Surface molecularly imprinted nanowire for protein specific recognition†

Ruizhuo Ouyang, Jianping Lei and Huangxian Ju*

Received (in Cambridge, UK) 17th June 2008, Accepted 3rd September 2008
First published as an Advance Article on the web 2nd October 2008
DOI: 10.1039/b810248a

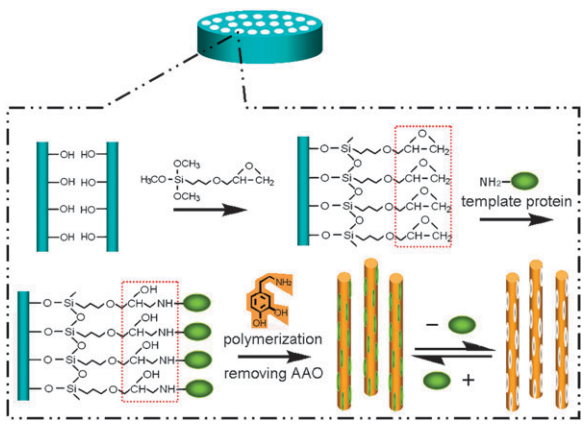
A surface molecularly imprinted nanowire is designed by chemical polymerization of dopamine in neutral aqueous solution, which shows high binding capacity and acceptable specific recognition behavior towards template proteins.

Molecular imprinting technology is one of the most promising approaches towards both rapidly and inexpensively producing biomimetic materials for applications in material science, molecular devices, separation science, catalysis and biomedical fields, especially in molecular recognition.[1] The imprinting of small organic or biomolecules such as drugs, steroids, sugars, amino acids and peptides is now well achieved and the resulting molecularly imprinted polymers (MIPs) have been considered as routine.[2] Although protein imprinted xerogels with integrated emission sites have been successfully applied in fluorimetric sensing of protein,[3] imprinting of large structures such as proteins and other biomacromolecules still presents challenges[1b,d,2a,4,5] due to molecular size, complexity, conformational flexibility and solubility factors. First, proteins are water-soluble, which is not always compatible with mainstream MIP preparation in organic solvents. Second, the highly cross-linked rigid networks of MIPs often result in low binding capacity and poor site accessibility due to slow diffusion-controlled equilibrium of template molecules in the interior of three-dimensional (3D) polymeric matrices.[6] Finally, the flexible structure and conformation of protein can be easily affected by the surrounding microenvironment of the imprinted cavity. In order to remove these problems, searching for new functional monomers with good biocompatibility and multifunctional groups is a necessary requirement.

Dopamine (DA, 3,4-dihydroxyphenylalanine), a small-molecule mimic of proteins,[7] has plenty of noncovalent functionalities such as amino-containing and hydroxy-containing groups as well as π–π bonds. These groups exactly match the characteristics of MIPs for efficient protein recognition.[1c,8] Thus it can be perceived as a perfect functional monomer for above purpose. Our previous work has reported the electrochemical copolymerization of DA and *o*-phenylenediamine for preparation of MIPs to enantioselectively recognize glutamic acid.[9] However, the electrochemical polymerization is greatly limited to conductive substrates, and the obtained molecularly imprinted membrane is also disadvantageous for high-capacity binding and molecular recognition. More recently, the self-polymerization of DA on a wide variety of materials in alkaline pH to form multifunctional polymer coatings has been reported.[7] In this communication, we report a facile approach for the preparation of a novel nanowire by chemical polymerization of DA in neutral pH (physiological condition) with anodic alumina oxide (AAO) membrane as a nanomold (Scheme 1).[10] The designed protein-imprinted polydopamine (PDA) nanowires show high rebinding capacity and specific recognition ability towards template proteins in aqueous media.

