estimated, temperature elevation by focusing a 1 W of CW_{1064} on D_2O was 2 K [13]. Notably, usage of D_2O as a solvent could suppress temperature elevation due to less absorption than that in H_2O. Additionally, a smaller absorption coefficient at 800 nm lowered the temperature elevation than that at 1064 nm. Concentration change due to the evaporation of the solvent during the experiment for longer than 60 min was estimated to be less than 5 % from the volume change. Thus, crystallization due to the concentration increase was assumed negligible, and the unsaturated solution used in the experiments remained unsaturated during observation. Finally, crystallization behavior was monitored by bright field transmission imaging with a CCD camera.

3 Results and discussion

3.1 Crystallization by femtosecond pulse laser trapping

Figure 1a–c show the representative glycine crystallization behavior due to fs pulse trapping for an unsaturated solution (SS = 0.9) at 600 mW. Focusing the fs laser to the air/solution interface did not cause immediate change, while crystallization at the focal spot was observed in 10 min after irradiation was started (Fig. 1a). A video of this crystallization process is available as an electronic supplemental material (Movie 1), allowing the visualization of real-time pulse laser trapping-induced crystallization. Notably, the solution was unsaturated, and crystallization never occurred spontaneously. A generated crystal typically became multi-crystalline during its growth. According to our results, small fragments were formed from the crystal surface due to cracking by fs irradiation. These fragments were trapped together with the mother crystal and evolved as multi-crystals (Fig. 1b). This study also

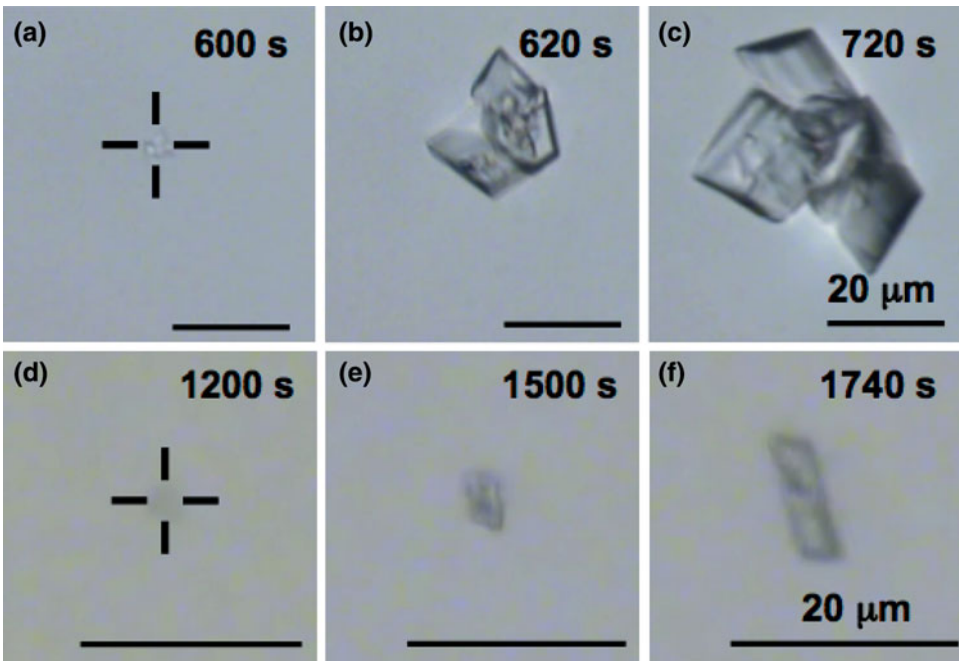

Fig. 1 Crystallization behavior of glycine under (**a**–**c**) fs and (**d**–**f**) CW_{800} laser trapping. Trapping laser irradiation time is given in the images. The *cross hairs* in (**a**) and (**d**) indicate the laser focus position. Laser power, 600 mW for both laser modes; SS, 0.9; Scale bar, 20 μm

considered possibilities of multi-nuclei formation caused by repeatedly irradiated pulses and of the successive trapping, fusion, and multi-crystal growth. Subsequently generated multi-crystals quickly grew to several tens of micrometer in length (Fig. 1) within a few hundred seconds of the fs laser irradiation.

According to our results, switching off the laser caused the diffusion of the crystal out from the focal spot and dissolution. This finding implies that glycine concentration at the distant areas from the trapping spot is still unsaturated. Even when the crystal remained around the focal spot after the trapping laser was switched off, it slowly dissolved into the solution in several minutes due to drop of the concentration. It should be noted that crystallization under present conditions differs from that caused by the second method using fs laser-induced break-down [5]. First, the fluence of applied laser pulse (0.81 Jcm^{-2}) is one order of magnitude lower than that required to induce the optical break-down and the subsequent cavitation bubble formation [14, 15]. Actually, microbubbles were not observed during the fs laser irradiation under the applied conditions. Second, crystallization by the cavitation bubble requires a supersaturated solution, and unsaturated solution used here does not allow crystal growth, even when nucleation is triggered.

