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Photocontrol of Biological Activities of Protein by Means of a Hydrogel

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Because proteins show high activity and are essential for biological function, proteins are important and useful biomolecules; however, it is hard to control activities whenever and wherever required. Although some photoactivated proteins have been reported to date, such proteins have required a protein-specific design and site-specific chemical modification. We have recently developed a method to encapsulate proteins within hydrogels that can be photocleaved with ultraviolet (UV) light, thus releasing the proteins; we refer to this method as "protein activation and release from cage by external light (PARCEL)." Biological activities of protein restricted by hydrogel encapsulation were recovered by applying external light to the protein-hydrogel. We also used these hydrogels to screen selective ligands as therapeutic agents for disease. This innovative technique for basic research in biology and biochemistry might also be useful in practical or clinical applications, such as biosensing, catalysis, and drug delivery.

Proteins have several remarkable advantages over conventional chemical catalysts, including high selectivity and efficiency; absence of undesirable side reactions; operability under mild conditions, such as in water at ambient temperature; and environmental friendliness. For these reasons, proteins are widely used in biotechnology, industry, and clinical trials.[1−3] However, proteins are denatured by heat and pH changes, are less soluble than some conventional catalysts, and also can suffer from autolytic degradation, all of which result in an overall decrease in the proteins' usability. Many trials have been performed in efforts to determine ways to utilize proteins more easily and universally while maintaining their optimal protein activity.[4,5] One strategy to prevent loss of biological activity of protein is to store proteins under conditions that reversibly render the proteins inactive; then, when protein activity is required, an activation stimulus is applied to the stored proteins. Many external stimuli can provide such activation, including physical stimuli (temperature, light, electric field), chemical stimuli (pH, specific ions, ionic strength), and biochemical stimuli (metabolites).[6] Of these stimuli, light is the most useful and promising to control biological activity of protein in vitro and in vivo, because light irradiation can be applied with spatial and temporal limitation for expression of protein activity at specific times or sites.[7] However, photocontrol of biological activity of protein is difficult, because it requires modification of proteins with a photoresponsive unit to control protein activity.[8−13] Furthermore, this modification must be site specific with regard to the catalytic center of the protein. Therefore, the development of a universal and simple preparation method for photocontrollable proteins is highly desirable.

To this end, we have developed a hydrogel-based protein immobilization scheme that allows proteins to maintain their activity for a long period of time (more than one year).[14−17] This hydrogel consists of a hydrophilic polymer swollen by water that is insoluble owing to physical and chemical cross-links and is able to encapsulate proteins without chemically bonding to them. In this study, we developed a photocleavable hydrogel that can encapsulate proteins and release them when subjected to ultraviolet (UV) irradiation. (We refer to this technique as "protein activation and release from cage by external light," abbreviated PARCEL.) Because the proteins were encapsulated by the hydrogel network physically (i.e., not by means of chemical bonding) and because the hydrogel network collapsed upon irradiation, we

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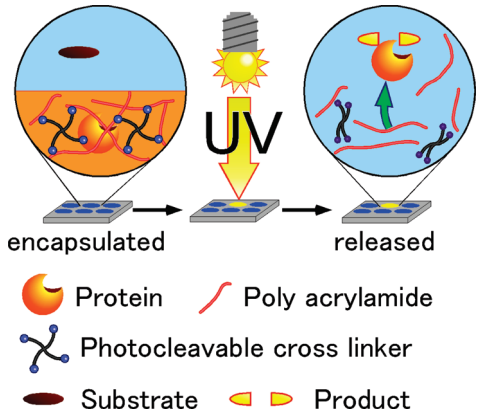

Figure 1. Representation of photocontrolled enzymatic reaction.

expected that a variety of encapsulated proteins would exhibit protein activity after being released from the hydrogel by UV irradiation, as depicted schematically in Figure 1.

