Thin Film Formation of a Protein by Laser Ablation Deposition Technique

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The ablation deposition technique was applied to a protein system for the first time. The sample polypeptide was silk fibroin with various excellent properties as a functional material. Chemical structures and surface morphology of thin films deposited by the technique were analyzed by infrared spectroscopy and atomic force microscopy, respectively. It is demonstrated that the ablation deposition is a powerful method to form a thin protein film.

In the past decade, laser-ablation deposition technique to form thin films has undergone explosive developments mainly in the field of inorganic material science.[1] Although the deposition technique has several advantages compared to conventional procedures to prepare thin films, applications of the technique to organic polymer systems have hitherto been limited to a few works.[2-5] In laser ablation of organic materials, whether the mechanism is photochemical[6,7] or photothermal,[8,9] aspects of chemical composition in ablation plume ejected seem to be quite complicated. Therefore, it is rather difficult to predict an applicability of the deposition technique to organic materials.

Recently, we examined the applicability to silk fibroin which is one of the most popular protein systems. Proteins have high potentials in the fields of molecular devices and bioelectronics. In particular, as a functional material silk fibroin has excellent properties in terms of thermal stability, fixing ability for enzymes, selective permeability for water, and fitness to biological tissues, etc..[10] Silk fibroin, however, has quite poor solubility to any solvents, hence it is important as well as indispensable to establish a convenient method to form the thin films. In addition, since the primary structure of fibroin is quite simple (ca. 80% of aminoacids are alanine and glycine), it would be a promising prototype for analyzing mechanisms underlying laser ablation of proteins. In the present report, we demonstrate that the ablation deposition is a powerful method to form a thin protein film.

Commercially supplied silk fibroin (Wako) was pressed to give a pellet of an ablation target. An excimer laser (Lambda Physik, EMG101, fwhm~20 ns) provided 351 or 248 nm pulses, which were introduced into a vacuum chamber (~ 10^{-3} mmHg) to induce ablation. Laser fluence was fixed to be 700 mJ/cm^2. Ablation plume was deposited onto a quartz or ZnSe substrate. Infrared spectra were measured with FT-IR spectrometer (Fuji Electric, Firis-100). An atomic force microscope (AFM, Digital Instruments, Nanoscope III) was used in the tapping mode. All the procedures were performed at room temperature.

The ablation threshold of the fibroin target was found to be ~500 mJ/cm^2 at 351 nm. The deposition with > ten thousands laser shots resulted in formation of colorless and transparent thin films. Figure 1 shows IR spectra of the target fibroin and thin films deposited onto ZnSe substrates. IR absorption spectroscopy is frequently applied in relevant research fields.[2-4,11] In addition, it is generally accepted that IR absorption spectroscopy is quite effective to determine not only primary structures of proteins but also secondary ones. As clearly seen in the figure, the spectrum of the thin film deposited at 351 nm was almost identical to that of the fibroin target over the wide spectral region. Namely, the bands of amido I at 1650 (C=O stretching), amido II at 1530 (N-H deformation and C-N stretching), and amido III at 1240 cm^{-1} (C-N stretching and N-H deformation) were well reproduced in the spectrum of the deposited film. Weak bands below ~1500 cm^{-1} were also in agreement with those of the target. These results clearly indicate that a thin fibroin film where the primary structure is almost maintained is successfully reconstructed by the ablation

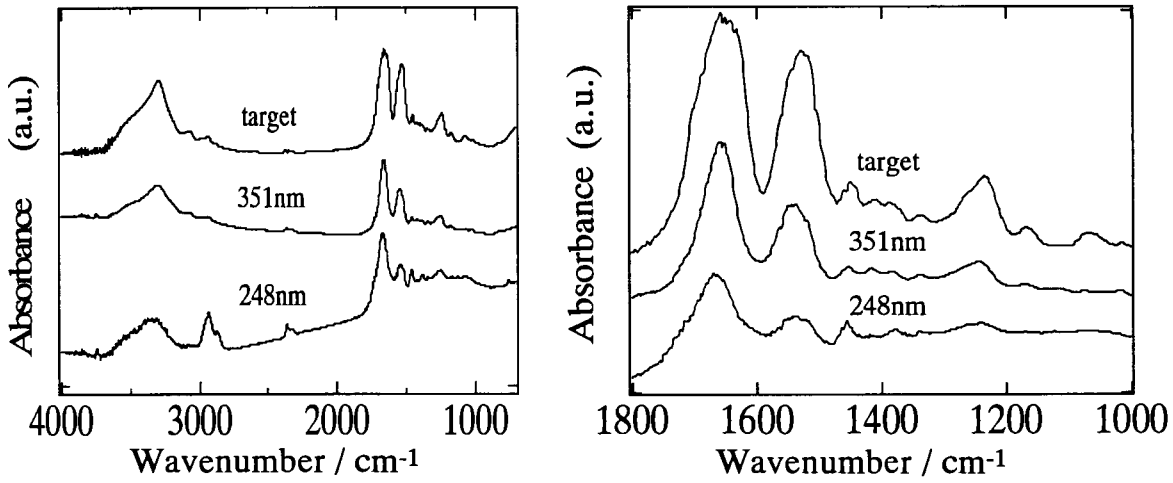

Figure 1. IR absorption spectra of target fibroin and deposited films. The right pannel indicates the expanded spectra in the lower wavenumber region. Laser wavelength is given in the figure.

deposition at 351 nm. Moreover, detailed analysis of slight shift of peak maxima of the three amido bands which are sensitive to the secondary structure suggested that a great part of the deposited film comprises random coil structures, although the structure of the target fibroin is the β-sheet type.

