J|A|C|S COMMUNICATIONS

Published on Web 04/08/2009

A Collagen-Mimic Dendrimer Capable of Controlled Release

Chie Kojima,† Sayako Tsumura, Atsushi Harada, and Kenji Kono*

Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

Received December 15, 2008; E-mail: kono@chem.osakafu-u.ac.jp

A collagen model peptide-attached dendrimer was synthesized as a potential functional collagen material. The peptides that clustered at the surface of the dendrimer formed a thermally reversible collagen-like triple helix. This dendrimer worked as a drug carrier with thermosensitive release capabilities, although it did not exhibit a lower critical solution temperature (LCST). From this dendrimer, the hydrogel could be made at low temperature.

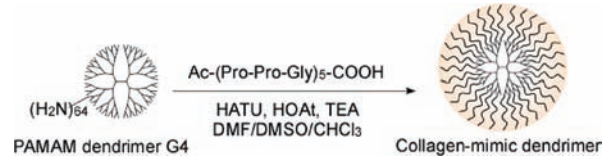
Collagen is the most abundant protein in mammals, and it is composed of glycine-proline-(hydroxy)proline [Gly-Pro-Pro(Hyp)] repeats to form a triple helix. It has been classically used as a biomaterial. For example, collagen gels are useful for long-term slow release drug delivery applications.[1] However, the release from collagen gels is generally uncontrollable, and thus, functional collagen materials are desired. Artificial collagen materials have been studied as an alternative to natural collagens extracted from animals, which can be contaminated with infectious pathogens and allergens. However, short collagen peptides cannot form a triple helix, which limits the ability to form artificial collagen material. Previously, various covalently knotted collagen model peptides have been studied.[1a,2] Goodman and co-workers[2b,c] used a dendrimer as a knot where the terminal groups were modified with the collagen model peptide (Gly-Pro-Nleu), in which Nleu is *N*-isobutylglycine.

Dendrimers, in contrast to linear polymers, have highly controllable sizes, topologies, and surface properties. Many researchers have designed new functional materials for a variety of applications.[3] Recently, the research into the biological applications of dendrimers, especially for drug and gene delivery, has progressed.[4] We have also reported that polyethylene glycol (PEG)-attached dendrimers are a potential drug carrier.[5]

In this work, we have designed a novel collagen-mimic drug carrier by combining collagen peptides with a dendrimer. Because the collagen-mimic dendrimers have a dendritic portion and a collagen part, it was expected that a functional collagen material with controlled release properties would result. We used ethylenediamine-core polyamidoamine (PAMAM) dendrimer of generation 4 (G4) and a collagen model peptide, $(Pro\text{-}Pro\text{-}Gly)_5$, to produce the collagen-mimic dendrimer. We characterized the collagen-like triple helix formation of the dendrimer. The drug release profiles using a model drug, rose bengal (RB), were investigated at different temperatures. Finally, a hydrogel was prepared using this dendrimer.

The dendrimer was synthesized according to Scheme 1 and characterized by ^{1}H NMR spectroscopy (Figure S1B in the Supporting Information). The spectrum contained signals derived from $(Pro\text{-}Pro\text{-}Gly)_5$ and from the PAMAM dendrimer. The integral ratio of the bound peptide to the dendrimer revealed that essentially every amino group of the dendrimer was attached to the peptide. From fluorescamine analysis, by which primary amines are detected

Scheme 1. Synthetic Pathway of the Collagen Model Peptide-Attached Dendrimer



(see the Supporting Information), less than one residual amino group per dendrimer was detected. This result is consistent with the NMR results.

