Enhancement of Light-induced Nucleation of Lysozyme in the Presence of Polyethylene Glycol (PEG) 4000

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Light-induced nucleation of protein was greatly enhanced by an addition of polyethylene glycol (PEG) 4000. PEG affects to increase reaction rate of the photochemical intermediate to produce lysozyme dimer which is responsible for the light-induced nucleation.

Crystallization of protein is important to reveal its 3D structure by X-ray diffraction crystallography. Recently, photophysical or photochemical light-induced crystallization of protein has been reported.[1–6] In our preliminary studies, we demonstrated photochemically induced nucleation of hen egg-white lysozyme in metastable solution.[7,8] Irradiation by a Xe lamp on supersaturated lysozyme solution in NaCl at pH 4.5, $\beta = 9$, from 10–60 s increased the number of lysozyme crystals in the droplet. The enzyme activity did not decrease within the time that nucleation was enhanced. The lattice parameters of the irradiated crystal are identical to that of the crystal without irradiation. Lysozyme produces intermediate residual tryptophanyl radicals. When the intermediate was scavenged by second photon, light-induced nucleation was inhibited. Therefore, residual tryptophanyl radical was concluded to be responsible for the light-induced nucleation. The light-induced nucleation was also observed in thaumatin system.[9] The nucleation mechanism is considered to be the same to lysozyme system because the same photochemical intermediate, residual tryptophanyl radical, was also observed in thaumatin system. We consider light-induced nucleation as a new method for preparing protein crystals in structural genomics and pharmaceutical industry.

Here, we report enhancement of the light-induced nucleation of lysozyme in the presence of polyethylene glycol 4000 (PEG4000). Though, PEG has been widely used as good crystallizing agent to reduce solubility, the effect of PEG in the light-induced nucleation is expected to be different effect from the understanding ever reported.

A 3-mL solution, containing 0.5-mg·mL^{-1} lysozyme, 1.4 M NaCl, and 0, 0.5, 1.0, and 2.0 wt % of PEG4000 in 50-mM sodium acetate (NaAc) buffer solution at pH 4.3 were placed in a $1.0 \times 1.0 \times 4.0$ cm dimension optical cell. The solutions were irradiated for 0, 30, 120, and 300 s by 300-W Xe lamp. 2 μL of the irradiated solution was mixed with equal amount of concentrated lysozyme solution (40 mg mL^{-1}) in the same buffer on a micro batch well to grow nucleus. Thus, the mixed final solutions contained 20-mg·mL^{-1} lysozyme, 0.7 M NaCl and 0, 0.25, 0.5, and 1.0% PEG4000. Sixteen wells simultaneous micro batch experiments were carried out for each experimental condition. To avoid undesired nucleation, the wells of micro batch plate were covered with paraffin oil and the solution was dropped through oil. The micro batch plate was kept at 20 °C in dark room.

Figure 1 shows photographs of the droplets observed 24 h after the mixing. No crystal was observed in the droplets without containing PEG4000 at any irradiation time. In the droplet containing 0.25% PEG4000 crystals appeared in the irradiated droplet and the numbers of the crystals increased with increasing in the irradiation time. The morphology of lysozyme crystal in the presence of PEG is reported to show longer form compared to lysozyme crystal obtained without PEG system.[10] With increasing in the PEG4000 concentration, the frequency of the appearance of the crystal increased. These results indicate that light-induced nucleation takes place, and strongly suggest that addition of PEG4000 enhances nucleation by light. Addition of PEG4000, however, is known to decrease the solubility of lysozyme; the enhancement of the nucleation by the irradiation is not concluded from this experiment.

To confirm whether the addition of PEG4000 enhance light-induced nucleation in truth, nucleation frequency should be compared at the same supersaturation, i.e., at the same driving force of nucleation. For this purpose, solubility was determined in the presence of PEG4000 by sitting drop experiment. The solubility was determined as 1.1 mg mL^{-1} at 1.4 M NaCl without PEG4000 and 0.37 mg mL^{-1} at 1.4 M NaCl with 0.2 wt %

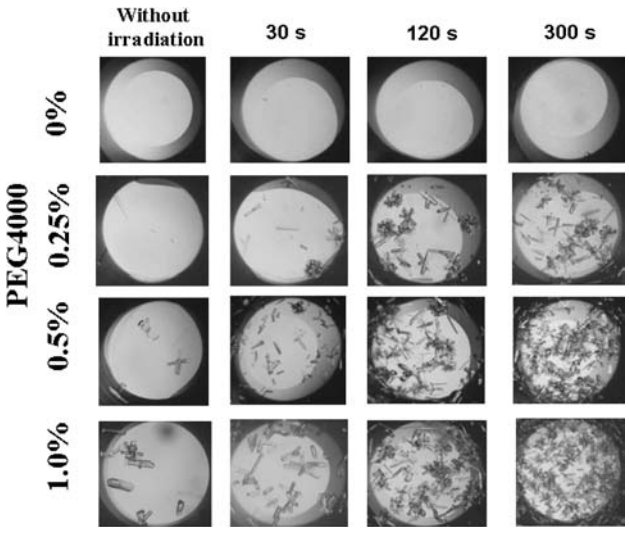

Figure 1. Photographs of the microbatch droplets of lysozyme solution in the presence of various concentration of PEG4000 with/without light irradiation observed at 24 h after the preparation. The diameter of the circle is 1 mm.