Next, we examined CW laser trapping crystallization under the same experimental conditions, namely only the oscillation mode of applied laser is different. Figure 1d–f shows representative crystallization behavior observed by focusing a CW_{800} to the air/solution interface. A video of the crystallization induced by CW laser trapping is available as an electronic supplemental material (Movie 2). The CW_{800} laser irradiation did not cause an immediate change; a crystal was observed at 20 min of irradiation (Fig. 1d). The generated crystal was trapped at the focal spot and gradually grew up to several tens of micrometers while trapped for hundreds of seconds (Fig. 1e, f). In contrast to fs laser trapping-induced crystallization, formed crystals were typically a single crystal.

Crystallization behavior by fs laser irradiation obviously differs from that by CW_{800}, particularly with respect to crystallization probability and crystallization time. Crystallization probability is defined here as the ratio of successful crystallization events to the total trials. If the crystal is formed within 30 min of trapping laser irradiation, this trial is counted as a successful event. The crystallization time is defined as the time required to identify generated crystal after laser irradiation is initiated. In contrast to 100 % crystallization probability of fs laser trapping at 600 mW, the CW_{800} trapping at the same power yielded only 50 % of crystallization probability (Fig. 2a). Additionally, fs laser could form a crystal, even at 200 mW, meanwhile, crystallization by CW_{800} could not be induced below 600 mW even when the solution was irradiated for 30 min. Crystallization threshold was determined to 200 and 600 mW for fs and CW_{800} irradiation, respectively. Moreover, crystallization time for fs pulse and CW_{800} obviously differed from each other at the same laser power (Fig. 2b). Crystallization time by the fs laser irradiation decreased with an increasing laser

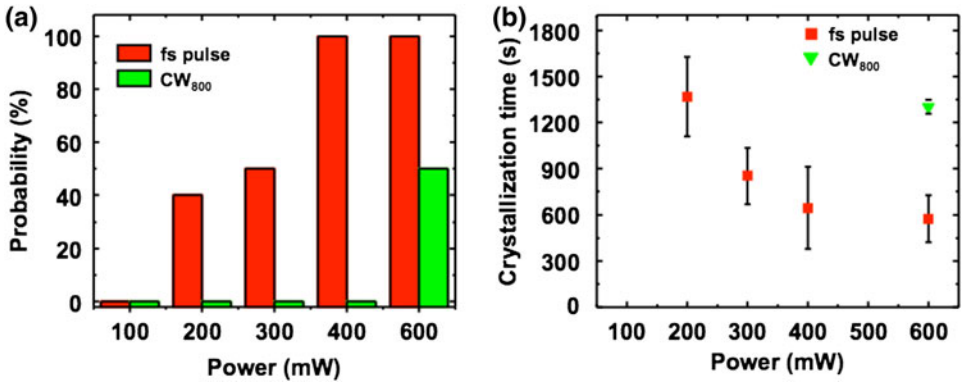

Fig. 2 **a** Crystallization probability and **b** crystallization time for fs and CW_{800} laser trapping. *Red* and *green bars* and *markers* correspond to fs and CW_{800} lasers, respectively. SS, 0.9

power and was almost a half (~10 min) of CW_{800} (~22 min). Besides crystal formation, fs and CW_{800} experiments also differed in crystal growth behavior. According to Fig. 1a–c, although crystal formed by fs pulse became ~40 μm in length within 2 min, the crystal formed by CW_{800} grew to ~11.6 μm within 9 min. By repeating the experiments, we can infer that crystal growth is faster for fs laser irradiation than for CW_{800} one.

Formation and growth of crystals in the unsaturated solution are atypical, since crystallization is generally achieved by simultaneously accomplishing high supersaturation and reorganization of molecules into an ordered crystalline structure [16, 17]. However, this is not the case for laser trapping crystallization. A previous study described the crystallization of glycine from the unsaturated solution by CW_{1064} trapping at the solution surface [8]. The glycine crystallization by CW_{1064} laser trapping is attributed to consecutive accumulation of glycine; its cluster formation and trapping of the glycine clusters at the focal spot. Consequently, local concentration at the focal spot has increased and overcome the critical concentration, eventually leading to a situation in which crystallization has been triggered and grown in the locally formed supersaturated region.

Crystallization and rapid growth of the crystal in an unsaturated solution by fs pulse trapping clearly demonstrate that the local concentration at and around the focal spot becomes supersaturated more efficiently by fs laser irradiation than that by CW_{800}. As the applied laser power was set to be the same for both fs and CW_{800}, photon numbers irradiated to the molecules within the pulse duration (i.e., 120 fs) for pulse laser (2.5×10^{23} photons) were 5 orders of magnitude larger than that for CW_{800} (2.4×10^{18} photons). The impulsively applied intense optical force and relaxation/dissociation between fs pulses is assumed here to be critical for the efficient accumulation of glycine clusters [18] and triggers the reorganization of assembled molecules into an ordered crystalline structure, eventually leading to crystallization.