EXPERIMENTAL SECTION

Materials. Tetra-poly(ethyl glycol)-amine (SUNBRIGHT PTE-050PA; M_n, 5328 g/mol) was purchased from NOF Corporation (Tokyo, Japan). Tetraethylmethylene diamine (TEMED), dichloromethane (DCM), acrylamide (AAm), acryloyl chloride (AC), triethylamine (TEA), *N*-ethyldiisopropylamine (DIEA), 1-methyl-2-pyrrolidone (NMP), ammonium persulfate (APS), tris (hydroxymethyl) aminomethane, hydrochloric acid, trypsin, papain, γ-glutamyltransferase from beef kidney, diethyl ether, sodium bicarbonate, and magnesium sulfate were purchased from Wako Pure Chemical Industries (Osaka, Japan). 1-Hydroxybenzotriazole (HOBt), 2-(1H-benzotriazole-a-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate (HBTU), and thermolysin were purchased from Peptide Institute (Osaka, Japan). Elastase pancreatic type I from porcin, 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenyl] butyric acid, and α-chymotrypsin from bovine pancrease type II were purchased from Sigma-Aldrich (St. Louis, MO). An EnzChek green fluorescence protease assay kit was purchased from Molecular Probes (Eugene, OR). An enzyme-linked immunosorbent assay (ELISA) kit for measuring human PCSK9 which contained lyophilized recombinant PCSK9 was purchased from CycLex (Nagano, Japan). Water was purified with a Milli-Q apparatus (Millipore, Bedford, MA).

Synthesis of Photo-Ac. The photolabile molecule 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitro-phenoxy] butyric acid (**1**) (1 mmol) was dissolved in dry DCM and stirred in a lightproof vial purged with N_2 gas. TEA (3 mmol) and AC (2.5 mmol), both dissolved in dry DCM, were added dropwise at 0 °C. The reaction was stirred at room temperature overnight. The reaction solution was washed with sodium bicarbonate (5 w/v % aq), dilute hydrochloric acid (1 v/v % aq), and water. The solution was evaporated, and the liquid product was dissolved in aqueous acetone (50 v/v % aq). This reaction mixture was stirred at room temperature overnight and then extracted with DCM to recover the liquid acrylated monomer. The DCM layer was washed with dilute hydrochloric acid (1 v/v % aq) and water, dried over magnesium sulfate, and evaporated to yield the photocleavable acrylate monomer referred to as Photo-Ac (**2**) (Scheme 1a). The structure of the compound (**2**) was confirmed by ^{1}H NMR.

^{1}H NMR (300 mHz DMSO-d_6): δ = 12.35 (br, $CH_2COO\mathbf{H}$), δ = 7.55 (s, Aromatic-**H**), δ = 7.15 (s, Aromatic-**H**), δ = 6.35, 5.95 (d, d, OC(=O)CH=C$\mathbf{H_2}$), δ = 6.25 (dd, OC(=O)C**H**=CH_2), δ = 5.25 (q, Aromatic-C**H**(CH_3)OC(=O)CH=CH_2), δ = 4.1 (t, Aromatic-O$\mathbf{CH_2}CH_2CH_2COOH$), δ = 3.9 (s, Aromatic-O$\mathbf{CH_3}$), δ = 2.4 (t, Aromatic-$OCH_2CH_2\mathbf{CH_2}COOH$), δ = 2.0 (m, Aromatic-$OCH_2\mathbf{CH_2}CH_2COOH$), and δ = 1.4 (d, Aromatic-CH$\mathbf{CH_3}$).

Synthesis of PEG-Photo-Ac (Cross-Linker with Photocleavable Moiety). Photo-Ac (**2**) (0.85 mmol) was dissolved in NMP, purged with N_2 gas, and stirred. The coupling agent HBTU (0.92 mmol), HOBt (0.92 mol), and DIEA (1.67 mmol) were added, and the mixture was stirred for 5 min; then, tetra-poly (ethylene glycol)-amine (**3**; tetra-PEG-amine; 0.042 mmol) in NMP was added. The reaction mixture was intermittently vortexed until all reactants were dissolved. The reaction was stirred at room temperature overnight. The product was precipitated in diethyl ether on ice and filtered. The collected substance was washed with diethyl ether and dissolved in water. The aqueous solution was dialyzed (SpectraPor6, CO 1000 g/mol) and freeze-dried to yield the tetra-acrylated PEG referred to as PEG-Photo-Ac (**4**) (Scheme 1a).[18] The structure of compound **4** was confirmed by ^{1}H NMR.