The thickness and pore diameter of the AAO membranes used for chemical polymerization of DA nanowire in neutral pH in the presence of ammonium persulfate (APS) as initiator were 60 μm and 200 nm, respectively. The molar ratio of DA to APS was a key parameter in the polymerization process. When the molar ratio was lower than 1 : 1, no polymer could be formed in the pores of AAO (Fig. S1A, ESI†). The AAO could even be partly or fully dissolved at high APS concentration. When the molar ratio was 1.5 : 1, the imprinted polymer in the pores showed wall-conglutinated nanotubes but with variable wall-thickness (Fig. S1B and C, ESI†). With the increasing molar ratio of DA to APS the formed nanotubes became more regular and the wall thickness increased until a wirelike nanostructure was formed. However, when the molar ratio was higher than 2 : 1, the imprinted polymer nanowires showed breakable structure (Fig. S1D, ESI†). Thus the optimal molar ratio of DA to APS was selected as 2 : 1, at which the obtained PDA nanowires showed desirable surface morphology (Fig. 1).

The obtained PDA nanowires showed excellent monodispersibility in water (Fig. 1A). Different from polypyrrole and polyacrylamide prepared with a similar procedure,[11] no



Scheme 1 Preparation and specific recognition of surface protein-imprinted PDA nanowires.

Key Laboratory of Analytical Chemistry for Life Science (Education Ministry of China), Department of Chemistry, Nanjing University, Nanjing, 210093, P. R. China. E-mail: hxju@nju.edu.cn; Fax: +86-25-83593593; Tel: +86-25-83593593

† Electronic supplementary information (ESI) available: Related reagents, instrumentation, praparation of protein-imprinted PDA nanowires, effect of molar ratio of DA to APS on the morphology of PDA nanowire, hydrophilicity of PDA nanowires, XPS characterization of AAO at different modifying stages, morphology of IPWs, binding amounts of target proteins as well as effects of pH and ionic strength, specificity of bovine and human Hb-IPWs. See DOI: 10.1039/b810248a

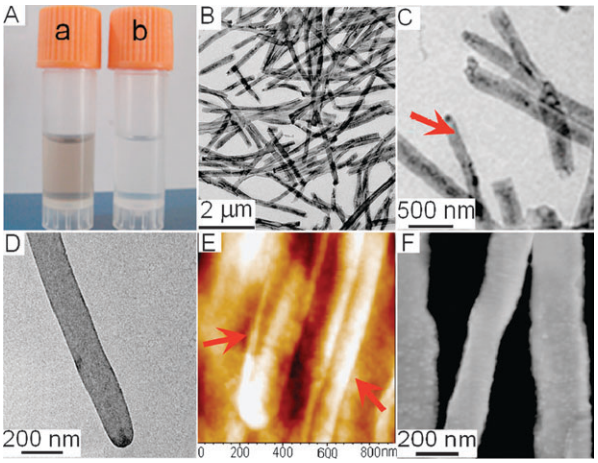


Fig. 1 Photographs of water in the presence (a) and absence (b) of PDA nanowires (A), and transmission electron microscopic (TEM) (B–D), atomic force microscopic (AFM) (E) and scanning electron microscopic (SEM) (F) images of PDA nanowires.

conglomerate structures of the PDA nanowires were observed. The TEM images indicated the formed PDA nanowires were uniform and their diameter was *ca.* 200 nm, the same as the pore size of AAO (Fig. 1B–D). Thus the size of PDA nanowires could be controlled by changing the pore size of AAO. The magnified image of a single nanowire marked with a red arrow in Fig. 1C showed a solid structure (Fig. 1D), suggesting that the obtained polymers were not nanotubes but the desired nanowires. The nanowires had lower possibility than the nanotubes for the nonspecific adsorption of protein due to the smaller surface area. Additionally, both AFM (Fig. 1E) and SEM (Fig. 1F) images also showed that the prepared PDA nanowires had good dispersibility and uniform surface, which is believed to be helpful for improving the binding capacity of protein.

The hydrophilicity of PDA nanowires was characterized by contact angle measurement. A bare glass slide without any treatment and the PDA nanowire-coated glass slide gave contact angles of 68.50 and 28.60°, respectively (Fig. S2, ESI†). The much lower contact angle of PDA nanowires indicated better hydrophilicity. This property was compatible for specific binding of biomolecules to the receptors. Thus, the PDA nanowires implied potential applications in recognition of biomolecules.