3.2 Temporally controlled crystallization triggering by a combination of continuous wave and femtosecond pulse laser trapping

By combining fs and CW_{1064} laser irradiations, this study also developed a spatiotemporally controlled single crystal fabrication method. A saturated solution (SS = 1.0) rather than an unsaturated one was used to form the local high-concentration region effectively. Formation of multi-crystals by continuous fs pulse irradiation was prevented using sequential irradiation of different modes of laser (Fig. 3a). Closely examining different combinations of irradiation conditions for both lasers revealed typical successful results. Preliminary CW_{1064} irradiation at 400 mW efficiently elevates the local supersaturation and subsequent 50 ms irradiation of fs pulses at 200 mW can trigger the nucleation at the focal point, respectively. Following to the fs irradiation, CW_{1064} was again switched on for trapping and growth of the generated crystal, leading to single crystal formation without damage to multi-crystals. Figures 3b–d show the outcome of representative

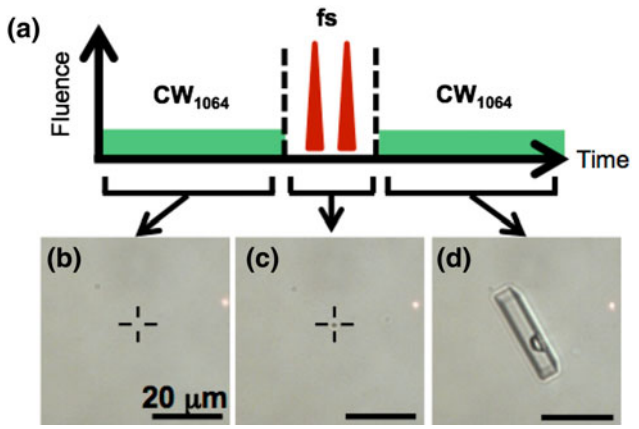

Fig. 3 **a** Schematic illustration of sequential CW, fs, CW laser irradiation scheme. **b** Nothing formed during CW_{1064} trapping, **c** a small crystal formed by fs triggering, **d** a grown crystal under CW_{1064} trapping. The cross hairs correspond to laser focus. Fs laser, 200 mW; CW_{1064}, 400 mW; SS, 1.0

spatiotemporally controlled single crystal fabrication. The preliminary CW_{1064} irradiation for 23 min did not form the crystal (Fig. 3b), while successive fs laser irradiation for 50 ms generated a crystal immediately (Fig. 3c).

Although the generation of a few μm crystal within 1 s for more than 80 % of fs triggered crystallization was observed, some crystals appeared slowly with a few seconds of an interval. Crystal growth is a stochastic phenomenon, and generated crystals must grow more than 1 μm to be recognized under the microscope. Therefore, the observed dispersion in crystal appearance time appears to be owing to the stochastic nature of a nucleation by 50 ms fs laser irradiation and subsequent crystal growth. Multi-crystals formation was successfully prevented by adjusting the fs laser irradiation time as short as possible. The generated crystal was captured by the CW_{1064} laser, which was introduced after the fs pulses and grew to longer than several tens μm (Fig. 3d). Notably, additional crystal generation was never observed without the fs irradiation. Moreover, a defect observed in the crystal (Fig. 3d) under the second CW_{1064} irradiation disappeared during successive crystal growth to the mm size by slow evaporation of solvent. Correspondingly, only one crystal was obtained from the focal spot by triggering the crystallization with high spatiotemporal controllability. Demonstrated crystallization by a combination of fs and CW laser trapping is markedly better than the other laser-induced crystallization methods in spatiotemporal controllability. In particular, temporal controllability of triggering the crystallization is improved by 1–2 orders higher than CW laser trapping crystallization method.

4 Conclusion

This study has demonstrated the laser trapping crystallization of amino acid by using fs laser pulses as a trapping light source. Experimental results indicate that fs laser trapping at the air/solution interface can crystallize glycine more efficiently than CW laser trapping. Higher crystallization probability, shorter crystallization time, lower laser power, and faster crystal growth are demonstrated as well. According to our results, short irradiation of fs laser pulses to locally prepared high supersaturation region by CW_{1064} irradiation can induce the nucleation immediately. Such irradiation triggers a single crystal formation with subsecond temporal and μm spatial controllability, which has never been achieved previously using other crystallization methods. This high spatiotemporal controllability allows us to synchronize crystallization to various time-resolved spectroscopic methods, including X-ray scattering and diffraction, IR, Raman scattering and fluorescence spectroscopies with 100 % crystallization probability. This method is highly promising for use in observing the primary processes of crystallization.

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