Human Serum Albumin Nanotubes Comprising Layer-by-layer Assembly with Polycation

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Cylindrical protein nanotubes comprising a layer-by-layer (LbL) assembly of human serum albumin (HSA) with polycations [polyallylamine hydrochloride (PAH), poly-L-arginine hydrochloride (PLA), and poly-L-lysine hydrobromide (PLL)] have been prepared using template synthesis with a porous anodic aluminum oxide (AAO) membrane. Visible absorption spectra of the multilayered thin film of the polycation and HSA on a quartz plate showed alternate LbL film formation of $(\text{polycation/HSA})_n$.

Template synthesis using vertically oriented pore arrays of anodic aluminum oxide (AAO) membrane enables construction of well-defined nanofibers and nanotubes from a range of materials.[1–6] In particular, a layer-by-layer (LbL) deposition technique, which is multilayer build-up utilizing the electrostatic interaction between oppositely charged molecules onto the pore walls, yields tailored hollow structures such as polyelectrolyte nanotubes.[7–9] Proteins are also natural polyelectrolytes having versatile bioactivities. Consequently, preparation of LbL protein nanotubes has attracted much interest because of their potential applications in biotechnology.[10–13] Human serum albumin (HSA) is the most abundant plasma protein in our bloodstream (4–5 g/dL);[14] therefore, HSA nanotubes would be of medical importance as biocompatible nanocarriers for drugs, DNA, proteins, and viruses. Another advantage of the LbL assembly technique is that it confers a preferred electrostatic charge on the inner and outer surfaces of the tube by the first and last layer materials used. It can create an active loading and release system of guest molecules into the pore.[15] Herein, we present the synthesis and structure of HSA nanotubes having different charges on their inner and outer surfaces. The nanotubes were fabricated by alternate LbL depositions of polycation [polyallylamine hydrochloride (PAH), poly-L-arginine hydrochloride (PLA), poly-L-lysine hydrobromide (PLL) (Figure 1)] and HSA into a porous AAO membrane, followed by release of the cylindrical core by wet-chemical etching of the template.

The isoelectric point (*p*I) of HSA is 4.8: the protein is negatively charged at physiological pH and positively charged below pH 4.8.[14] A citric acid buffer (CB, pH 3.8) solution and phosphate buffer (PB, pH 7.0) solution of HSA were alternately filtered through the AAO membrane (200 nm pore-size, Anodisc, Whatman Japan K.K.) using a syringe pump.[13] The LbL deposition for three cycles of each component produced three bilayers (total six layers) of HSA onto the pore walls: $(\text{HSA/HSA})_3$. Instead of the CB (pH 3.8) solution of HSA, a PB solution (pH 7.0) of PAH, PLA, or PLL was used. The obtained hybrid AAO membrane was fixed on a silicon wafer with epoxy resin and immersed in a 10% phosphoric acid to dissolve the alumina template.[16]

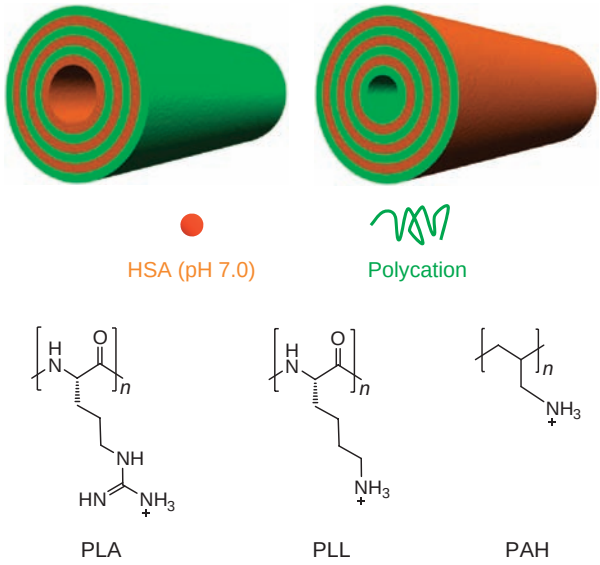

Figure 1. Schematic illustrations of HSA nanotubes having different charges on the outer surface and inner pore (o/i) (left picture: positive (+; green)/negative (−; orange), right picture: −/+). Structures of polycations used for template synthesis of HSA nanotube.