^{1}H NMR (DMSO-d_6): δ = 8.0 (br, C(=O)N**H**CH_2CH_2O), δ = 7.55 (s, Aromatic-**H**), δ = 7.15 (s, Aromatic-**H**), δ = 6.35, 5.95 (d, d, OC(=O)CH=C$\mathbf{H_2}$), δ = 6.25 (dd, OC(=O)C**H**=CH_2), δ = 5.2 (q, Aromatic-C**H**(CH_3)OC(=O)CH=CH2), δ = 4.1 (m, Aromatic-O$\mathbf{CH_2}CH_2CH_2COOH$), δ = 3.9 (s, Aromatic-O$\mathbf{CH_3}$), δ = 3.7 (m, $NHCH_2$ $CH_2C\mathbf{H_2}O$), δ = 3.5 (br, $[C\mathbf{H_2}C\mathbf{H_2}O]_n$, $n \approx 28$), δ = 3.3 (s, O$\mathbf{CH_2}$C), δ = 3.2 (m, NH$\mathbf{CH_2}$ CH_2CH_2O), δ = 2.4 (m, Aromatic-$OCH_2CH_2\mathbf{CH_2}$COONH), δ = 1.9 (m, Aromatic-O$\mathbf{CH_2}CH_2CH_2$COONH), δ = 1.6 (m, $NHCH_2$ $C\mathbf{H_2}CH_2O$), and δ = 1.4 (d, Aromatic-CH$\mathbf{CH_3}$).

Synthesis of PEG-Ac (Cross-Linker without Photocleavable Moiety). The tetra-PEG-amine (0.01 mmol) was dissolved in dry DCM and stirred in a lightproof vial purged with N_2 gas. TEA (0.12 mmol) and AC (0.1 mmol) in dry DCM were added dropwise at 0 °C. The reaction was stirred at room temperature overnight. The product was precipitated in diethyl ether on ice, and the suspension was filtered. The collected substance was washed with diethyl ether and dissolved in water. The aqueous solution was dialyzed (SpectraPor6, CO 1000 g/mol) and freeze-dried to yield the tetra-acrylated PEG referred to as PEG-Ac (**5**). The structure of compound **5** was confirmed by^{1}H NMR.

^{1}H NMR (DMSO-d_6): δ = 8.0 (br, C(=O)N**H**CH_2CH_2O), δ = 6.15 (dd, OC(=O)C**H**=CH_2), δ = 6.05, 5.95 (d, d, OC(=O)CH=C$\mathbf{H_2}$), δ = 3.7 (m, $NHCH_2$ $CH_2C\mathbf{H_2}O$), δ = 3.5 (br, $[C\mathbf{H_2}C\mathbf{H_2}O]_n$, $n \approx 28$), δ = 3.3 (s, O$\mathbf{CH_2}$C), δ = 3.2 (m, NH$\mathbf{CH_2}$ CH_2CH_2O), and δ = 1.6 (m, $NHCH_2$ $C\mathbf{H_2}CH_2O$).

Preparation of Photocleavable Hydrogel Precursor. A 3:1 mixture of aqueous AAm solution and 0.3 M APS was used to prepare the hydrogel. The aqueous AAm solution was composed of 6 M AAm and 0.03 M PEG-Photo-Ac (**4**).

Preparation of Enzyme Solution. Solutions of 100 μg/mL enzyme (trypsin, chymotrypsin, thermolysin, and papain) were