X-Ray photoelectron spectroscopic (XPS) characterization was performed to analyze the composition of the PDA nanowires. The XPS survey spectrum of PDA showed a nitrogen-to-carbon signal ratio (N/C) of 0.105 (Fig. 2A), which was in the N/C range of PDA polymer coatings formed on different materials,[7] and close to the theoretical value for dopamine (N/C = 0.125). The fitted C 1s spectrum (signal at 288.29 eV, Fig. 2B) confirmed the oxygen-containing groups within the obtained PDA structure existed in the form of hydroxy groups, which showed a peak at 533.64 eV in the fitted O 1s spectrum (Fig. S3, ESI†). Similar to the polymerization process in the presence of oxygen,[7] the oxidation of DA monomer in presence of APS and rearrangement of the oxidation product led to an intermolecular cross-linking polymer structure. The abundant functional groups and well-defined network were beneficial to efficient protein imprinting and rebinding.

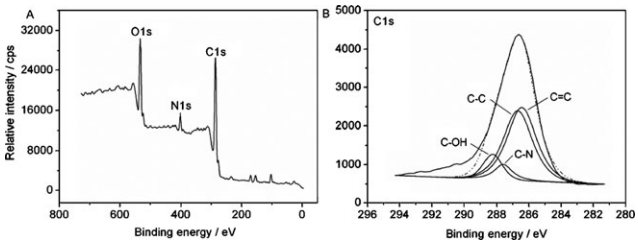


Fig. 2 XPS survey (A) and fitted C 1s (B) spectra of PDA prepared without presence of template.

Considering these advantages of PDA nanowires, a method for template protein immobilization on the inner pore walls of AAO was then designed, with which the surface protein-imprinted PDA nanowires (IPWs) could be prepared for protein recognition using homologous proteins, bovine and human hemoglobin (Hb), as template molecules (Scheme 1).

First, terminal epoxy groups were introduced into the inner pore walls of AAO with an epoxysilane-related sol–gel process, which reacted spontaneously with free amino sites of protein[11] to attach template protein molecules to the inner walls of AAO.[12] In comparison with the XPS survey spectrum of non-modified AAO (Fig. S4A, ESI†), the presence of the Si 2p peak at 103.05 eV in epoxysilane modified AAO (Fig. S4B, ESI†) indicated that epoxy groups were successfully introduced to the pore walls of AAO. Similarly, the efficient coupling of template protein on the inner wall of silica nanotubes was clearly confirmed by the appearance of an N 1s peak at 401.40 eV (Fig. S4C, ESI†). This method simplified greatly the immobilization procedure of protein for preparation of MIPs, as compared with that using glutaraldehyde as a linker.[12a]

Both bovine and human Hb-IPWs were then prepared by immersing the protein-coupled alumina membranes in a solution containing DA and APS at a molar ratio of 2 : 1, respectively. After polymerization, the alumina membranes and template protein were removed. The resulting PDA nanowires were characterized by TEM, SEM and AFM (Fig. S5, ESI†), which also showed good monodispersibility and uniform surface morphology. The PDA nanowires with "footprints" of the template protein on the surface could reversibly rebind template protein molecules (Scheme 1). The optimal conditions of ionic strength and pH for obtaining the maximun binding amount of IPWs were at 50 mM and 7.35 (Fig. S6, ESI†), at which either bovine or human Hb-IPWs have high binding capacity towards individual template molecules, while the nonimprinted PDA nanowires (NIPWs) show low amount of bound protein molecules due to the nonspecific adsorption of protein on NIPWs (Fig. 3 and Fig. S7, ESI†). These results indicated that the imprinting procedure well imparted the imprinted cavity with a well defined size and positions of functional groups. Furthermore, a remarkable linear binding for two individual template proteins was achieved in the concentration range of IPWs up to 3.0 mg mL^{-1}. In this range the selectivity ratio of IPWs to NIPWs towards template protein was assessed at the same amount of nanowires according to the following equations:

$$K_B = C_s/C_l \quad (1)$$

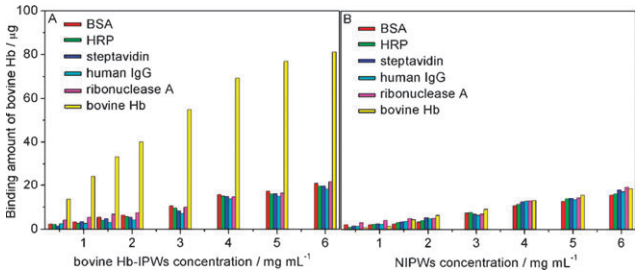


Fig. 3 Binding profiles of bovine Hb-IPWs (A) and NIPWs (B) to bovine Hb and other reference proteins.

