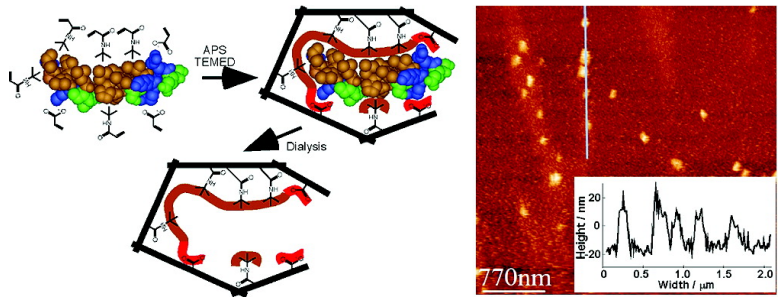
Communication

Peptide Imprinted Polymer Nanoparticles: A Plastic Antibody

Yu Hoshino, Takashi Kodama, Yoshio Okahata, and Kenneth J. Shea

J. Am. Chem. Soc., **2008**, 130 (46), 15242-15243 • DOI: 10.1021/ja8062875 • Publication Date (Web): 23 October 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/23/2008

Peptide Imprinted Polymer Nanoparticles: A Plastic Antibody

Yu Hoshino,[†] Takashi Kodama,[‡] Yoshio Okahata,[‡] and Kenneth J. Shea*,[†]

Department of Chemistry, University of California, Irvine, California 92697, and Department of Biomolecular Engineering, Tokyo Institute of Technology, Yokohama 226-8501, Japan

Received August 8, 2008; E-mail: kjshea@uci.edu

Synthetic polymer nanoparticles (NPs) capable of binding to specific biomacromolecules are of significant interest as substitutes for antibodies. Such particles can be utilized as inexpensive and stable functional materials for medicine, drug targeting, separations, biosensors, diagnostics, and antidotes for toxins and viruses. The nonbiological approach of molecular imprinting creates populations of specific recognition sites in robust network polymers by polymerization of cross-linking and functional monomers in the presence of a target molecule.[1] Recently, this approach has been extended to biologically interesting targets including peptides and proteins.[2] Traditional imprinting is achieved with polymers that are prepared in bulk; methods to imprint specific binding sites for biomacromolecules on protein size NPs have not been reported. Here we describe an approach for preparing imprinted polymer NPs that have a specific binding affinity for the bee toxin melittin (Mel), a 26 amino acid peptide. The NPs are comparable in size to protein clusters with average affinities that approach those of antibodies.

The mild conditions of precipitation polymerization using acrylamides such as *N*-isopropylacrylamide (NIPAm) were optimal for biomacromolecular imprinting. These formulations have been shown to yield a 10−100 nm monomodal distribution of polymer NPs in aqueous solution using very low concentrations of surfactant.[3] The monomers used for polymer synthesis included NIPAm as the backbone monomer in combination with acrylamide (AAm), acrylic acid (AAc), *N*-(3-aminopropyl) methacrylamide hydrochloride (APS), and *N*-*tert*-butylacrylamide (TBAm), as hydrogen-bonding, negative-charged, positive-charged, and hydrophobic functional monomers and *N*,*N*′-methylenebisacrylamide (BIS, 2 mol %) as a cross-linker (Figure 1a). Employing combinations of the above monomers, we synthesized a small combinatorial library of NPs (Table 1, Supporting Information (SI)). Significantly, the polymer synthesis does not require organic solvent or a heating step that would be expected to cause denaturation of many biomacromolecules.