Scanning electron microscopy (SEM) images of the sample showed formations of very uniform and flexible nanotubes of $(\text{HSA/HSA})_3$, $(\text{PAH/HSA})_3$, and $(\text{PLA/HSA})_3$ with respective outer diameters of 330 ± 16 nm (Figure 2a), 349 ± 15 and 321 ± 14 nm (Figures 2b and 2c, respectively). Although we used a 200-nm pore template, the tubules' diameters were greater than the value because the pore diameter in the middle of the AAO membrane was over 300 nm. The maximum tube length (ca. 60 μm) corresponds to the template thickness. The nanotube wall thickness was ca. 110 nm. Therefore, the inner diameter was estimated as ca. 120 nm. In contrast, the PLL/HSA formed soft nanotubes (338 ± 22 nm outer diameter) and the edges of the hollows were not sharp (Figure S1).[16] Polyethyleneimine with HSA did not yield a stable tube. These results imply that the HSA nanotube structure is dependent on the properties of the complement polycation.

In neutral water (pH 7.0), the $(\text{HSA/HSA})_3$ nanotubes retain negative charges of the pore; however the positive charges on the outer surface partly become negative because of the low *p*I value of HSA. On the other hand, the $(\text{PAH/HSA})_3$ and $(\text{PLA/HSA})_3$ nanotubes can maintain their negative charges of the pores and positive charges on the exterior (Figure 1, left picture). Interestingly, preparation with positively charged HSA (pH 3.8) as the first layer and followed by three-bilayers of (HSA/PLA) also yielded similar nanotubes (338 ± 18-nm outer diameter)

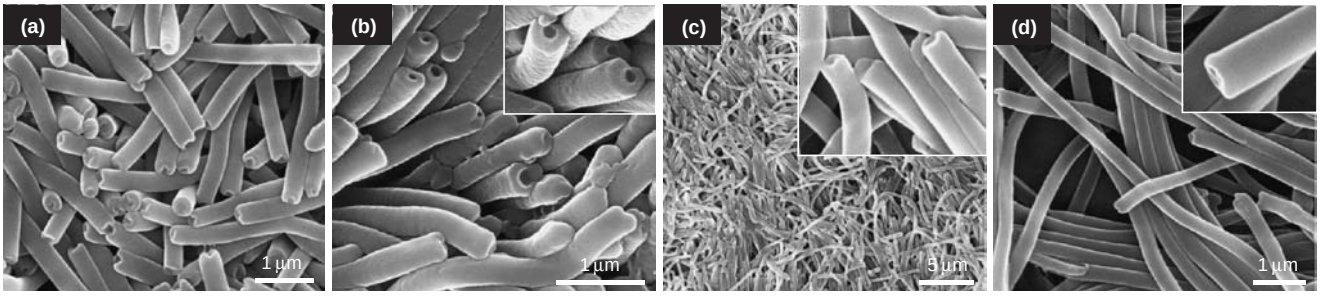

Figure 2. FE-SEM images of (a) $(HSA/HSA)_3$ nanotubes (outer surface charge/inner pore charge: $-/-$), (b) $(PAH/HSA)_3$ nanotubes $(+/-)$, (c) $(PLA/HSA)_3$ nanotubes $(+/-)$, and (d) $HSA(HSA/PLA)_3$ nanotubes $(-/+)$.

(Figure 2d). This $HSA(HSA/PLA)_3$ nanotube (total seven layers) has a positively charged interior and negatively charged outer surface (Figure 1, right picture) at pH 7.0. The LbL deposition technique can provide a desired cylindrical hollow structure of HSA in which the inner and outer surface charges can be modulated by the first and last layer component used.

To confirm the LbL film growth of HSA and the polycations, a $(PAH/HSA)_n$ thin film was prepared on a planar quartz substrate using a general dipping procedure.[17,18] The cleaned quartz plate was immersed repeatedly into the PAH and HSA solution, which yielded PAH/HSA multilayers.[16] Formation of the alternate LbL assembly was monitored using visible absorption spectroscopy. An iron–tetraphenylporphyrin derivative[19] was incorporated into HSA to enhance the absorption.[20] The initial layer of PAH showed no spectrum in the 350–700-nm region. On the other hand, the second layer of HSA exhibited marked absorbance at 428 nm, which is attributed to the porphyrin Soret band. The absorbance at 428 nm increased linearly with the number of HSA layers (Figure 3). The combination of PLA/HSA and PLL/HSA exhibits a similar LbL assembly. These results clearly show alternate film formations of the $(polycation/HSA)_n$.