where K_B is the binding equilibrium constant, C_s is the amount of template protein bound to the nanowires and C_l is the free protein concentration in the solution, and

$$\alpha = K_{BI}/K_{AN} \quad (2)$$

where α is selectivity ratio, K_{BI} and K_{AN} are the binding and adsorption equilibrium constants of protein on IPWs and NIPWs, respectively. Both IPWs displayed much higher binding constant towards the corresponding proteins than NIPWs (Table S1, ESI†), with selectivity ratios of 9.44 and 9.93 for bovine and human Hb-IPWs, respectively. Meanwhile, the bovine and human Hb-IPWs showed the maximum binding amounts of 25.33 ± 0.51 and 27.21 ± 0.60 mg g^{-1}, respectively (Fig. S8, ESI†). The high selectivity ratio and binding capacity of the IPWs were attributed to the sterically complementary cavity structure with excellently defined binding sites such as amino-containing, hydroxy-containing groups and π–π bonds and van der Waals forces available for interaction with the template protein, which led to the outstanding recognition behavior towards the target molecules.

Five proteins including bovine serum albumin (BSA, isoelectric point (IP) 4.7), streptavidin (IP 5.0), horseradish peroxidase (HRP, IP 7.2), human IgG (IP 9.0) and ribonuclease A (IP 9.6) were used to investigate competitive binding experiments with bovine and human Hb-IPWs. In the presence of the competing protein, the relative rebinding of template proteins to the IPWs still reached above 80% (Fig. S9, ESI†), which was much higher than the 60% of polyacrylamide nanowires.[12a] Additionally, the binding constants of IPWs towards the target proteins are 7–9 times higher than the other five reference proteins, indicating a high selectivity (Table S1, ESI†).

Homologous proteins, bovine and human Hb, were chosen as templates to further evaluate the specific recognition ability of the designed imprinted nanowires. The relative binding amounts of the template proteins against homologous proteins were impressively 4.1 and 4.5 times higher for bovine and human Hb-IPWs, respectively (Fig. S10, ESI†), despite their similar amino acid sequences and 3D structures. These results also indicated the high specificity and selectivity of the imprinted nanowires towards template proteins, which could contribute to the difference relative to homologous proteins in polar interactions[1c] and steric complementarity with the well-defined network of the imprinted cavity.

In summary, a facile method has been proposed to prepare a surface protein-imprinted nanowire for protein specific recognition by immobilization of template protein molecules on the pore walls of an AAO nanomold and then chemical polymerization of DA in physiological conditions. Profiting from the surface molecular imprinting technology, the IPWs have good site accessibility towards the target protein molecules, which makes the imprinting of large biomolecules very easy. The designed protein-IPWs have excellent monodispersibility, good hydrophilicity, and high rebinding capacity and showed specific recognition ability towards target protein molecules. Dopamine as a promising functional monomer for chemical polymerization overcomes the difficulties in protein imprinting to a great extent due to its unique biocompatibility and multifunctional groups. This work should provide a new insight into the preparation of surface molecularly imprinted nanomaterials for recognition of many other important biomacromolecules and biosensing application.

This work was supported by the National Science Funds for Creative Research Groups (20521503), the Major Research Plan (90713015), Key Program (20535010) and General Program (20705012) from the National Natural Science Foundation of China. We are also thankful for the help from Dr Huanghao Yang at the First Institute of Oceanography and Dr Jianshu Wang at the Modern Analytical Center of Nanjing University.

