Photodimerization of Anthryl Moieties in a Poly(methacrylic acid) Derivative as Reversible Cross-linking Step in Molecular Imprinting

Jun Matsui,* Yoshifumi Ochi, and Katsuyuki Tamaki*
Department of Chemistry, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501

(Received September 16, 2005; CL-051189; E-mail: matsui@konan-u.ac.jp)

A methodology for reversible molecular imprinting is presented. A molecularly imprinted polymer (MIP) is prepared by photo-cross-linking poly(methacrylic acid) (PMAA) with anthryl moieties as photo-cross-linking sites in the presence of an antimalarial drug cinchonidine as a template molecule, resulting in enhanced binding capacity to cinchonidine, compared with non-imprinted polymers. MIP is then "initialized" by exposure to shortwave UV that cleaves the anthracene-dimer-based cross-links, resulting in the reduced binding capacity comparable to the non-imprinted polymers.

Molecular imprinting is a methodology to produce a polymer with functional groups arranged for recognition of specific target molecules.[1] Polymers obtained by molecular imprinting (molecularly imprinted polymers, MIPs) have often been compared to enzymes and antibodies, and utilized in various applications ranging from analysis[2] to catalysis.[3] In typical MIP syntheses, functional monomers, complexed with a template molecule, are polymerized (cross-linked) to preserve the arrangement of the monomers complementary to the template molecule. Even after removal of the template molecule from the resultant network polymer, it is believed that the complementarity can be still preserved in terms of both shape and functionality, allowing the polymer to specifically bind the template molecule. Cross-linking manners are greatly important for constructing binding sites, and have been the central theme for establishing the imprinting techniques.[4] Although radical polymerization has been the most commonly used in the MIP synthesis, the reaction is not quite appropriate for controlling and monitoring cross-linking degree. Furthermore, once the radical polymerization proceeds, the structure and property of resultant MIPs can not be changed due to inert cross-links. To date, however, few cross-linkers controllable by external stimuli have been reported.[5]

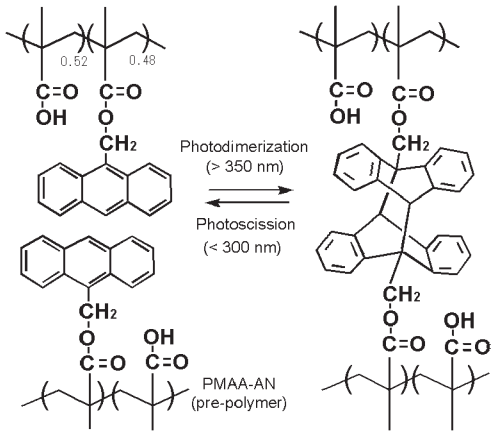

Figure 1. Photo-cross-linking/photoscission of poly(methacrylic acid) prepolymers with anthryl moieties.

In this study, aiming at performing reversible molecular imprinting upon external stimuli, we examine [4 + 4] photodimerization of anthryl groups as cross-linking step (Figure 1). It is known that anthracene and its derivatives form a dimer upon exposure to longwave UV and the dimer is restored to an original monomer by irradiation of shortwave UV.[6] Various polymeric materials have been reported utilizing this reaction,[7,8] and our design of molecular imprinting is based on these studies demonstrating interesting reversible properties. Another point in our design is a use of a linear polymer with cross-linkable moieties (a pre-polymer); it is appropriate to use pre-polymers as MIP precursors rather than monomers for constructing network structure by dimerization-based cross-linking. We previously reported that poly(methacrylic acid) (PMAA) with styryl moieties can be used for preparing cinchonidine-selective MIPs,[9] in which carboxyl moieties of PMAA are supposedly immobilized as complementary to cinchonidine by cross-linking.[10] In this study, we designed a PMAA-based pre-polymer bearing anthryl moieties to utilize their reversible dimerization for cross-linking.