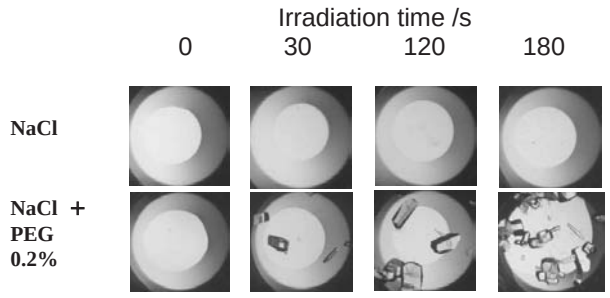

Figure 2. Photographs of the lysozyme solutions on the microbatch plate at the same supersaturation $\beta = 3.5$. with/without PEG4000. Photographs were taken 24 h after the irradiation. Diameter of the center circle is 1 mm.

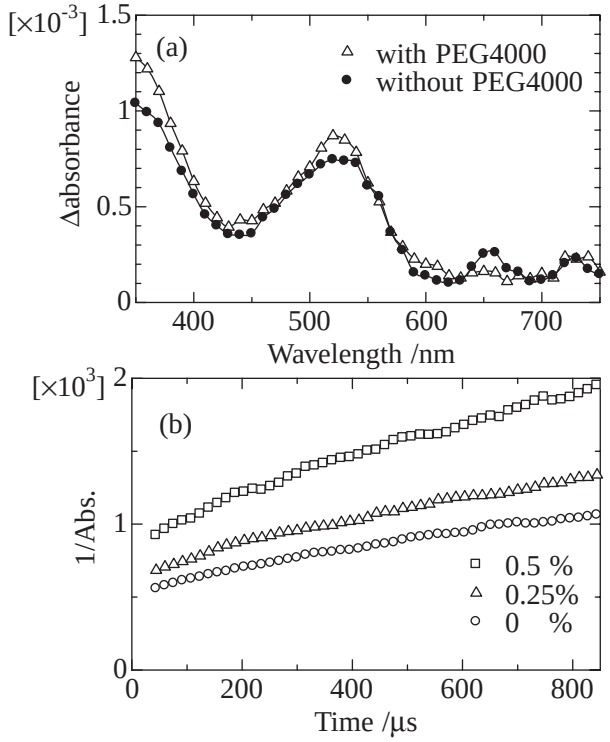

Figure 3. (a) Transient absorption spectra of lysozyme in the presence of PEG4000 (△) and without PEG4000 (●). (b) Reciprocal plot of the time profile at 520 nm, concentrations of PEG4000 are (○) 0%, (△) 0.25%, and (□) 0.5%.

PEG4000 at 20 °C. We prepared $\beta = 3.5$ ($\beta = C/Ce$) supersaturated solution for with/without PEG4000. The concentrations were 3.85 mg mL^{-1} for without PEG4000 and 1.30 mg mL^{-1} lysozyme with 0.2 wt % PEG (both solutions contain 1.4 M NaCl). Each solution was irradiated for 0, 30, 120, and 180 s and then the solutions were mixed with concentrated lysozyme solution on the micro batch plate to grow nucleus. Figure 2 shows the photographs of the droplets. In the droplets without PEG4000, no crystal was observed at any irradiation time. On the other hand, crystal appeared in the irradiated droplets containing PEG4000 and the numbers of the crystals increased in the irradiation time. Thus, the addition of PEG4000 enhances light-induced nucleation even at the same supersaturation.

Finally, we will discuss the mechanism of light-induced nucleation by the addition of PEG4000. To clarify the function of PEG4000 to the intermediate, transient absorption experiment was carried out. Experimental procedure was described in the literature.[7] Figure 3a shows the transient absorption spectrum of lysozyme with/without PEG4000 (0.2 wt %) 64 μs after the laser excitation. Observed spectrum was residual tryptophanyl radical having peak at 520 nm.[11,12] As the spectrum is identical to transient spectrum without PEG4000, PEG4000 does not affect initial photochemical reaction. Figure 3b shows the reciprocal plots of the time profile at 520 nm by changing PEG4000 concentration. All the plots show linear dependence against the time, which means that the radical decays through second-order process. With increasing in the PEG4000 concentration, the slope increased. This result indicates that dimer formation rate becomes larger by the addition of PEG4000. Since the addition of PEG4000 enhances light-induced nucleation, the dimer formation seems to relate to the light-induced nucleation.

From these results, we propose the mechanism of light-induced nucleation. First step of normal nucleation process begins from two monomers form $n = 2$ smallest cluster. The smallest cluster result from weak molecular interaction (van der Waals interaction or hydrogen bond) is the most unstable cluster due to surface free energy disadvantage. Thus, the $n = 2$ cluster formation is the rate-determining step among the nucleation process. On the other hand, if the photochemical dimer behaves the same as a $n = 2$ cluster to grow trimer, tetramer to the critical size of the nucleus, the nucleation process have an advantage starting from stable species. Therefore, the enhancement of the nucleation can be explained. Now, we are preceding several experiments to detect the dimer.

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