Time-modulated Release of Multiple Proteins from Enzyme-responsive Multilayered Capsules

Yuki Itoh,[1,2] Michiya Matsusaki,[1] Toshiyuki Kida,[1] and Mitsuru Akashi*[1,2,3]
[1]*Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871*
[2]*Center of Excellence (COE) Program for 21st Century, Osaka University*
[3]*Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi 332-0012*

(Received November 20, 2007; CL-071289; E-mail: akashi@chem.eng.osaka-u.ac.jp)

The time-modulated release of two kinds of proteins in response to specific enzymes was achieved by using multilayered capsules incorporating these proteins in separate positions of the capsule, which were prepared via layer-by-layer assembly of chitosan, dextran sulfate, and poly(L-lysine).

The development of self-assembled polymeric particles has been actively pursued to construct novel drug delivery systems with versatile functions such as biodegradability and stimuli responsibility.[1] We have reported various kinds of nanoparticles, which were prepared by the self-assembly of synthetic and naturally occurring polymers, as promising drug carriers.[2] Recently, the release of multiple drugs and genes from a single carrier has attracted much attention, because multiple drugs can induce strong synergistic effects.[3] For example, Wang et al. reported that the delivery of paclitaxel and RNA by core–shell nanoparticles more remarkably enhanced their inhibitory effects against cancer-cell growth than the delivery of each of them.[3c] Some researchers also reported synergistic effects of multiple growth factors for blood vessel and cartilage regeneration.[3a,3b] However, in conventional release systems, it was not possible to control the individual release rate of multiple drugs from a single carrier, because their release rates depended on only their diffusion properties from the carrier. To construct individually controlled release systems for multiple drugs, new methodologies such as the incorporation of multiple drugs into separate sites in a single carrier and the setting of an independent driving force for the release of each drug are required.

In this context, we directed our attention to hollow capsules prepared by the layer-by-layer (LbL) assembly of oppositely charged biodegradable polymers. LbL assembly is a useful technology to fabricate nanometer-sized multilayers composed of macromolecules such as synthetic and biodegradable polymers.[4] The hollow capsules prepared by LbL assembly have a semipermeable membrane, and their diameter, membrane thickness, and membrane components are easily controlled.[5] Many researchers have reported the controlled drug release from multilayered capsules due to changes in the physicochemical properties of the membrane polymers upon changes in pH or ionic strength;[6] however, to the best of our knowledge, there has been no report on the individually controlled release of multiple drugs from a single capsule. Recently, we developed enzymatically degradable capsules composed of chitosan (CT) and dextran sulfate (Dex) multilayers as a novel drug carrier, in which the controlled release of encapsulated FITC–albumin was successfully carried out by the enzymatic degradation of the CT membranes with chitosanase.[7] The enzymatic degradation of the capsule membrane is an ideal method to easily control the release of a drug encapsulated inside the capsule. Here, if two types of drugs are separately incorporated inside the capsule and into the capsule membrane, respectively, we can individually control their release by exploiting the enzymatic degradation of the capsule membrane (Figure 1a). In this letter, we report the first example of the time-modulated release of two proteins from a single capsule by a combination of the incorporation of these proteins into separate positions of the capsule and the enzymatic degradation of the capsule membrane.

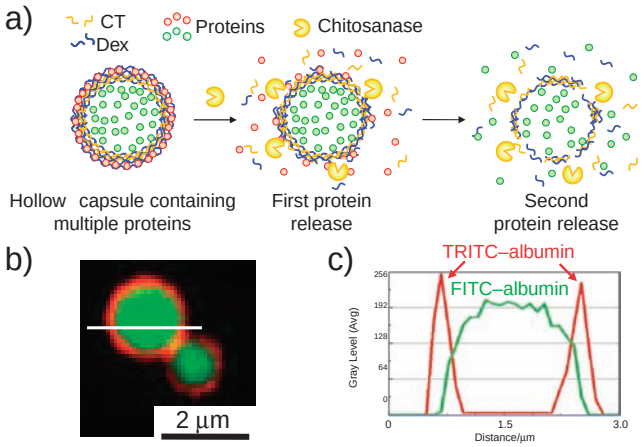

Figure 1. a) Schematic illustration of the multiple protein release from the biodegradable capsules via enzymatic degradation. b) Confocal microscopic image, and c) line scan image of the capsules containing FITC–albumin inside the capsule, and TRITC–albumin in the capsule membrane.