Figure 1A shows the circular dichroism (CD) spectra of the collagen-mimic dendrimer, the acetylated $(Pro\text{-}Pro\text{-}Gly)_5$ peptide, and natural type-I collagen. The $(Pro\text{-}Pro\text{-}Gly)_{10}$ peptide was used as a control. The positive Cotton effect at 225 nm, which corresponds to the collagen-like triple helix, was observed in the spectra of the natural collagen and the $(Pro\text{-}Pro\text{-}Gly)_{10}$ peptide.[6] The collagen-mimic dendrimer also exhibited the positive Cotton effect, although it was not observed in the acetylated $(Pro\text{-}Pro\text{-}Gly)_5$ peptide. The molar ellipticity of the dendrimer was 28% of that for the $(Pro\text{-}Pro\text{-}Gly)_{10}$ peptide at 20 °C because of the shorter peptide length. The Cotton effect of the dendrimer was enhanced in the presence of ethanol (Figure S2), confirming that a collagen-like triple helix was formed.[6] The $(Pro\text{-}Pro\text{-}Gly)_{10}$ peptide formed the collagen-like triple helix after incubation at 4 °C for more than 1 day but not without the incubation (Figure S3). The collagen-mimic dendrimer, in contrast, exhibited the positive Cotton effect to the same degree with and without incubation at 4 °C (Figure S3). These results suggest that the clustering of the collagen peptide at the surface of the dendrimer induced the formation of the collagen-like triple helix. Goodman and co-workers[2b,c] and Higashi et al.[7] also reported that their peptide-modified dendrimers exhibited more highly helical structures than the free peptides, which supports our results.

Collagen undergoes a transition to become gelatin at a melting temperature that depends on the sequence.[1,2,6] We next investigated

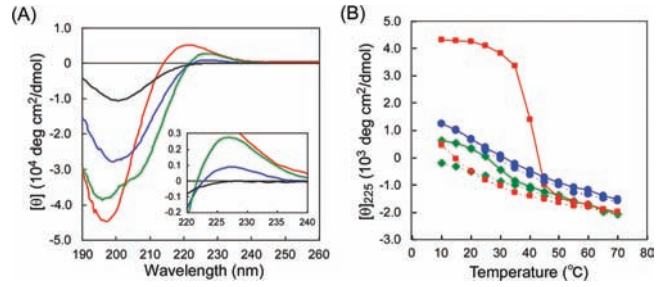


Figure 1. CD analysis of the collagen-mimic dendrimers (blue), the acetylated $(Pro\text{-}Pro\text{-}Gly)_5$ peptide (black), the $(Pro\text{-}Pro\text{-}Gly)_{10}$ peptide (green), and type-I collagen (red). (A) CD spectra. A portion of the spectra is enlarged in the inset. (B) Thermal reversibility upon heating (solid lines) and cooling (dashed lines).

† Present affiliation: Nanoscience and Nanotechnology Research Center, Osaka Prefecture University, Osaka, Japan.

10.1021/ja809639c CCC: $40.75

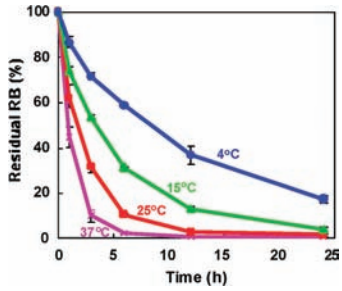


Figure 2. Release profiles of RB from the collagen-mimic dendrimer at different temperatures. Residual RB (%) was calculated from the 557 nm absorbance of the complex solution in the dialysis bag.

