Glucose Responsive Two-step Release of Hydrogel-immobilized Protein

Kazutoshi Murota, Seiji Sakamoto, and Kazuaki Kudo*
Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505

(Received February 26, 2008; CL-080216; E-mail: kkudo@iis.u-tokyo.ac.jp)

We designed a two-step-release system that enables protein release from hydrogel in response to glucose. In the system, one of the heterodimeric coiled-coil forming peptides was initially fixed to a hydrogel via a boronate–diol interaction. The peptide was specifically released from the hydrogel by externally incoming glucose. As a result, a protein immobilized in another hydrogel was librated owing to the displacement by the peptide at its hydrogel-bound coiled-coil moiety.

Hydrogels that release preloaded bioactive molecules in response to external stimuli are useful materials in the field of drug delivery and release systems.[1] Concerning this, we have recently reported a new protein immobilizing/releasing method based on hetero-stranded coiled-coil formation of peptides.[2] In the system, one of the hetero-stranded coiled-coil peptide chains (1αK) was grafted on a polymer hydrogel and the other chain (1αE) was fused to a target protein. By mixing these two components, the protein was immobilized into the hydrogel by noncovalent and highly specific peptide–peptide interaction. Moreover, protein bound to the gel was selectively and specifically released by the addition of 1αE or 1αK peptide.

In this communication, we propose a novel two-step-release method with the intention of extending the variation of stimuli-responsive systems (Figure 1a). In our previous report,[2] the peptide for the release of the immobilized protein was externally added. On the contrary, in the two-step-release method, the peptide is initially fixed into a hydrogel via a stimuli-responsive linker. In the design, the peptide is specifically released from the hydrogel by incoming stimulus, and is liberated into the solution. In the next step, the released peptide acts on the counterpart peptide fixing a protein in the other hydrogel, leading to the subsequent release of the protein.

The design of coiled-coil forming peptide sequences was described in our previous paper.[3] Briefly, the peptides 1αK and 1αE contain hydrophobic (Leu and Val), basic (Lys), and acidic (Glu) residues along with a buried Asn residue to direct a heterodimeric parallel coiled-coil structure (Figure 1b). They form heterodimeric parallel coiled-coil in a buffer with an association constant of $9 \times 10^6\ M^{-1}$. As a model protein, green fluorescent protein (GFP) was chosen, because its inherent fluorescence can be directly used for the quantitative estimation of the immobilized and released amount.[4] In the protein design, the 1αE sequence was fused to N-terminus of GFP (1αE–GFP).

For a peptide-immobilizing interaction, we employed a reversible reaction between boronates and diols. Phenylboronic acids are known to form cyclic hydroxoboronate esters with molecules having 1,2- or 1,3-diol units such as glycols and sugars.[5] In this study, phenylboronic acid moieties were introduced into a polymer network of the gel. For the peptide immobilization, a glycol moiety was incorporated at the C-terminal end of 1αEY peptide through a disulfide bond (CTGO–1αEY). We expected that the diol-modified peptide binds to the gel via diol–boronate interaction and in turn, the peptide will be easily displaced by externally added glucose molecules due to the difference in their affinity for the boronate anion.[6]

The hydrogel for storing CTGO–1αEY was prepared by conventional radical polymerization of 4-(1,6-dioxo-2,5-diaza-7-octenyl)phenylboronic acid (700 nmol),[7] *N*,*N*′-diacryloylethylenediamine (17.5 nmol), and acrylamide (7 μmol) in 17%