In conclusion, we prepared protein nanotubes comprising LbL assemblies of HSA with polycation using template synthesis with a porous AAO membrane. Especially, PLA and PAH were effective polycations as electrostatic glue for holding each protein layer. This method to synthesize a cylindrical structure is applicable to almost any protein. These protein architectures might present a new area of smart nanotube development to create products that are useful for biochemical and biomedical applications.

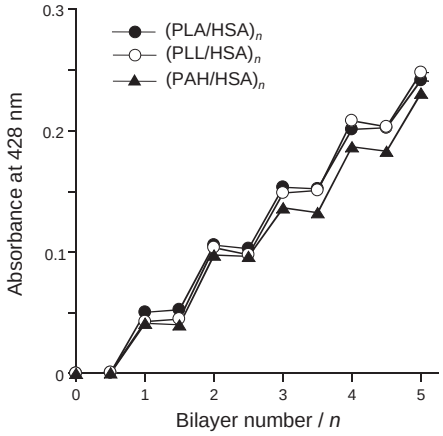

Figure 3. UV absorbance at 280 nm of LbL $(polycation/HSA)_n$ thin film on the planar quartz substrate.

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References and Notes

1 a) C. R. Martin, *Science* **1994**, *266*, 1961. b) S. Hou, C. C. Harrell, L. Trofin, P. Kohli, C. R. Martin, *J. Am. Chem. Soc.* **2004**, *126*, 5674.
2 a) M. Steinhart, J. H. Wendorff, A. Greiner, R. B. Wehrspohn, K. Nielsch, J. Schilling, J. Choi, U. Gösele, *Science* **2002**, *296*, 1997. b) M. Steinhart, R. B. Wehrspohn, U. Gösele, J. H. Wendorf, *Angew. Chem., Int. Ed.* **2004**, *43*, 1334.
3 L. Zhi, T. Gorelik, J. Wu, U. Kolb, K. Mullen, *J. Am. Chem. Soc.* **2005**, *127*, 12792.
4 J. Jang, S. Ko, Y. Kim, *Adv. Funct. Mater.* **2006**, *16*, 754.
5 R. O. Al-Kaysi, C. J. Bardeen, *Chem. Commun.* **2006**, 1224.
6 C. Barrett, D. Iacopino, D. O'Carroll, G. De Marzi, D. A. Tanner, A. J. Quinn, G. Redmond, *Chem. Mater.* **2007**, *19*, 338.
7 S. Ai, G. Lu, Q. He, J. Li, *J. Am. Chem. Soc.* **2003**, *125*, 11140.
8 D. H. Kim, P. Karan, P. Göring, J. Leclaire, A.-M. Cambinade, J. Majoral, U. Gösele, M. Steinhart, W. Knoll, *Small* **2005**, *1*, 99.
9 S. Hou, J. Wang, C. R. Martin, *J. Am. Chem. Soc.* **2005**, *127*, 8586.
10 G. Lu, S. Ai, J. Li, *Langmuir* **2005**, *21*, 1679.
11 S. Hou, J. Wang, C. R. Martin, *Nano Lett.* **2005**, *5*, 231.
12 S. J. Son, J. Reichel, B. He, M. Schuchman, S. B. Lee, *J. Am. Chem. Soc.* **2005**, *127*, 7316.
13 G. Lu, T. Komatsu, E. Tsuchida, *Chem. Commun.* **2007**, 2980.
14 T. Peter, *All about Albumin: Biochemistry, Genetics and Medical Applications*, Academic Press, San Diego, **1996**.
15 a) H. Yui, Y. Shimizu, S. Kamiya, I. Yamashita, M. Masuda, K. Ito, T. Shimizu, *Chem. Lett.* **2005**, *34*, 232. b) N. Kameta, M. Masuda, G. Mizuno, N. Morii, T. Shimizu, *Small* **2008**, *4*, 561.
16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
17 Y. Lvov, K. Ariga, I. Ichinose, T. Kunitake, *J. Am. Chem. Soc.* **1995**, *117*, 6117.
18 J. Chen, S.-W. Huang, W.-H. Lin, R.-X. Zhuo, *Small* **2007**, *3*, 636.
19 2-(2-Methylimidazol-2-yl)octanoyloxymethyl-5,10,15,20-tetrakis($\alpha,\alpha,\alpha,\alpha$-*o*-pivalamidophenyl)porphinatoiron(II)–carbonyl complex.
20 T. Komatsu, Y. Matsukawa, E. Tsuchida, *Bioconjugate Chem.* **2002**, *13*, 397.

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