A pre-polymer, PMAA-AN, was prepared by esterification of poly(methacrylic acid) with 9-chloromethylanthracene (9-CMA) in the presence of DBU according to a previously reported procedure.[9] Purification was conducted by repeated reprecipitation in acetone/DMF, and the formation of the ester bond was confirmed by an IR spectrum (1724 cm^{-1}). Quantifying unreacted (9-CMA) by a reversed phase HPLC, the ratio of the anthryl unit was estimated to be ca. 48%. Prior to molecular imprinting, reversibility of the cross-linking of PMAA-AN was examined. UV absorption spectra were recorded with the solution of PMAA-AN in DMF (3.3 mg mL^{-1}) filled in a quartz cell (Hellma, Cells with Detachable Windows, $l = 0.1$ mm). Upon UV light irradiation (365 nm, 4 W), the peaks at 351, 368, and 389 nm, which are derived from the anthryl moiety, were gradually diminished (Figure 2). After 120-min exposure, no significant absorption was observed, suggesting that practically all the anthryl moieties were converted to the dimers. Also in ^{1}H NMR spectra, broad peaks derived from anthyl moieties ($\delta = 7.0$–7.5) decreased in intensity and new peaks appeared ($\delta = 6.5$–7.0), suggesting the formation of the dimerization-based cross-links.[7] Subsequently, the shortwave UV light (254 nm, 16 W) was applied to the cross-linked mixture. Upon 5-min irradiation, the original peaks of the anthryl moiety were recovered up to ca. 22% of the original absorbance. However, further restoration was not observed. The result suggests that the scission of cross-links took place partially and the considerable number of cross-links remain intact. A reason of the unquantitative restoration is currently unknown, although similar phenomena have been reported in other cross-linking systems based on dimerization

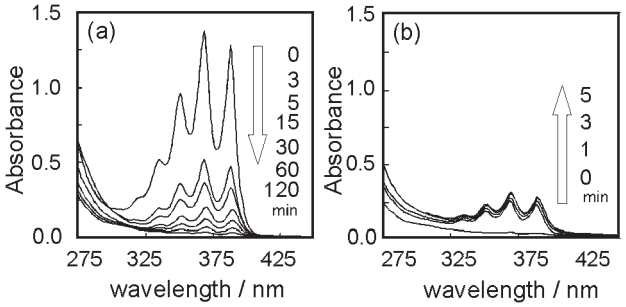

Figure 2. UV–vis spectra of PMAA-AN in DMF as a function of irradiation conditions; (a) 365 nm, (b) 254 nm.

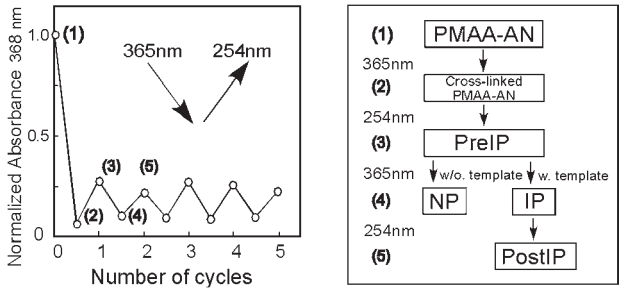

Figure 3. Number of reversible cycles of PMAA-AN monitored by UV–vis spectroscopy at 365 nm (left). Parenthesized numbers indicate the stages of the synthetic scheme from the pre-polymer to the post-cleaved imprinted polymer (right).

of anthryl moieties.[7]

Repeated dimerization/scission processes were examined by alternative irradiation at 365 nm (20 min) and 254 nm (10 min), recording the absorbance at 368 nm (Figure 3, left). The results suggest that the cross-linking process is fully reversible after the second cycle. Therefore, we planned to conduct reversible molecular imprinting with a partially cross-linked material (PreIP), which was obtained by irradiating PMAA-AN at 365 nm and subsequently at 254 nm (Figure 3, right).

For preparation of PreIP, in a screw-capped glass test tube, PMAA-An (530 mg) was dissolved in chloroform (30 mL) and, after sparging with nitrogen gas, the mixture was exposed to 365 nm light until no absorption derived from anthyl groups was observed. The mixture was subsequently irradiated at 254 nm until no further recovery of anthryl groups was observed in absorption spectra, poured into methanol, and centrifuged to obtain PreIP. Imprinting was conducted by irradiating (365 nm) fine particles of PreIP (32 mg) suspended in chloroform (2.0 mL) in the presence of cinchonidine (8.3 mg) as template. Resultant material was thoroughly washed with methanol–acetic acid, then with methanol until no release of cinchonidine was detected by a UV–vis spectrophotometer. After being dried under reduced pressure, the obtained imprinted-polymer (IP) was subjected to a batch binding test, where IP (8.0 mg) was immersed in acetonitrile solution of cinchonidine (100 μM, 10 mL) with continuous shaking for 18 h. After centrifugation, the supernatant was analyzed by a reversed-phase HPLC to estimate the amount of cinchonidine bound to the polymer. The batch binding test was also conducted with PreIP and a non-imprinted polymer (NP) that was prepared without addition of the template. The amount of cinchonidine bound to each polymer is shown in Figure 4. IP exhibited a binding capacity nearly twice as large