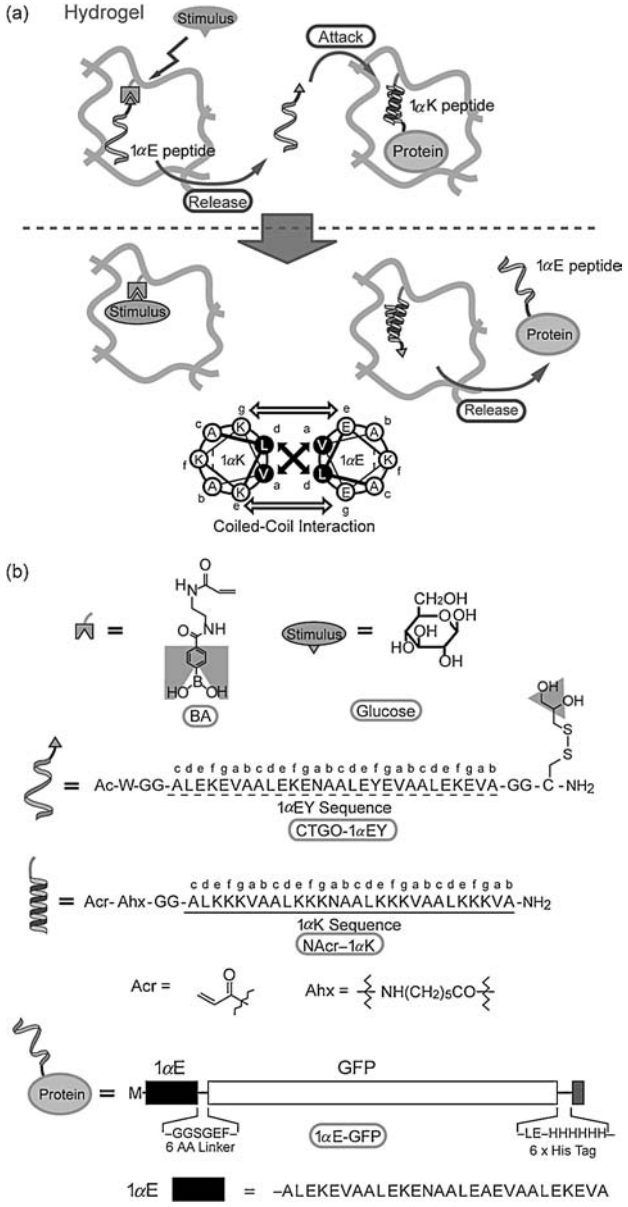

Figure 1. (a) Schematic illustration of a two-step-release system. (b) Design of molecules for two-step-release system.

2,2,2-trifluoroethanol/buffer (pH 7.4) at 25 °C (total volume: 12 μL). The gel was incubated with 200 μM CTGO–1αEY in the buffer (pH 7.4 or 9.0) for 20 h and washed with the same buffer to remove the free peptide. The amount of immobilized peptide estimated from fluorescence intensity of Trp residue was 0.20 and 0.43 nmol for pH 7.4 and 9.0, respectively. This difference qualitatively coincides with the pH dependence of the stability of the boronate esters.[7]

Release of CTGO–1αEY in response to applied glucose was examined. The CTGO–1αEY-containing gel was immersed in buffer (pH 9.0) with or without 300 μM glucose for 3 h. The released peptide amount was determined by measuring fluorescence intensity of the supernatant. In the presence of glucose, 62% of the immobilized peptide was released, whereas 35% was released in the absence of the glucose. The glucose responsive enhancement might be due to the diol–glucose exchange reaction.

The 1αE–GFP-immobilized hydrogel was prepared according to our previous paper.[2] It was confirmed that both the coiled-coil forming ability of 1αE region and fluorescence property of GFP moiety tolerate the basic conditions of pH 9.0.

The protein releasing study was performed. The amount of released 1αE–GFP was determined by fluorescence intensity after incubation of 1αE–GFP-containing gel in buffer (pH 9.0) (Figure 2). In the control experiments without CTGO–1αEY-containing gel, the addition of glucose to the 1αE–GFP gel showed no effect on the protein releasing behavior (bars A and B). This result indicates that glucose does not perturb the heteromeric coiled-coil interaction between 1αE–GFP and polymer-bound 1αK peptide. The coexistence of CTGO–1αEY-containing gels brought about a slight increase in the released 1αE–GFP amount presumably owing to the aforementioned passive liberation of the peptides from CTGO–1αEY-containing gels (bar C). The amount of ejected 1αE–GFP was significantly raised to ca. 40% when glucose was present (bar D). The estimated amount of released CTGO–1αEY for this case is 0.7 nmol. The final amount of the liberated protein is in good agreement with that obtained from a one-step releasing experiment using 0.7 nmol of 1αE–peptide and the 1αE–GFP-immobilized hydrogel.[8]