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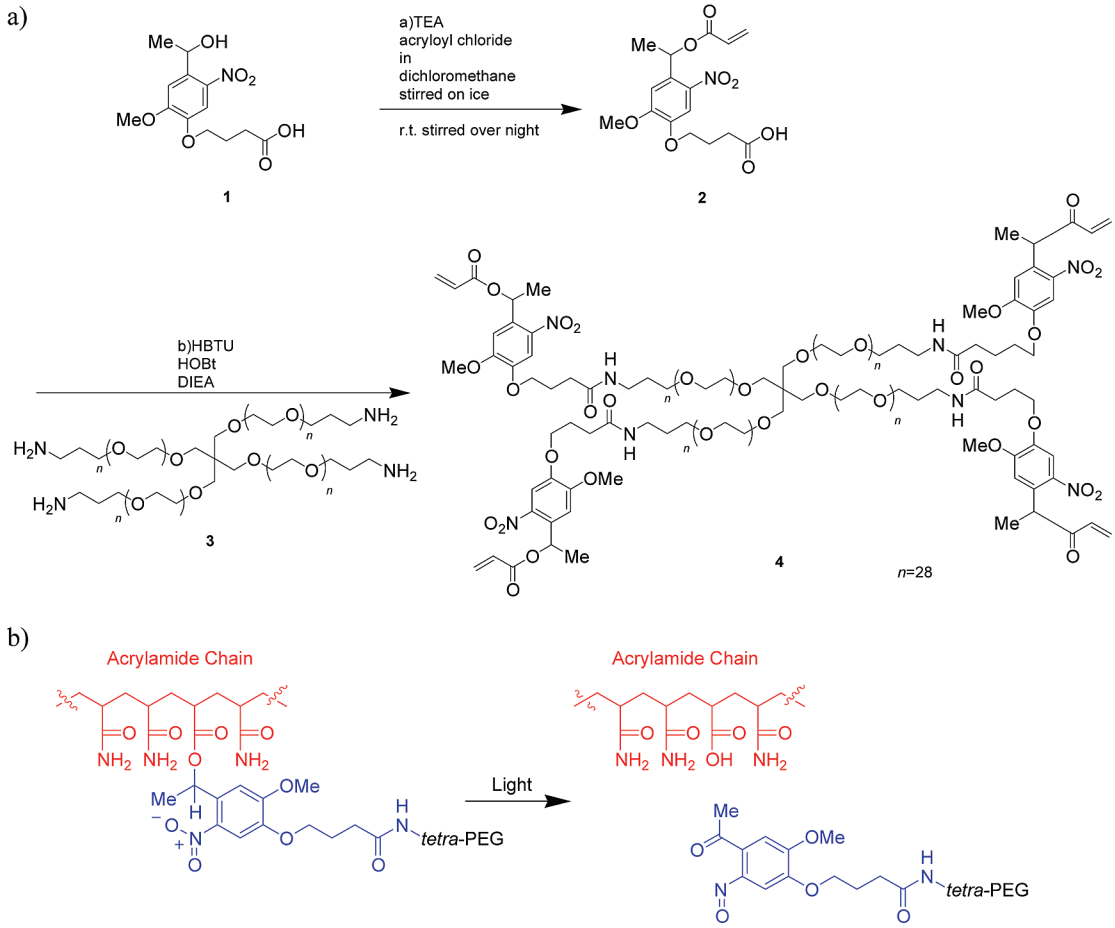
Scheme 1. (a) Synthesis of the Photocleavable Cross Linker 4. (b) Photocleavage Reaction in the Hydrogel



prepared by dissolution in 50 mM Tris/HCl (pH 7.0). To inhibit autodigestion, 20 mM $CaCl_2$ was added to the trypsin solution. A solution of 1 μg/mL elastase was prepared by dilution in 20 mM sodium phosphate (pH 8.0). A 10 mg/mL γ-GTP solution was prepared by dissolution in 20 mM sodium phosphate (pH 8.0).

Preparation of Hydrogel-Encapsulated Enzymes. Hydrogel-encapsulated enzymes were prepared in 96-well micro assay plates (BD Falcon 96-well assay plate 353241, BD, Franklin Lakes, NJ) that were pretreated with plasma (O_2, 100 cc, 200 W, 3 min, Yamato Plasma Cleaner PDC210, Yamato Scientific. Co., Ltd., Tokyo, Japan) to acquire wettability. First, 20 μL of hydrogel precursor and 10 μL of enzyme solution were dispensed into the wells and shaken for 1 min. Then, 10 μL of 0.1 M TEMED in 1 M Tris/HCl buffer (pH 7.0) was added to the mixture to induce gelation, and the mixture was stored overnight at 4 °C. After gelation, the gel was washed three times with 1 M Tris/HCl buffer (pH 7.0). The hydrogel thickness was estimated to be approximately 1.3 mm.