Melittin was chosen as the target and imprint molecule for this work (Figure 1b). Melittin (Mel), the major component of bee venom from *Apis mellifera*, is a well-studied biotoxin. It is a 26 amino acid peptide with known strong cytolytic and antimicrobial activity.[4] Mel has also been used as a model target for development of general toxin inhibiters.[5] Mel (600 nM) was added to the monomer solution prior to polymerization to synthesize Mel imprinted polymer (MIP) NPs. Control (nonimprinted) polymers (NIPs) were prepared in an identical manner but in the absence of mellitin. Initiation was achieved by addition of ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine. Following polymerization, Mel and any unreacted monomers were removed by extensive dialysis. Removal of target peptide (>99%) from the NPs was confirmed by HPLC and the fluorescent signal from tryptophan

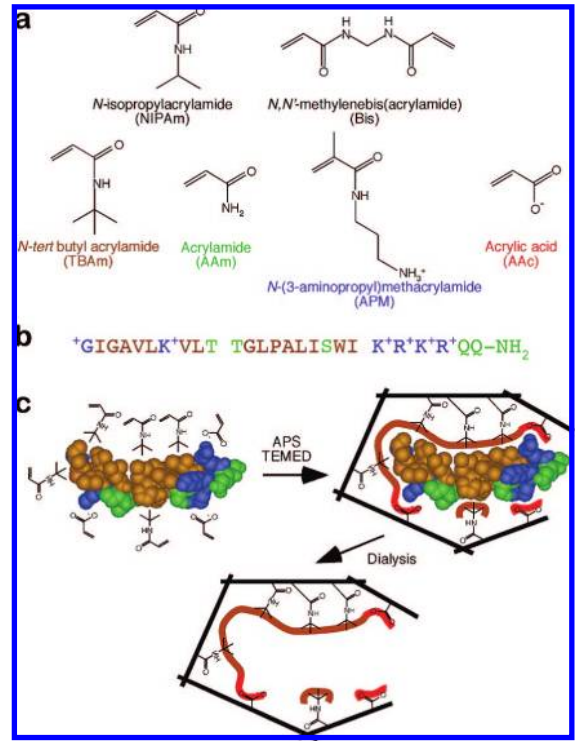


Figure 1. (a) Monomers used for NP synthesis. (b) Amino acid sequence of Mel. (c) Schematic representation of the Mel imprinting process. Hydrophobic, positive/negative charged, and hydrophilic residues are printed in brown, blue/red, and green. The PDB ID is 2MLT.

(Supporting Figures 1 and 2). Any remaining traces of melittin is not expected to influence subsequent studies since it would comprise <0.03% of the NP and would be trapped in the interior of the NP. The hydrodynamic diameter of the NPs was measured by DLS, and the yields of polymer NPs ranged from 50 to 90% (Table 1, SI).

A 27 MHz quartz crystal microbalance (QCM) was used to quantify interactions between the small library of MIP and NIP NPs and Mel (Figure 2a).[6] Figure 2b shows representative time courses of the frequency change (ΔF) of a QCM surface functionalized with Mel. NIP and MIP nanoparticle solutions were injected at the indicated intervals. The greatest increase of mass on the QCM electrode was found upon injection of MIP NPs that were comprised of 5% AAc and 40% TBAm (**9**, **12**, Table 1 SI). Little mass change was observed upon injection of the same concentration of NPs but polymerized *without* Mel (NIP, **14**) (Figure 2b). The optimum formulation of MIP NPs was achieved with 5% AAc 40% TBAm (**9**) and 5% AAm 5% AAc 40% TBAm (**12**). Other formulations in the library had little affinity to Mel (Figure 2c). We also examined the interaction between plasma proteins and MIP NPs; the imprinted NPs had little or no interaction with albumin and fibrinogen (Figure

[†] University of California.
[‡] Tokyo Institute of Technology.