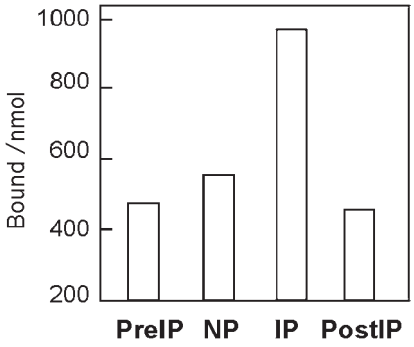

Figure 4. The amount of cinchonidine bound to the pre-imprint polymer (PreIP), non-imprinted polymer (NP), imprinted polymer (IP) and post-initialized polymer (PostIP).

as that of PreIP and NP, suggesting that the template induced the formation of cinchonidine-binding sites within the network polymer. Finally, IP was suspended in DMF and exposed to 254-nm light to erase the molecular memory effect by scission of the cross-links. The resultant polymer PostIP exhibited a binding capacity similar to PreIP, showing that the polymer lost the cinchonidine-binding ability introduced by the imprinting process.

In conclusion, poly(methacrylic acid) with anthryl moieties was examined as a potential precursor in synthesizing MIPs. Although optimization of the cross-link density and detailed characterization of the resultant materials should be conducted before establishing the utility of this pre-polymer, the controllability and reversibility of the photoresponsive cross-linking would be promising for opening new applications of MIPs; for instance, our methodology could be applied to construct MIP array chips with mask patterns, and MIP sorbents with affinity tunable and restorable after repeated use.

References

1 a) *Molecular Imprinting-From Fundamentals to Applications*, ed. by M. Komiyama, T. Takeuchi, T. Mukawa, H. Asanuma, Wiley-VCH, Weinheim, Germany, **2002**. b) B. Sellergren, *Molecularly Imprinted Polymers: Man-Made Mimics of Antibodies and their Application in Analytical Chemistry*, Elsevier, Amsterdam, **2001**. c) S. C. Zimmerman, N. G. Lemcoff, *Chem. Commun.* **2004**, 5.
2 a) R. Levi, S. McNiven, S. A. Piletsky, S.-H. Cheong, K. Yano, I. Karube, *Anal. Chem.* **1997**, *69*, 2017. b) K. Haupt, M. Mosbach, *Chem. Rev.* **2000**, *100*, 2495.
3 M. Tada, T. Sasaki, Y. Iwasawa, *J. Phys. Chem. B* **2004**, *108*, 2918.
4 S. A. Piletsky, I. Mijangos, A. Guerreiro, E. V. Piletska, I. Chianella, K. Karim, A. P. F. Turner, *Macromolecules* **2005**, *38*, 1410.
5 H. Hiratani, C. Alvarez-Lorenzo, J. Chuang, O. Guney, A. Y. Grosberg, T. Tanaka, *Langmuir* **2001**, *17*, 4431.
6 D. Cao, H. Meier, *Angew. Chem., Int. Ed.* **2001**, *40*, 186.
7 Y. Zheng, M. Micic, S. V. Mello, M. Mabrouki, F. M. Andreopoulos, V. Konka, S. M. Pham, R. M. Leblanc, *Macromolecules* **2002**, *35*, 5228.
8 a) T. Torii, H. Ushiki, K. Horie, *Polym. J.* **1993**, *25*, 173. b) Y. Chujo, K. Sada, R. Nomura, A. Naka, T. Saegusa, *Macromolecules* **1993**, *26*, 5611.
9 J. Matsui, N. Minamimura, K. Nishimoto, K. Tamaki, N. Sugimoto, *J. Chromatogr., B* **2002**, *804*, 223.
10 a) J. Matsui, I. A. Nicholls, T. Takeuchi, *Tetrahedron: Asymmetry* **1996**, *2*, 1357. b) J. Haginaka, C. Kagawa, *Anal. Sci.* **2003**, *19*, 39.

Published on the web (Advance View) December 10, 2005; DOI 10.1246/cl.2006.80