First, we prepared the $(Dex/CT)_2/Dex/TRITC–albumin/Dex/CT/Dex$ capsules, where FITC–albumin was incorporated inside the capsules and TRITC–albumin was embedded into the Dex/CT multilayer membrane, via the LbL assembly of Dex/CT onto FITC–albumin-entrapped mesoporous silica, followed by HF etching.[2c,7–9] We confirmed that HF hardly affected the structure of albumin by CD spectra.[9] Here, TRITC–albumin and FITC–albumin were used as models for two types of proteins. Confocal microscopic observation of the capsules clearly showed that FITC– and TRITC–albumins were located inside the capsule and in the capsule membrane, respectively (Figures 1b and 1c). The amounts of FITC– and TRITC–albumin incorporated in the capsules (1 mg) were 76 and 46 μg, respectively.

The release properties of the FITC– and TRITC–albumins from the capsules triggered by enzymatic degradation of the capsule membrane were then evaluated.[9] We employed chitosanase

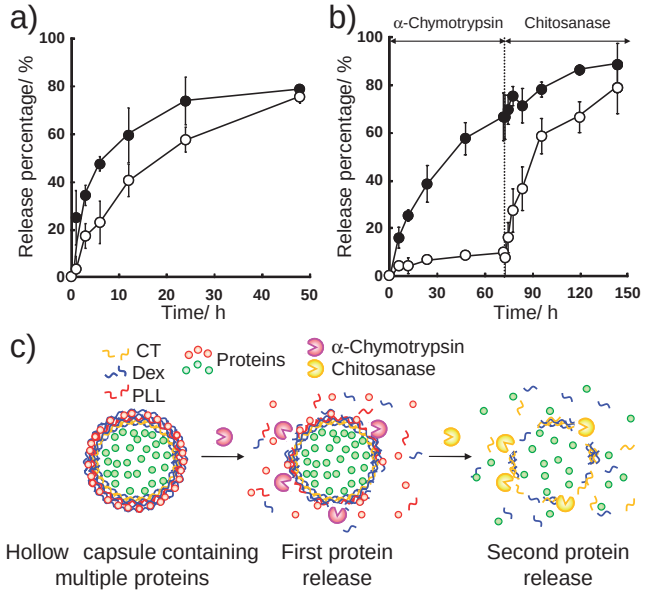

Figure 2. Release profiles of TRITC–albumin (filled circles) and FITC–albumin (open circles) from a) $(Dex/CT)_2/Dex/TRITC–albumin/Dex/CT/Dex$ and b) $(Dex/CT)_2/(Dex/PLL)_2/Dex/TRITC–albumin/Dex/PLL/Dex$ capsules encapsulating FITC–albumin at 37 °C ($n = 3$). These capsules were incubated with a) chitosanase alone or b) α-chymotrypsin plus chitosanase. c) Schematic illustration of the individual and stepwise release of multiple proteins from biodegradable capsules in response to two types of enzymes.

as model enzyme for enzymatic degradation of the CT membrane. The capsules were added into the chitosanase solution [0.1 M acetic acid buffer (pH 5.6) including 0.01 mg mL^{-1} chitosanase] at 37 °C. The TRITC–albumin trapped in the capsule membrane was quickly released, and more than 50% of the TRITC–albumin was released within 6 h. In contrast the FITC–albumin encapsulated inside the capsule was released more slowly and more than 20 h of incubation was required for the release of one half of the original amount (Figure 2a). On the other hand, these albumins were barely released without the enzyme.[9] For a quantitative analysis, the release profiles were fitted to a pseudo-first-order equation.[9] The observed rate constants for the release of TRITC– and FITC–albumins were ca. 0.11 and 0.06. The chitosanase adsorbed onto the surface of the capsule seemed to degrade the outermost CT layer first, and then the outermost Dex layer was peeled off from the capsule surface with the progress of enzymatic degradation of the CT layer.[10] Therefore, the TRITC–albumin trapped in the capsule membrane was released first, and then the FITC–albumin encapsulated inside the capsule was released thereafter. However, it was difficult to achieve the individual and stepwise release of two kinds of proteins by this system with a single enzyme.