the thermal stability of the collagen-mimic dendrimer (Figure 1B). The molar ellipticities of the natural collagen and the $(Pro\text{-}Pro\text{-}Gly)_{10}$ peptide decreased sharply at ~40 and 30 °C, respectively. On the other hand, that of the collagen-mimic dendrimer gradually decreased with increasing temperature.[8] Interestingly, the positive Cotton effect of the collagen-mimic dendrimer recovered with decreasing temperature. This behavior was not observed in the natural collagen and the $(Pro\text{-}Pro\text{-}Gly)_{10}$ peptide under our experimental conditions. This thermal property was also analyzed by differential scanning calorimetry (DSC) and variable-temperature ^{1}H NMR measurements. An insignificant endothermal signal of the collagen dendrimer was not observed in the DSC analysis, suggesting that the phase transition occurred only in a small part of this compound. ^{1}H NMR experiments on the collagen-mimic dendrimer and the $(PPG)_{10}$ peptide were performed at different temperatures (Figure S4). Signals corresponding to an assembled peptide are different from those for an unassembled peptide.[9] From the NMR spectrum of the $(PPG)_{10}$ peptide at room temperature, this peptide almost completely formed collagen-like triple helixes under our conditions. The spectra were drastically changed at 40 °C and became irreversible after cooling. On the other hand, the spectra of the collagen-mimic dendrimer had signals that gradually decreased with heating and then almost completely recovered after cooling. Therefore, this thermal reversibility was unique to the collagen-mimic dendrimer.

We next investigated the release of a model drug, RB, from the collagen-mimic dendrimer at different temperatures. We previously reported the preparation of the PEG-attached dendrimer encapsulating RB and the investigation of the release behavior.[5b,10] Although that dendrimer does not have thermosensitive properties, the release rate of RB was slightly lower at 4 °C than at 37 °C (Figure S5). It may be because the low molecular mobility at 4 °C slightly enhanced the binding affinity and/or suppressed the permeability of the dialysis membrane. The release of RB from the collagen-mimic dendrimer was more effectively suppressed at lower temperature (Figure 2). From the molecular ellipticity ratio of the collagen-mimic dendrimer to the $(PPG)_{10}$ peptide, the extent of formation of the triple helix of the collagen dendrimer was 58, 39, 14, and 0% at 4, 15, 25, and 35 °C, respectively (Figure 1B). The collagen-like triple helix formation at lower temperatures may improve the binding properties of RB to the dendrimer and/or suppress the permeability of RB at the dendrimer surface. Therefore, the collagen-mimic dendrimer may be a suitable drug carrier in response to temperature. Many thermosensitive drug carriers based on thermosensitive polymers such as poly(*N*-isopropylacrylamide) (PNIPAM) have previously been reported. These compounds have an LCST, at which they undergo a transition from soluble forms to insoluble ones.[11] In contrast, the collagen-mimic dendrimers did not exhibit a phase transition under our experimental conditions but just changed the helix formation. Therefore, the thermosensitivity of this dendrimer may occur in a different manner than for other thermosensitive materials.

Since collagen is a main component of the extracellular matrix, we prepared the hydrogel using this dendrimer. The hydrogel could be made by cooling an aqueous solution of the collagen-mimic dendrimer, which melted at 35 °C (Figure S6). The enhanced triple helix formation at low temperature might contribute to the gel formation.

In conclusion, we synthesized a collagen-mimic dendrimer and characterized it by ^{1}H NMR and CD spectrometry. The collagen peptides attached to the dendrimer induced a collagen-like triple helix. This helix was thermally reversible, in contrast to natural collagen. This dendrimer can act as a thermosensitive drug carrier and form a hydrogel, which can be used as a potential cellular matrix for controllable drug release. Because the melting point of the collagen peptide depends on the sequence and the length of the peptide, the optimized peptide may be able to promote the release of encapsulated drug molecules at a desired temperature.[1a,8] A detailed characterization remains to be undertaken. Our study of this kind of dendrimer is ongoing, with the aim of elucidating the influences of dendrimer generation and peptide length and sequence on dendrimer function.

Acknowledgment. This work was supported by grants from the Cosmetology Research Foundation and in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT).

Supporting Information Available: Experimental procedures, ^{1}H NMR spectra, CD spectra, temperature-dependent profiles for release of RB from the PEG-attached dendrimer, and photographs of the hydrogel. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Koide, T. *Connect. Tissue Res.* **2005**, *46*, 131–141. (b) Wallacea, D. G.; Rosenblattb, J. *Adv. Drug Delivery Rev.* **2003**, *55*, 1631–1649. (c) Sano, A.; Maeda, M.; Nagahara, S.; Ochiya, T.; Honma, K.; Itoh, H.; Miyata, T.; Fujioka, K. *Adv. Drug Delivery Rev.* **2003**, *55*, 1651–1677.

