Photocontrolled Attachment of Anti-Azobenzene Antibody onto Azobenzene-Linked Quartz Surfaces

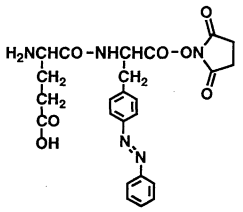
Kazuo Kawashima, Masahiko Sisido,*† and Kunihiro Ichimura
Research Laboratory of Resources Utilization, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 227
†*Department of Bioengineering Science, Faculty of Engineering, Okayama University, 3-1-1 Tsushimanaka, Okayama 700*

(Received March 2, 1995)

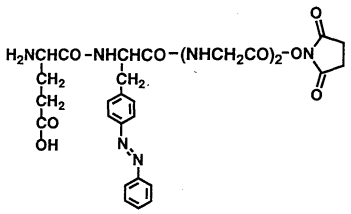
Peptides carrying azobenzene group were covalently linked to surfaces of quartz plates and the plates were reacted with a monoclonal antibody against *trans*-azobenzene group. The amount of antibody attached was estimated by an AFM observation of gold-labeled anti-mouse IgG antibodies that were bound to the anti-azobenzene antibody. The number of the gold particles decreased significantly when the peptides on the quartz plate were irradiated with UV light prior to the attachment of the anti-azobenzene antibody.

Monoclonal antibodies have been widely used as tools in organic chemistry.[1] We have reported previously that monoclonal antibodies against a nonnatural amino acid, L-*p*-phenylazophenylalanine showed on/off switching for the binding and release of the peptide antigen, when the azobenzene group was photoisomerized between *cis* and *trans* state reversibly.[2,3] The on/off switching has been applied to control electron mediation between two redox enzymes.[4] In this letter we report that the photoswitching can be applied to control the attachments of antibodies and other small particles onto a surface linked with peptides carrying an azobenzene group.

A quartz plate was reacted with aminopropyltriethoxysilane by a conventional procedure and the amino groups on the surface were reacted with the hapten peptide N-hydroxysuccinimide esters (**1** and **2**). The corresponding fully protected peptide esters [Boc-Glu(O^{t}Bu)-azoAla-ONSu and Boc-Glu(O^{t}Bu)-azoAla-Gly$_2$-ONSu, azoAla=L-*p*-phenylazophenylalanine, Boc=*t*-butyloxy-carbonyl, ONSu=N-hydroxysuccinimide, O^{t}Bu=*t*-butyl ester] were prepared by conventional liquid-phase method.[2,3] The Boc and ^{t}Bu groups were removed by 3N HCl in dioxane for 60 min and the deprotected peptide esters were reacted with the aminosilylated quartz plate in THF.



Glu-azoAla-ONSu (1)



Glu-azoAla-Gly-Gly-ONSu (2)

The quartz plates were then immersed into a solution of anti-azobenzene antibody[1] (Z1H01, 1.0x10^{-7} M) for 60 min at room temperature and rinsed with phosphate buffer (pH=7.0) containing polyoxyethylene sorbitan monolaurate (Tween20) to remove the antibodies that are nonspecifically adsorbed onto the surface. After drying the plates under vacuum, atomic force microscope (AFM) observation was attempted to visualize the antibodies attached onto the surfaces. However, because of the intrinsic roughness of the quartz surfaces, we could not observe the antibody molecules. In other experiments using a mica plate covered with a monolayer of hapten-containing phospholipids, a single molecule of antibody has been observed by AFM.[5]

To detect the antibodies on the surfaces, a goat anti-mouse IgG antibody labeled with fluorescein isothiocyanate [ZYMED, FITC-Goat anti-mouse IgG(Gamma)] was reacted. A FITC labeled antibody solution (4x10^{-8} M) was put onto the plates for 60 min and the plates were rinsed with the PBS(Tween) and then with distilled water. Fluorescence measurements were carried out after the plates were dried under vacuum. The quartz plate linked with the hapten peptide **1** showed higher fluorescence intensity of FITC than that without the hapten peptide, indicating the presence of anti-azobenzene antibody on the surface. However, it was impossible to evaluate absolute quantity of anti-azobenzene antibodies from the fluorescence measurement.

To evaluate the number of anti-azobenzene antibodies on the surface, an anti-mouse IgG goat antibody labeled with a gold microparticle was attached on the surface and the number of gold particles was counted with AFM (Seiko, SFA300). The plates linked with the peptide haptens and reacted with the anti-azobenzene antibody, was immersed into the gold-labeled antibody solution (ZYMED, Gold-Goat x mouse IgG, 20 nm, Blot Grade) for 60 min and rinsed with PBS(Tween) and then with distilled water. The same procedure was applied to the plate irradiated with UV light before the contact with the anti-azobenzene antibody. The UV irradiation was carried out with a 200W Hg/Xe lamp through a bandpass filter (350±20 nm) (Toshiba UV-D36B) for 5 min. Under these conditions, about 85 % of azobenzene groups turns to *cis* form in solution, but the content of *cis* form in the peptides on the plates could not be determined.