10.1021/ja8062875 CCC: $40.75

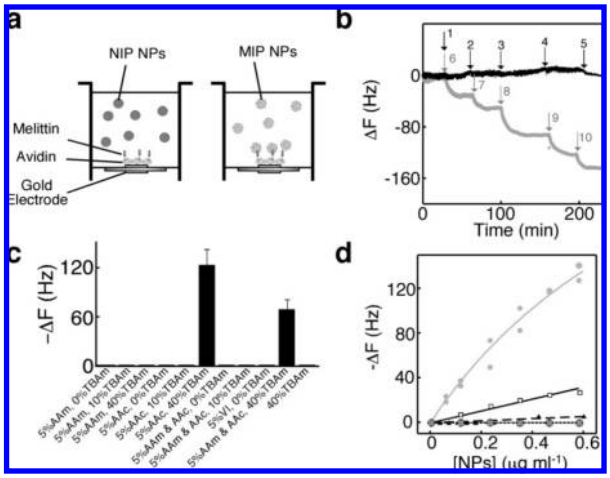


Figure 2. Interaction between polymer NPs and Mel and plasma proteins observed by a QCM in water with 6.9 μM SDS, 25 °C. (a) Schematic of QCM experiments to monitor interactions between NIP/MIP (left/right) NPs and Mel immobilized on QCM electrode. (b) Representative time course of the frequency change (ΔF) of Mel immobilized on a 27 MHz QCM following injection of NIP **14** and MIP **9** solutions. Solutions of NIP (black line) and MIP (gray line) were injected at the time points indicated by the black and gray arrows into the QCM cell. Final concentrations after each arrow are 0.11 (1), 0.18 (2), 0.24 (3), 0.35 (4), 0.42 (5), 0.12 (6), 0.23 (7), 0.35 (8), 0.46 (9), and 0.58 (10) μg mL^{-1}. (c) QCM screening of the library of polymer NPs for their interaction with Mel (the mean frequency change $\pm$ standard deviation ($n = 3$)) after injection of 0.6 μg mL^{-1} NPs into a Mel-immobilized QCM. (d) Binding isotherms of MIP NPs to Mel (gray circles), BSA (white circles), fibrinogen (gray crosses), and γ-globulin (white squares) immobilized on the QCM surface and NIP NPs to Mel (black triangles) immobilized QCM.

2d). γ-Globulin had a slightly higher affinity to the MIP nanoparticle but still much less than that of Mel. These results establish that the optimal polymer formulations were found for imprinting by Mel during polymerization to produce NPs with complimentary binding sites. A calculated apparent dissociation constant ($K_{d(app)}$) of 7.3−25 pM was obtained by nonlinear fitting of the binding plot to the Langmuir isotherm under an assumption that all particles in solution are 54 nm spheres and the polymer density is $0.08 < \rho < 0.27$.[3] This dissociation constant is comparable to that of a natural antibody ($K_d = 17$ pM).[7]

The binding results demonstrate the importance of optimizing monomer composition to achieve successful imprinting of target binding sites on polymer NPs. The target molecule, Mel, has 26 residues of which 6 are positively charged (Figure 1b). The sequence is amphiphilic, since six amino acids at the C-terminus of the peptide are hydrophilic, while the remainder is comprised of a high proportion of apolar residues. The successful monomer combination for Mel imprinting contains both 40% of hydrophobic monomers (TBAm) and 5% of negatively charged functional monomers (AAc). Other MIP NPs, even those MIP NPs containing 5% and AAc 10% TBAm, do not interact with Mel at the same concentration. Successful MIP NPs are capable of interacting with Mel by both electrostatic and hydrophobic interactions and enable Mel to be incorporated as a template into the polymerizing NPs with high efficiency (Figure 1c). Several observations are consistent with this proposal. For example, the fluorescence of tryptophan in Mel is quenched in proportion to added TBAm. The diminution of fluorescence intensity occurs in the μM region (Supporting Figure 3), indicating that part of the target molecule interacts with TBAm

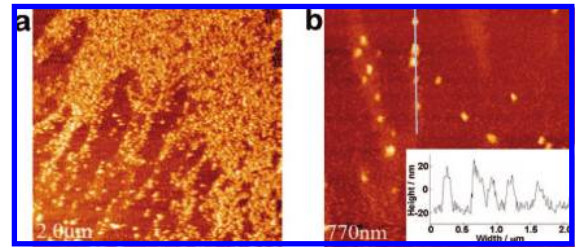


Figure 3. AFM images of MIP NPs synthesized with 5% AAc (a) and 40% TBAm (b). A height profile of cross section (blue line) is shown in insert.

monomers in the prepolymerization solution. In addition, a prepolymerized monomer solution containing 40% TBAm became cloudy upon addition of Mel.