UV Stimulation for Photocleavage. Spot UV Curing Equipment SP-7 (Ushio Inc., Tokyo, Japan) was used as a light source for UV irradiation. The wavelength of the irradiating light was 365 nm at an intensity of 20 mW/cm^2.

Enzymatic Activity Measurements Using Fluorescence. Enzymatic reactions were performed in the 96-well micro assay plate (Figure 1). The hydrogel-encapsulated enzyme coated the bottom of the wells, and we measured the fluorescence signal of the wells before and after UV irradiation. BODIPY FL casein was used as a substrate for fluorescence assays. Before fluorescence measurement, 60 μL of 1 M Tris/HCl buffer (pH 7.0) and 100 μL of 10 μg/mL BODIPY FL casein were added to each well. Then the plate was incubated at 37 °C for 1 h, and the fluorescence signal (Ex 485 nm, Em 535 nm) was measured with a multiplate reader (Appliscan, Thermo Electron Corporation). For light intensity assays, an incubation time of 2 h was used to maximize the measurable fluorescence signal, because we observed low signals in the wells that were irradiated with low-intensity light.

Quantitative Analysis of Bound Antibody (anti PCSK9). The bound anti-PCSK9 was quantitatively analyzed using an ELISA kit, which employed a quantitative sandwich enzyme immunoassay technique. Hydrogel-encapsulated PCSK9 was prepared at a concentration of 40 ng/mL PCSK9. A solution of 100 μL of horseradish-peroxidase-conjugated anti-PCSK9 monoclonal antibody (contained in the kit as ready to use) was added to one hydrogel-coated well. After UV irradiation, the assay plate was shaken for 1 h to release PCSK9 from the hydrogel network and to allow interaction with the antibody. The supernatant (100 μL) in the well was moved to another well that was coated with anti-PCSK9 polyclonal antibody. The PCSK9-anti-PCSK9 complex was bound to the polyclonal antibody. After incubating the mixture for 1 h and washing, the substrate tetra-methylbenzidine (contained in the kit) was added to the well. Then, 0.5 M sulfuric acid was added to stop the reaction, and the absorbance at 450 nm was measured with a multiplate reader.

RESULTS AND DISCUSSION

Hydrogel Design. To prepare the photocleavable hydrogel, we designed a protein-compatible hydrogel that contained a photocleavable functional group. A 2-nitrobenzyl ether-derived moiety was selected as the photocleavable functional group because it is easily cleaved by mild photoirradiation at 365 nm, a wavelength that does not seriously damage encapsulated proteins.[18−20] A photocleavable acrylic linker (**2**) was synthesized by acrylation of a photolabile linker (**1**), and four molecules of **2** were subsequently attached with carboxyl group to a single tetra-PEG-amine molecule (**3**; molecular weight 5328) to create a photocleavable cross-linker (**4**) as described below (Scheme 1a).[18,21,22] Cross-linker **4** was combined with AAm at a ratio of 2:1 (w/w), because the estimated pore size (9−12 nm) of the resulting hydrogel was appropriate for protein encapsulation.[23] The polymerization reaction took place in a solution containing the target protein, and thus, the protein was encapsulated within the newly formed hydrogel network. Since chemical cross-linking within the hydrogel network occurred only at the cross-linker sites, the network collapsed completely upon cleavage of the cross-linker's photocleavable group by UV irradiation (Scheme 1b, Figure S1 in the Supporting Information). As the hydrogel network collapsed, the encapsulated proteins were easily released.

Photoinduced Enzymatic Activity. To investigate possible interactions between the protein and the hydrogel network, we encapsulated enzymes within the developed hydrogel and released them by UV irradiation. We expected that if enzymatic activity were irreversibly inhibited by the encapsulation, the enzymatic activity observed would be substantially decreased or nonexistent. In contrast, strong activity was observed when the enzyme was released from the hydrogel. To further examine the influence of hydrogel network composition on enzymatic activity, we prepared two different hydrogels: one hydrogel contained the photocleavable cross-linker, and the other hydrogel had the same structure except without the 2-nitrobenzyl ether-derived (photocleavable) moiety. Trypsin was encapsulated into both types of hydrogels, and we compared the enzymatic activity of these gels before and after UV irradiation (Figure 2). Trypsin contained in both hydrogels showed very low activity before UV irradiation. However, after UV irradiation, strong enzymatic activity was observed only for the enzymes encapsulated in the photocleavable hydrogel. These results demonstrate that trypsin encapsulated in the photocleavable hydrogel could be released upon the UV irradiation.