The AFM pictures of the gold particles attached on the plates linked with the peptide **1** are shown in Figure 1. Figure 1a shows the AFM picture of the plate with *trans* azobenzene groups (without UV irradiation) and Figure 1b is that with UV irradiation prior to the reaction with anti-azobenzene antibody. Similar AFM pictures with the peptide **2** are shown in Figures 2a and 2b, respectively. The average numbers of gold particles on various surfaces are counted and listed in Table 1.

The numbers of gold labeled antibodies bound onto the surfaces linked with peptides carrying *trans*-azobenzene groups (Figures 1a and 2a) are much larger than those with *cis*-rich-azobenzene groups (Figures 1b and 2b). The results indicate that anti-azobenzene antibodies were bound to the hapten peptide in the *trans* form, and the bound antibodies are visualized by the attachment of gold labeled secondary antibodies. The numbers of gold particles on the UV-irradiated surfaces cannot be neglected especially on the surface linked with the peptide **2**. This may be interpreted in terms of the remaining *trans* azobenzene under the photostationary state.

It may be argued that the polarity of the quartz surfaces becomes higher when the peptides become *cis* form, and the

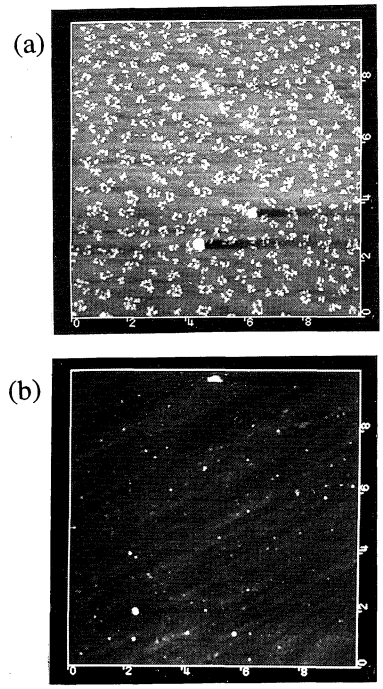


Figure 1. AFM pictures of gold-labeled anti-IgG antibodies bound to anti-azobenzene antibodies reacted on a hapten-linked quartz surfaces. Hapten=Glu-azoAla (**1**), (10μmx10μm). (a) Anti-azobenzene antibody was reacted before UV irradiation, (b) the antibody was reacted after UV irradiation.

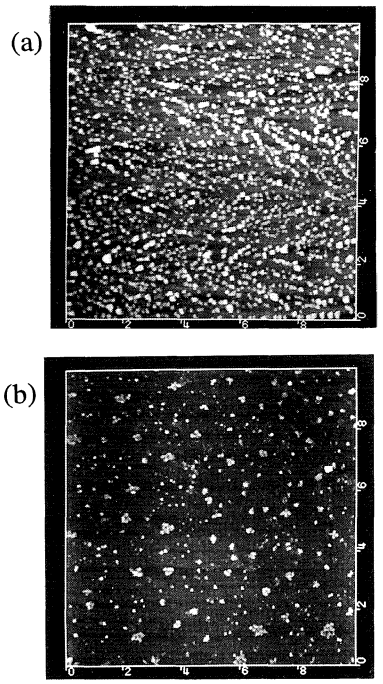


Figure 2. AFM pictures of gold-labeled anti-IgG antibodies bound to anti-azobenzene antibodies reacted on a hapten-linked quartz surfaces. Hapten=Glu-azoAla-Gly-Gly (**2**), (10μmx10μm). (a) Anti-azobenzene antibody was reacted before UV irradiation, (b) the antibody was reacted after UV irradiation.

Table 1. Number of gold particles on the surfaces covered with various hapten peptides and reacted with various antibodies

Hapten peptide/Antibody	No. of gold particles/(2μmx2μm)	
	In the dark	Uv-irradiated
1/anti-azobenzene	38±8	3±2
2/anti-azobenzene	78±10	18±4
1/anti-pyrene	5±3	9±4
2/anti-pyrene	12±5	14±5
1(Fully protected)/anti-azobenzene	16±5	5±5

antibody adsorption may be suppressed on the polar surfaces. To examine this possibility, anti-pyrenyl antibody (P1I03)[6,7] was used instead of anti-azobenzene antibody. The number of gold particles on the surfaces linked with the peptide in the *trans* form and that on the surface with the *cis*-rich peptide were counted (Table 1). The number of gold particle was small in both cases, indicating that the attachment of anti-azobenzene antibody is really caused by a specific binding to the *trans* azobenzene groups.

The binding experiment was also carried out on the quartz surface linked with a fully protected hapten peptide, Boc-Glu(O^{t}Bu)-azoAla-Gly$_2$. In this case, a smaller number of anti-azobenzene antibody was detected on the surface with *trans* peptides than that with free peptides. After UV-irradiation, the number became very small.

To conclude, the above experimental data indicate that *trans* azobenzene-specific binding took place on the quartz surface linked with the hapten peptide carrying a *trans* azobenzene group. Only very small number of the antibody was attached on the surface with *cis*-rich azobenzene group. The photoregulated antibody binding will find applications to the photopatterning of gold particles for microwiring and to the photopatterning of various protein molecules for sequential arrangement of enzymes.[8]

References and Notes

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