AFM images of MIP NPs **9** show the NPs are well dispersed over a wide area of the mica surface (Figure 3a, b). The diameter of particles obtained from the height profile was in the range 30−40 nm (Figure 3b insert). The size is comparable to that of IgM and suggests that MIP NPs may be capable of being transported by diffusion in viscous mucus as well as blood capillaries.

In summary, high affinity and selective polymer NPs for the biotoxin melittin were synthesized by an imprinting precipitation polymerization. The optimum NP formulation was selected by QCM evaluation of a small combinatorial library of functional monomers. The binding affinity and size of the MIP NPs are similar to those of natural antibodies. These results are a starting point for the preparation and evaluation of synthetic polymer antibodies for key boimacromolecules.

Acknowledgment. We thank T. Ozeki at Initium, Inc. for help with QCM measurement as well as D. Griffiths and A. Hou at Horiba Instruments, Inc. for help in DLS measurement. Y.H. was supported by a JSPS postdoctoral fellowship.

Supporting Information Available: Experimental procedures and supporting data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) *Molecularly Imprinted Materials: Science and Technology*; Yan, M., Ramstrom, O., Eds.; CRC Press: 2004. (b) *Molecularly Imprinted Polymers*; Sellergren, B. Ed.; Elsevier: Amsterdam, 2001. (b) Shea, K. *Trends Polym. Sci.* **1994**, *2*, 166–173. (c) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812–1832. (d) Haupt, K. *Anal. Chem.* **2003**, *75*, 376A–383A. (e) Zimmerman, S. C.; Lemcoff, N. G. *Chem. Commun.* **2004**, 5–14. (f) Mosbach, K. *Sci. Am.* **2006**, *295*, 86–91.

(2) (a) Hansen, D. E. *Biomaterials* **2007**, *28*, 4178–4191. (b) Bossi, A.; Bonini, F.; Turner, A. P. F.; Piletsky, S. A. *Biosens. Bioelectron.* **2007**, *22*, 1131–1137. (c) Kempe, M.; Glad, M.; Mosbach, K. *J. Mol. Recognit.* **1995**, *8*, 35–39. (d) Hjertén, S.; Liao, J. L.; Nakazato, K.; Wang, Y.; Zamaratskaia, G.; Zhang, H. X. *Chromatographia* **1997**, *44*, 227–234. (e) Hart, B. R.; Shea, K. J. *J. Am. Chem. Soc.* **2001**, *123*, 2072. (f) Rachkov, A.; Minoura, N.; Shimizu, T. *Anal. Sci.* **2001**, *17*, 609–612. (g) Nishino, H.; Huang, C. S.; Shea, K. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 2392–2396. (h) Miyata, T.; Jige, M.; Nakaminami, T.; Uragami, T. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 1190. (i) Li, Y.; Yang, H. H.; You, Q. H.; Zhuang, Z. X.; Wang, X. R. *Anal. Chem.* **2006**, *78*, 317–320.

(3) (a) Debord, J. D.; Lyon, L. A. *Langmuir* **2003**, *19*, 7662–7664. (b) Ogawa, K.; Nakayama, A.; Kokufuta, E. *Langmuir* **2003**, *19*, 3178–3184.

(4) (a) Habermann, E. *Science* **1972**, *177*, 314–322. (b) Bechinger, B. *J. Membr. Biol.* **1997**, *156*, 197–211.

(5) (a) Britt, A. M.; Burkhart, K. K.; Billingsley, M. L. *Pharmacology* **1995**, *50*, 307–312. (b) Blondelle, S. E.; Houghten, R. A.; Pèrez-Payá, E. *J. Biol. Chem.* **1996**, *271*, 4093–4099.

(6) (a) Hoshino, Y.; Kawasaki, T.; Okahata, Y. *Biomacromol.* **2006**, *7*, 682–685. (b) Ebara, Y.; Okahata, Y. *J. Am. Chem. Soc.* **1994**, *116*, 11209–11212.

(7) Grünigen, R. V.; Schneider, C. H. *Immunology* **1989**, *66*, 339–342.

JA8062875