Thus, we attempted to prepare capsules composed of the multilayered membrane including two types of enzyme-degradable polymers which could be degraded individually by two different enzymes (Figure 2c). For the controlled release of two different proteins with a time interval difference, we prepared $(Dex/CT)_2/(Dex/PLL)_2/Dex/TRITC–albumin/Dex/PLL/Dex$ capsules, where FITC–albumin was incorporated inside the capsules and TRITC–albumin was embedded into the Dex/PLL multilayer membrane, via the LbL assembly of Dex/CT and Dex/PLL using the same procedure as described above.[9] The amounts of FITC– and TRITC–albumin contained in the capsules (1 mg) were 56 and 41 μg, respectively. α-Chymotrypsin and chitosanase were chosen as the first and second enzymes, respectively. First, the capsules were added into an α-chymotrypsin solution [0.1 M acetic acid buffer (pH 5.6) including 0.01 mg mL^{-1} α-chymotrypsin] at 37 °C (Figure 2b). TRITC–albumin was predominantly released from the capsule membrane by the enzymatic degradation of the PLL layer, and approximately 60% of the TRITC–albumin was released after 72 h, whereas less than 10% of the FITC–albumin was released from the interior of the capsule even after 72 h. This result clearly indicated that the preceding release of TRITC–albumin trapped in the capsule membrane and the suppression of the non-specific release of FITC–albumin encapsulated inside the capsule were successfully achieved by the construction of a capsule membrane composed of two enzyme-responsive multilayers which differed from each other in the type of enzyme trigger. After 72 h, chitosanase was added into the reaction solution (chitosanase concentration = 0.01 mg mL^{-1}), and the FITC–albumin was released upon the enzymatic degradation of the CT layer. The release of TRITC–albumin after the addition of chitosanase may have been caused by release from the desorbed capsule membrane containing FITC–albumin. These results show that a novel individual and stepwise release system of multiple proteins with a time interval difference from a single capsule was constructed for the first time by manipulating the capsule membrane components to independently respond to two different enzymes.

In conclusion, we successfully prepared capsules incorporating two kinds of proteins in separate positions of the capsule via LbL assembly, and achieved the time-modulated release of these two proteins. This multiple protein release system was fabricated by using the appropriate enzymatically degradable polymers, as components of the capsules, to respond to specific enzymes in the target disease areas. The release rate of the encapsulated proteins can be more precisely controlled by manipulating the capsule membrane thickness, and work along this line is now in progress.

References and Notes

1 a) J. Panyam, V. Labhasetwar, *Adv. Drug Delivery Rev.* **2003**, *55*, 329. b) Y. Bae, S. Fukushima, A. Harada, K. Kataoka, *Angew. Chem., Int. Ed.* **2003**, *42*, 4640.
2 a) M. Akashi, I. Kirikihira, N. Miyauchi, *Angew. Makromol. Chem.* **1985**, *132*, 81. b) M. Matsusaki, K. Hiwatari, M. Higashi, T. Kaneko, M. Akashi, *Chem. Lett.* **2004**, *33*, 398. c) Y. Itoh, M. Matsusaki, T. Kida, M. Akashi, *Chem. Lett.* **2004**, *33*, 1552. d) T. Kida, M. Mouri, M. Akashi, *Angew. Chem., Int. Ed.* **2006**, *45*, 7534.
3 a) T. Richardson, M. Peters, A. Ennett, D. Mooney, *Nat. Biotechnol.* **2001**, *19*, 1029. b) T. Holland, Y. Tabata, A. Mikos, *J. Controlled Release* **2005**, *101*, 111. c) Y. Wang, S. Gao, W. Ye, H. Yoon, Y. Yang, *Nat. Mater.* **2006**, *5*, 791.
4 a) G. Decher, *Science* **1997**, *277*, 1232. b) D. T. Haynie, L. Zhang, J. S. Rudra, W. Zhao, Y. Zhong, N. Palath, *Biomacromolecules* **2005**, *6*, 2895.
5 a) E. Donath, G. B. Sukhorukov, F. Caruso, S. A. Davis, H. Möhwald, *Angew. Chem., Int. Ed.* **1998**, *37*, 2201. b) F. Caruso, R. A. Caruso, H. Möhwald, *Science* **1998**, *282*, 1111.
6 A. A. Antipov, G. B. Sukhorukov, *Adv. Colloid Interface Sci.* **2004**, *111*, 49.
7 Y. Itoh, M. Matsusaki, T. Kida, M. Akashi, *Biomacromolecules* **2006**, *7*, 2715.
8 A. Yu, Y. Wang, E. Barlow, F. Caruso, *Adv. Mater.* **2005**, *17*, 1737.
9 Supporting Information is available electronically on the CSJ-Journal Web site. http://www.csj.jp/journals/chem-lett/.
10 T. Serizawa, M. Yamaguchi, M. Akashi, *Macromolecules* **2002**, *35*, 8656.

Published on the web (Advance View) February 2, 2008; doi:10.1246/cl.2008.238