TOPICS

Molecular Recognition and Immobilization of Biomolecules Induced by Photo-Irradiation on the Surface of an Azopolymer

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アゾポリマー表面への生体分子の分子認識光固定

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The development of genomics has produced a large demand for DNA micro array technology. To gain a more profound understanding of biological science, in-depth analysis of the functions of proteins, sugar chains and cells will become considerably important for the next generation. Tools for biological analysis, such as protein arrays, will be widely used for elucidating biological function. Technology for immobilizing biological materials onto substrates will play a crucial role in the development of such tools. We designed a new method for immobilizing biological molecules, which utilizes deformation on the surface of a polymer film induced by photo-irradiation.

Figure 1 shows the principle of the immobilizing process. Biological molecules on an azopolymer containing an azobenzene moiety are irradiated with visible light. The surface deforms around the shape of biomolecule by a mass transfer of the azopolymer. The transfer is induced by the plasticization of azopolymer through a photo-isomerization process. It can be argued that the shape of the biomolecule is recognized by the azopolymer through a photo response. An increase in the contact area between the molecules and the surface enhances the immobilizing force.

It is well known that an azopolymer including a push-pull type azo moiety shows a photo-induced modification of the surface relief structure.[1] We demonstrated that a hexagonally arrayed structure of polystyrene microspheres a monolayer thick was transcribed onto the azopolymer surface as an indented structure.[2] In the present study, we found that the microspheres were more strongly fixed to the surface of the azopolymer with increased irradiation power and time. The immobilized spheres thus prepared remained on the surface after ultrasonic washing.

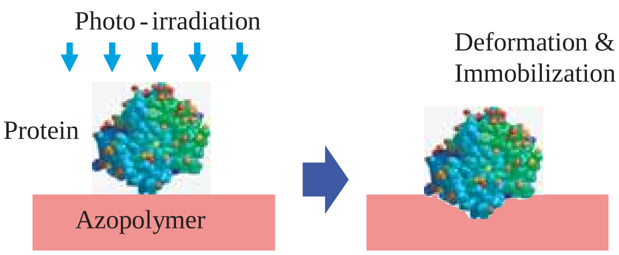

Fig. 1 A schematic principle for the immoblization of biomolecules induced by photo-irradiation.

Furthermore, we examined the immobilization of biomolecules. Linearly focused laser light was irradiated onto a λ-DNA solution sandwiched between the surface of the azopolymer and a glass cover. After washing and staining with cyanine dye, images of linearly immobilized DNA were obtained with fluorescent and optical microscopes. Dye-labeled immunoglobulin solutions with various concentrations were spotted on the azopolymer ($1\mu l$) followed by drying and photo-irradiation with a LED light source. After washing with buffer solution and drying, the immobilized immunoglobulin was detected by the fluorescent emission. The immunoglobulin could be detected down to nearly 10 pg. The surface of the sample was measured by AFM and approximately mono-layered immunoglobulin was confirmed to be immobilized on the azopolymer. The sample surface was treated with a surfactant to wash off the immunoglobulin, and was again measured by AFM after verifying that fluorescence was not emitted from the spot. An indentation of a few nanometers scale was observed on the surface. These results indicate that the deformation was induced by photo-irradiation and that immobilization was enhanced by the increase in contact area.

Human and bovine serum albumin (HSA and BSA, respectively) solutions were immobilized by photo-irradiation on the azopolymer surface. The immobilized sample was treated with anti-HSA mouse-antibody solution followed by dye-labeled anti-mouse IgG goat antibody. After drying, fluorescent emission was only detected from the HSA spot. The selective antigen-antibody reaction was able to be performed on the immobilized biomolecules by photo-irradiation.

The novel photo-induced immobilization of biomolecules exhibits the following advantages compared to conventional techniques: a huge variety of biomolecules can be immobilized, it allows position-controllable immobilization, and there is less damage to biomolecular function. Therefore, the immobilizing method has application to biological chips, biological sensors and biological reactors.

References

1) Batalla, E., et al. : Appl. Phys. Lett., **66**(1995), 136
2) Kawata, Y., et al. : Opt. Commun., **161**(1999), 6

(Report received on Jul. 21, 2004)