(2) (a) Goodman, M; Bhumralkar, M.; Jefferson, E. A.; Kwak, J.; Locardi, E. *Biopolymer* **1998**, *47*, 127–142. (b) Kinberger, G. A.; Cai, W.; Goodman, M. *J. Am. Chem. Soc.* **2002**, *124*, 15162–15163. (c) Kinberger, G. A.; Taulane, J. P.; Goodman, M. *Tetrahedron* **2006**, *62*, 5280–5286.

(3) (a) *Dendrimers and Other Dendritic Polymers*, Fréchet, J. M. J., Tomalia, D. A., Eds.; Wiley Series in Polymer Science; John Wiley & Sons, Ltd.: Chichester, U.K., 2001. (b) Tomalia, D. A. *Prog. Polym. Sci.* **2005**, *30*, 294–324.

(4) (a) Boas, U.; Heegaard, P. M. H. *Chem. Soc. Rev.* **2004**, *33*, 43–63. (b) Lee, C. C.; MacKay, J. A.; Fréchet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, *12*, 1517–1526. (c) Svenson, S.; Tomalia, D. A. *Adv. Drug Delivery Rev.* **2005**, *57*, 2106–2129. (d) Klajnert, B.; Bryszewska, M. *Dendrimers in Medicine*; Nova Science Publishers, Inc., New York, 2007.

(5) (a) Kojima, C.; Kono, K.; Maruyama, K.; Takagishi, T. *Bioconjugate Chem.* **2000**, *11*, 910–917. (b) Kojima, C.; Toi, Y.; Harada, A.; Kono, K. *Bioconjugate Chem.* **2007**, *18*, 663–670. (c) Kono, K.; Kojima, C.; Hayashi, N.; Nishisaka, E.; Kiura, K.; Watarai, S.; Harada, A. *Biomaterials* **2008**, *29*, 1664–1675.

(6) Kishimoto, T.; Morihara, Y.; Osanai, M.; Ogata, S.; Kamitakahara, M.; Ohtsuki, C.; Tanihara, M. *Biopolymer* **2005**, *79*, 163–172.

(7) Higashi, N.; Koga, T.; Niwa, M. *Adv. Mater.* **2000**, *12*, 1373–1375.

(8) Persikov, A. V.; Ramshaw, J. A. M.; Brodsky, B. *J. Biol. Chem.* **2005**, *280*, 19343–19349.

(9) Malacini, G.; Feng, Y.; Goodman, M. *J. Am. Chem. Soc.* **1996**, *118*, 10359–10364.

(10) (a) Haba, Y.; Harada, A.; Takagishi, T.; Kono, K. *Polymer* **2005**, *46*, 1813–1820. (b) Kono, K.; Fukui, T.; Takagishi, T.; Sakurai, S.; Kojima, C. *Polymer* **2008**, *49*, 2832–2838.

(11) (a) Kono, K.; Takagishi, T. *Methods Enzymol.* **2004**, *387*, 73–82. (b) Kono, K. *Adv. Drug Delivery Rev.* **2001**, *53*, 307–319. (c) Chilkoti, A.; Dreher, M. R.; Meyer, D. E. *Adv. Drug Delivery Rev.* **2002**, *54*, 1093–1111. (d) Chilkoti, A.; Dreher, M. R.; Meyer, D. E.; Raucher, D. *Adv. Drug Delivery Rev.* **2002**, *54*, 613–630. (e) Haba, Y.; Harada, A.; Takagishi, T.; Kono, K. *J. Am. Chem. Soc.* **2004**, *126*, 12760–12761. (f) Haba, Y.; Kojima, C.; Harada, A.; Kono, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 234–237.

JA809639C