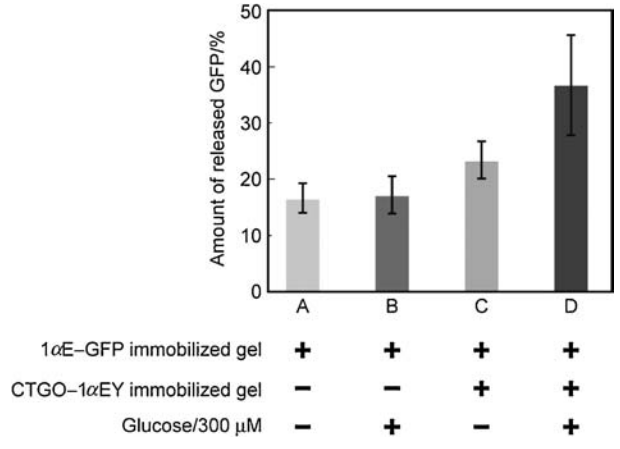

Figure 2. The amount of released 1αE–GFP after the gels were incubated for 18 h in 200 μL of 150 mM NaCl, 20 mM Tris-HCl buffer (pH 9.0). The amounts of pre-loaded 1αE–GFP and CTGO–1αEY in the gels were about 0.13 and 1.2 nmol, respectively.

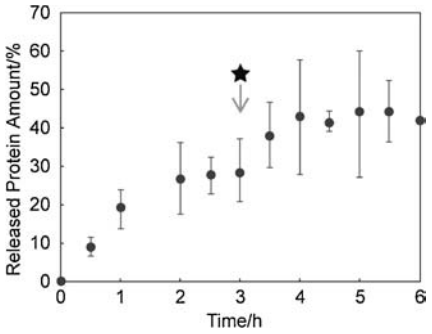

Figure 3. Release profile of 1αE–GFP from hydrogel in the presence of CTGO–1αEY gels in 200 μL of the buffer. The asterisk indicates the time when 2 μL of 30 mM glucose was added.

Release profile of 1αE–GFP was also monitored (Figure 3). The peptide-containing hydrogel and the protein-immobilized hydrogel in the buffer were equilibrated for 2 h and the total cumulative release amount of protein was 28%. Addition of glucose to the mixture brought about an immediate increase in the amount of released protein up to ca. 43%, indicating that the two-step-releasing was realized. The time required for the completion of the initial equilibration is comparable to that observed in the externally-added-peptide triggered process.[8] This means that the passive release of the CTGO–1αEY peptide is considerably faster compared to the subsequent peptide-induced protein liberation process. The time course after the addition of glucose, including the absence of the apparent induction period, indicates that the glucose-induced peptide-releasing process occurred rapidly.

In summary, we have successfully constructed a two-step-release system for hydrogel-immobilized protein. The advantage of this method is its potential expandability to other stimulus-responsive protein-releasing system without any further modification on the protein structure. This finding will contribute to the development of stimuli-sensitive hydrogel materials.

We thank Prof. H. Mihara and Dr. T. Takahashi (Tokyo Inst. of Tech.) for DNA sequencing. This work was partially supported by the Global COE Program for Chemistry Innovation.

References and Notes

1 S. Chaterji, I. K. Kwon, K. Park, *Prog. Polym. Sci.* **2007**, *32*, 1083.
2 K. Murota, S. Sakamoto, K. Kudo, *Chem. Lett.* **2007**, *36*, 1320.
3 S. Sakamoto, A. Ito, K. Kudo, S. Yoshikawa, *Chem.—Eur. J.* **2004**, *10*, 3717.
4 G. S. Waldo, B. M. Standish, J. Berendzen, T. C. Terwilliger, *Nat. Biotechnol.* **1999**, *17*, 691.
5 G. Springsteen, B. Wang, *Tetrahedron* **2002**, *58*, 5291.
6 The stability constant ($\log K_a$) for ethylene glycol with phenylboronic acid is 0.44 whereas that for glucose in 2.04: T. D. James, K. R. A. S. Samankumara, S. Shinkai, *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910.
7 A. Matsumoto, S. Ikeda, A. Harada, K. Kataoka, *Biomacromolecules* **2003**, *4*, 1410.
8 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/.

Published on the web (Advance View) April 26, 2008; doi:10.1246/cl.2008.582