Enzymatic Activity Control by Light Intensity. Because the enzyme was released from the hydrogel and strong enzymatic activity was obtained after UV irradiation, we expected that enzymatic activity could be modulated by varying the intensity of the incident UV light. Four different UV intensities (25, 50, 100, and 200 mJ) were used to study the effects of UV intensity on enzyme activity. Figure 3 shows the relationship between the light intensity and the enzymatic activities observed after the irradiation. Predictably, the strongest enzymatic activity was obtained when

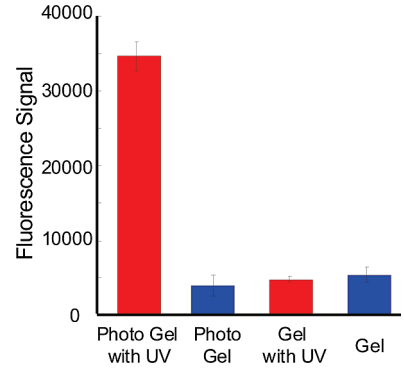


Figure 2. Regulation of enzymatic activity using the photocleavable hydrogel: "Photo gel" refers to the photocleavable hydrogel, and "gel" refers to a hydrogel that lacked photocleavable moieties. All samples were measured in triplicate, and error bars represent means ± SD.

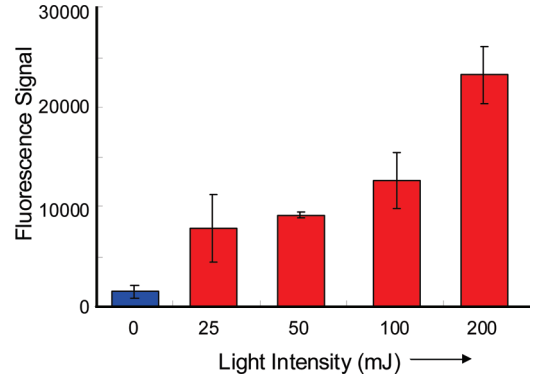


Figure 3. Effect of UV light intensity on tryptic activity. All samples were measured in triplicate, and error bars represent means ± SD.

the strongest intensity of UV light was used (200 mJ), and the activity decreased with decreasing light intensity. We estimated that about 40% of the enzyme encapsulated in the hydrogel was released and exhibited activity under 200 mJ irradiation. This increased activity was observed only in the area of the hydrogel that was irradiated. These results suggest that the rate of enzyme release could be regulated by adjusting the UV intensity to control the extent of photocleavage that occurred within the hydrogel. Consequently, this encapsulation technique allowed us to control enzymatic activity through changes in UV intensity.

Stability of the Encapsulated Enzyme. Many researchers have reported that the stability of enzymes is improved by encapsulating them in hydrogels, because the water content in hydrogels is similar to that in living organisms and also because hydrogel networks isolate individual enzymes, thus preventing autodigestion.[24−26] We expected to observe similar effects upon encapsulating enzymes in our photocleavable hydrogel; that is, we expected that the stability of trypsin would be improved by encapsulation in the hydrogel and that tryptic activity would be maintained for a long time. To confirm this hypothesis, we

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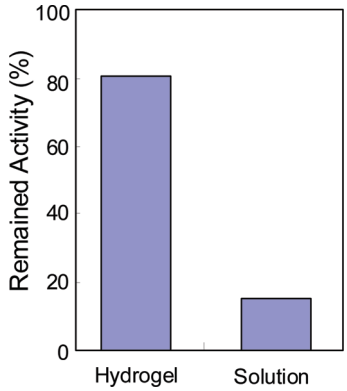

