Immobilization of Protein on Micropatterns by the Use of Photoremovable Activated Ester

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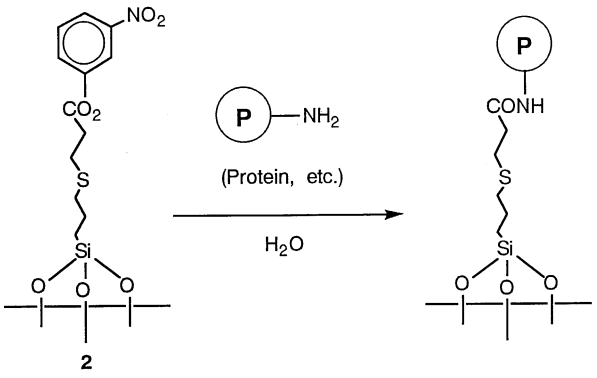
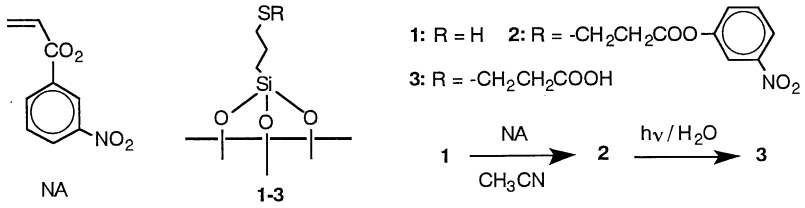
3-Nitrophenoxycarbonyl group was introduced to a silylated glass surface with 3-mercaptopropyltrimethoxysilane (MPS) through the Michael addition of corresponding acrylic acid ester to the mercapto group of the MPS. The nature of this ester group, reactivity to amino group giving amide bond (activated ester) under dark and to water giving a corresponding carboxylic acid upon UV-irradiation, was utilized to prepare micropatterns of protein on the glass surface.

Micropatterning of material surfaces has been a current subject from viewpoints of microelectronics, microoptics, molecular devices, and also biotechnology. Immobilization of functional molecules on a minute area is a challenging technique for studying their chemical behavior in confined space or at interphase.[1] Protein and other biomaterial have attracted much interest as target molecules due to their highly specific chemical response as seen in immunoassay.[2] Microfabrication with these substances, however, will be essentially different from that with other polymeric or inorganic materials, since treatments of biomaterials including immobilization are strictly limited by their deactivation such as denaturation. As an organic interface between those substance and material surface, use of molecular-order thin layer such as self-assembled monolayers will be advantageous to avoid the problem caused by direct contact with material surface in addition to some reasons: thinness (< 2 - 3 nm), wide applicability for various materials, and capability for further chemical modification.[3] These properties of the monolayers are based on covalent bonding to materials, which insure chemical and physical stability towards contact with various solvents in chemical fabrications. For instance, LB film or polymer cast film is less stable towards chemical treatment with solution, which will limit their use as a binding layer. Introduction of a functionality which enables both micropatterning and immobilization to the thin film also will be an important subject. We report in this communication, chemical behavior of 3-nitrophenoxycarbonyl group introduced in thin films of an organic silane as a photoremovable activated ester and spatially-controlled immobilization of protein and some aminopolymers.[4,5]

Thin layer of 3-mercaptopropyltrimethoxysilane (MPS) (**1**) was prepared on quartz substrate.[6] 3-Nitrophenoxycarbonyl group was introduced by the Michael addition of 3-nitrophenyl acrylate (NA) to the mercapto group.[7] The resulted glass plate bearing the thin layer (**2**) was placed in water in a quartz cuvette together with a copper mesh (40 μm width) as a photomask, and was irradiated with a KrF laser (30 mJ cm^{-2} $pulse^{-1}$, 3000 pulses). Contact angle with water of the irradiated area (30°) was smaller than that of unirradiated one (40°) to indicate formation of hydrophilic group. Furthermore, pH dependence of the wettability of the irradiated surface was observed for buffer solutions with various pH. The contact angle with solution of pH 10 was (22°) lower than that with a solution of pH 4.5 (39°). On the other hand, little pH dependence was observed for the unirradiated surface (40 ± 3°). These behaviors can be due to the presence of acidic group at the surface.[8] As a model reaction, 3-nitrophenyl hexanoate was irradiated at 248 nm in acetonitrile/water (1/1 v/v). Hydrolytic photodecomposition of the ester giving corresponding acid was observed. These results strongly suggested hydrolysis of 3-nitrophenoxycarbonyl group giving carboxylic group (thin layer **3**) in the thin layer upon photoirradiation and not to photodegradation of acylalkylthio group (Scheme 1).[6b]

The glass plate irradiated through a mesh was immersed in a phosphate buffer solution (pH7.4) of bovine serum albumin (BSA) monomer (Sigma) for 1 h. After the removal of excess BSA with the phosphate buffer solution for 4 h, the plate was stained with tetramethylrhodamineisothiocyanate (TRITC) for observation by fluorescence microscopy. The unirradiated area was observed as a fluorescent pattern where BSA was immobilized. This can be attributable to reaction of 3-nitrophenoxycarbonyl group of **2** with amino group of BSA to form amide bond as reported by Bodanzky for condensation between





Scheme 1.

3-nitrophenyl ester of an N-protected aminoacid (phthalylglicine) and a C-protected aminoacid (ethyl glycinate),[4] and, therefore, simple adsorption of BSA can be ruled out. Whereas, the irradiated area showed little fluorescence, where immobilization or adsorption of BSA was suppressed by hydrophilicity.[6a] Similar results were obtained for other amino-polymers such as polyallylamine or polyethyleneimine. The present method does not require any coupling reagents in immobilization process since **2** itself has selective reactivity toward amino group. Thus, the present approach will be fruitful in preparing micropatterns of various functional proteins and biomolecules, leading to development of future biosensors.

References and Notes

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7 Conditions for the conversion of **1** to **2** such as solvent or use of catalyst were not optimized.

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