On the other hand, in the case of the film deposited at 248 nm, a band around 2900 cm^{-1} assigned obviously to C-H stretching mode appeared additionally, and the small peaks were hardly observed in the lower wavenumber region than 1500 cm^{-1}. This implies that a large part of primary structures of fibroin was destroyed due to relatively high photon energy of 248 nm light.

In Figure 2 are shown AFM images of the thin films deposited onto quartz plates at 351 and 248 nm. Also in this case, the surface morphology exhibited perceptible dependence of the excitation wavelength. In both of the frames, several particle-like projections were observed on the films which covered the substrates entirely, however, their size differed from each other. In the film deposited at 351 nm, the size of particles lay in the order of 1 - 2 μm, while it was relatively small (< 0.5 μm) at 248 nm.

The excitation wavelength dependencies of the chemical structure and the surface morphology described above are tentatively explained as the following in terms of ablation mechanisms. Silk fibroin contains aminoacids with aromatic rings (i.e. tryptophane ~ 0.2 mol%). The 351 nm laser light slightly excites the aromatic rings and impurities involved in the sample fibroin. Presumably, on the analogy of the ablation mechanism of doped polymers[12,13] and aromatic vinyl polymers,[14] ablation of fibroin at 351 nm proceeds photothermally. In this case, the aromatic moieties and the unknown impurities are considered to play a role as the efficient light-to-heat energy converter.[12] Large debris of the polypeptide chains ejected photothermally with less damages are deposited onto the substrate to reconstruct the fibroin film with the surface morphology of Figure 2 (a). On the other hand, fibroin has a strong absorption band originating from peptide bonds below 250 nm. Light excitation of this band monophotonically leads to the main chain scission of peptide bond (-CHR-CONH-CH_2- ----hν----> -CHR-CO・ + ・NH-CH_2-) followed by numerous radical chain reactions and rearrangements.[15] Namely, the various small fragments formed by the 248 nm photochemical reactions are deposited to give the different IR spectrum from that of fibroin. The relatively small particle-like projections seen in the film deposited at 248 nm would correspond to aggregates of the various small fragments formed by these reactions.

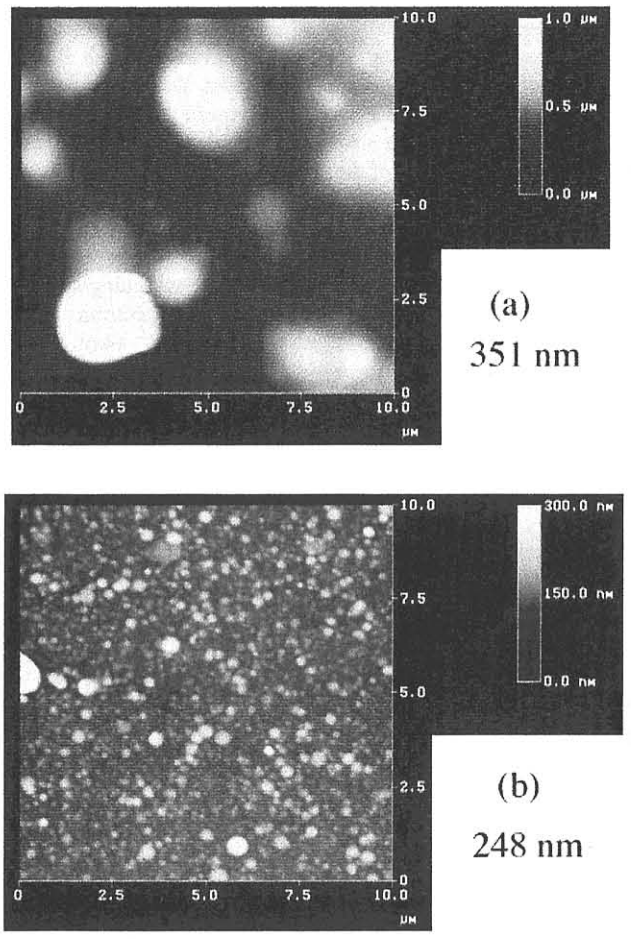

Figure 2. AFM images of thin films deposited by 351 (a) and 248 nm (b) laser ablation .

In conclusion, the ablation deposition at 351 nm is quite effective to form a thin film of silk fibroin which is a typical bioprotein. The morphological aspect revealed by AFM is reconcilable with the ablation mechanism deduced from the results of the IR spectroscopy. Toward applications of protein films to molecular devices, it is indispensable to control the secondary structures and such study is currently in progress.

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