Figure 4. Stability of encapsulated trypsin: Trypsin was stored at 37 °C for 12 h within the hydrogel network or in solution. The remaining tryptic activity was calculated by determining the ratio of the measured activity after UV irradiation versus the initial observed activity, which was measured just after preparation of the enzyme samples.

compared the activity of trypsin stored by means of hydrogel encapsulation with that of trypsin stored in Tris/HCl buffer. Both samples were incubated for 12 h at 37 °C. Figure 4 shows the tryptic activity observed in solution and that observed in the hydrogel after UV irradiation. Although almost all tryptic activity in solution was lost after 12 h of storage, about 80% of the encapsulated trypsin activity remained. These results show that trypsin was protected within the hydrogel and that tryptic activity was maintained even after 12 h of storage. Notably, in this study, trypsin was stored at an optimum temperature for enzymatic reaction (37 °C). In contrast, if trypsin was encapsulated within the hydrogel and stored under refrigeration (4 °C), we would expect its activity to be maintained for an even longer time period.[27] These experiments showed that the hydrogel prevented protein degradation, and that the place, rate, and time of enzyme release could be regulated by adjusting the external UV light stimuli.

Versatility of the Encapsulation Technology. Trypsin was physically encapsulated within the hydrogel network; that is, no chemical modification of trypsin was necessary to achieve encapsulation. We presumed that this encapsulation and release technique could be applicable to a variety of enzymes. To confirm the versatility of our technique, we encapsulated other enzymes with different physical properties from those of trypsin. We chose five enzymes: a metalloprotease (thermolysin), a cysteine protease (papain), two serine proteases (chymotrypsin and elastase), and an aminotransferase (γ-GTP). The molecular weights and pI values of these enzymes are in the range of 25−86 kDa and 6.2−8.8, respectively. Each enzyme was encapsulated within a hydrogel network, and we compared the enzymatic activity before and after UV irradiation for each enzyme. Fluorescence (BODIPY) labeled casein was used as a substrate for all the encapsulated enzymes because its enzymatic reaction product is strongly fluorescent. Figure 5 compares the activities among the five encapsulated enzymes before and after UV irradiation. All the enzymes were released upon irradiation, as evidenced by the dramatic increase in activity observed for all enzymes following irradiation of each hydrogel-encapsulated enzyme. Although the activities of the

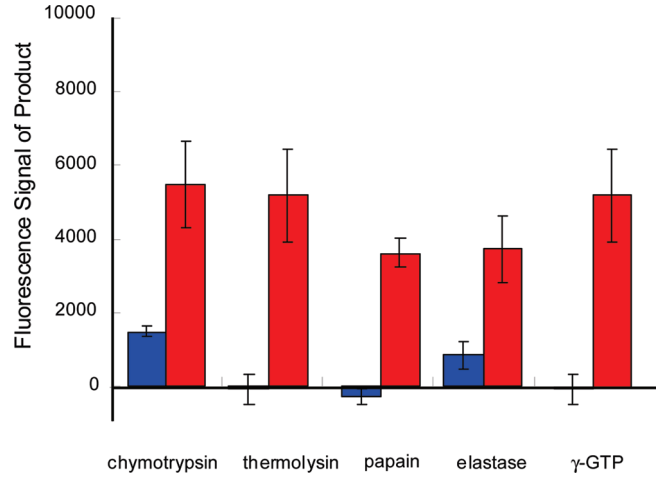

Figure 5. Enzymatic activities of five different enzymes encapsulated within hydrogel networks. Blue bars represent enzymatic activities before irradiation, and red bars represent those observed after irradiation. Chymotrypsin, thermolysin, and papain were encapsulated at a concentration of 1 μg per hydrogel, elastase was contained at 10 μg per hydrogel, and γ-GTP was contained at 1 mg per hydrogel.

encapsulated enzymes were negligible or nonexistent before irradiation, strong activities were obtained after UV irradiation. Notably, a substantial amount of enzymatic activity was observed for chymotrypsin and elastase before irradiation. We believe that because the molecular sizes of chymotrypsin and elastase were smaller than those of the other enzymes studied, these smaller enzymes were more likely to escape the hydrogel network prior to irradiation.[28] We expect that if the mesh size of the hydrogel was decreased by changing the mixing ratio of AAm and tetra-PEG-amine or by changing the length of the cross-linker, those smaller enzymes would be encapsulated more efficiently. Nevertheless, the results in Figure 5 show that almost all the enzymes tested were successfully encapsulated in the present hydrogel network and were released upon UV irradiation. As we expected, this photocontrol of enzymatic activity did not require the direct introduction of a photoresponsive unit to the encapsulated compound. Therefore, we concluded that this system was universal and applicable to a variety of biomolecules.