Notes and references

1 (*a*) H. Asanuma, T. Hishiya and M. Komiyama, *Adv. Mater.*, 2000, **12**, 1019; (*b*) L. Ye and K. Mosbach, *Chem. Mater.*, 2008, **20**, 859; (*c*) N. W. Turner, C. W. Jeans, K. R. Brain, C. J. Allender, V. Hlady and D. W. Britt, *Biotechnol. Prog.*, 2006, **22**, 1474; (*d*) K. Haupt, *Chem. Commun.*, 2003, 171.
2 (*a*) K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495; (*b*) O. Hayden, P. A. Lieberzeit, D. Blaas and F. L. Dickert, *Adv. Funct. Mater.*, 2006, **16**, 1269; (*c*) J. Matsui, T. Sodeyama, K. Tamaki and N. Sugimoto, *Chem. Commun.*, 2006, 3217.
3 Z. Tao, E. C. Tehan, R. M. Bukowski, Y. Tang, E. L. Shughart, W. G. Holthoff, A. N. Cartwright, A. H. Titus and F. V. Bright, *Anal. Chim. Acta*, 2006, **564**, 59.
4 (*a*) J. Svenson, N. Zheng and L. A. Nicholls, *J. Am. Chem. Soc.*, 2004, **126**, 8554; (*b*) B. H. Schmidt, K. Mosbach and K. Haupt, *Adv. Mater.*, 2004, **16**, 719; (*c*) C. J. Tan and Y. W. Tong, *Anal. Chem.*, 2007, **79**, 299; (*d*) T. Y. Guo, Y. Q. Xia, G. J. Hao, M. D. Song and B. H. Zhang, *Biomaterials*, 2004, **25**, 5905; (*e*) D. F. Tai, C. Y. Lin, T. Z. Wu and L. K. Chen, *Anal. Chem.*, 2005, **77**, 140; (*f*) M. Haruki, Y. Konnai, A. Shimada and H. Takeuchi, *Biotechnol. Prog.*, 2007, **23**, 1254; (*g*) F. L. Dickert and O. Hayden, *Anal. Chem.*, 2002, **74**, 1302; (*h*) M. Ogiso, N. Minoura, T. Shinbo and T. Shimizu, *Biomaterials*, 2006, **27**, 4177.
5 D. E. Hansen, *Biomaterials*, 2007, **28**, 4178.
6 (*a*) A. Bossi, S. A. Piletsky, E. V. Piletska, P. G. Righetti and A. P. F. Turner, *Anal. Chem.*, 2001, **73**, 5281; (*b*) C. D. Ki, C. Oh, S. G. Oh and J. Y. Chang, *J. Am. Chem. Soc.*, 2002, **124**, 14838.
7 H. Lee, S. M. Dellatore, W. M. Miller and P. B. Messersmith, *Science*, 2007, **318**, 426.
8 (*a*) F. L. Dickert and O. Hayden, *Adv. Mater.*, 2000, **12**, 311; (*b*) G. Q. Fu, H. Yu and J. Zhu, *Biomaterials*, 2008, **29**, 2138.
9 R. Z. Ouyang, J. P. Lei, H. X. Ju and Y. D. Xue, *Adv. Funct. Mater.*, 2007, **17**, 3223.
10 (*a*) K. B. Jirage, J. C. Hulteen and C. R. Martin, *Science*, 1997, **278**, 655; (*b*) S. A. Miller, V. Y. Young and C. R. Martin, *J. Am. Chem. Soc.*, 2001, **123**, 12335; (*c*) S. B. Lee, D. T. Mitchell, L. Trofin, T. K. Nevanen, H. Söderlund and C. R. Martin, *Science*, 2002, **296**, 2198; (*d*) D. T. Mitchell, S. B. Lee, L. Trofin, N. Li, T. K. Nevanen, H. Söderlund and C. R. Martin, *J. Am. Chem. Soc.*, 2002, **124**, 11864.
11 Z. F. Fu, C. Hao, X. Q. Fei and H. X. Ju, *J. Immunol. Methods*, 2006, **312**, 61.
12 (*a*) Y. Li, H. H. Yang, Q. H. You, Z. X. Zhuang and X. R. Wang, *Anal. Chem.*, 2006, **78**, 317; (*b*) H. H. Yang, S. Q. Zhang, F. Tan, Z. X. Zhuang and X. R. Wang, *J. Am. Chem. Soc.*, 2005, **127**, 1378.