Application of the Method in Screening for Therapeutic Agents. We used our newly developed hydrogel method to screen candidates for a therapeutic agent. Using the developed hydrogel method, we immobilized a biomolecule related to the target disease in a single well of a micro assay plate and determined the change of ligand concentrations before and after UV irradiation. Prior to UV irradiation, the encapsulated biomolecule could not interact with the ligand, and thus, only nonselective interaction was observed between the gel matrix and the ligand; we considered the state of the well to represent a "healthy model". Upon UV irradiation, the immobilized biomolecule was released from the hydrogel; we considered this state of the well to represent a "disease model". In the disease model, the released biomolecule interacted selectively with the ligand, and the concentrations of bound ligand changed dramatically. The ligand exhibited the greatest change in bound concentration before and after UV irradiation and was considered a strong candidate for a therapeutic

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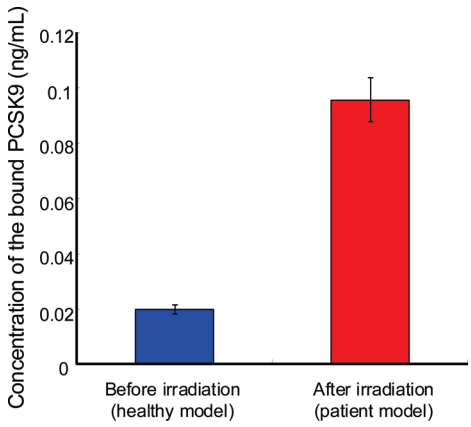

Figure 6. Selective binding assay of anti-PCSK9 to PCSK9: The concentration of the bound antibody was measured by ELISA before and after UV irradiation. All samples were measured in triplicate, and error bars represent means ± SD.

agent for the target disease. This screening method was simple, accurate, cheap, and fast, because two different models (i.e., the healthy and disease models) were constructed within in the same well of the micro assay plate.

To verify the effectiveness of the hydrogel as a disease model, we choose proprotein convertase subtilisin/kexin-type 9 (PCSK9), a glycoprotein that is a biomarker of arteriosclerosis.[29−31] An antibody of PCSK9 (anti-PCSK9) was used as a model compound of a selective ligand to bind PCSK9 in this study. We prepared a hydrogel-encapsulated PCSK9 and used an ELISA kit to measure the concentrations of the bound anti-PCSK9 before and after UV irradiation of the hydrogel. As expected, the high affinity of anti-PCSK9 to PCSK9 caused the concentration of the bound anti-PCSK9 to increase dramatically after UV irradiation (Figure 6). This result indicates that this technique can be used as a disease model and would be amenable to screening of a selective ligand. We expect that by the use of the screening method described here, other suitable therapeutic agents for arteriosclerosis could be identified from a sample solution containing many potential candidates.

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CONCLUSION

In this study, we developed a new photocleavable hydrogel to control protein function by means of UV light stimulation. The photocleavable hydrogel enabled protein encapsulation and release under physiological conditions. Because chemical modification was not necessary to encapsulate proteins within the hydrogel network, we presumed that this technique was amenable to a variety of biomolecules and also to other functional molecules. This is the first technique which enables us to directly control the function of a variety of proteins whenever and wherever required in vitro by controlling the irradiation time and placement. The hydrogel prevented protein degradation, and the biological activity of protein was maintained for a long period of time. Therefore, this technique is an innovative development in the field of controllable activity of protein, and we expect that this technique can be applied not only to research areas that already utilize proteins but also to those areas where proteins currently are not used owing to their delicate nature.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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