Molecularly Imprinted Polymer as 9-Ethyladenine Receptor Having a Porphyrin-Based Recognition Center

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Received January 5, 2000

Design and synthesis of artificial host molecules using porphyrins have been eagerly studied for understanding biological phenomena.[1] Recent tactics for the molecular design are based upon pre-organization, considering complementality of the host molecules to a given guest molecule in terms of chemical, topological characteristics, and compromising rigidity to maintain the complementarity and flexibility to enable induced-fit via cooperative multiple interaction.[2] The synthesis is conducted by using precursors bearing functional groups required or modifying a porphyrin to construct a three-dimensional binding site on a porphyrin plane, in which a guest molecule is bound via coordination by a porphyrin metal center or hydrogen bonding/electrostatic interaction by modifiers linked with the porphyrin.

In addition to the covalent bond-based synthesis strategy, other tactics utilizing self-organization of molecules have come to be recognized useful for building macro host structures.[3] Molecular imprinting (MI) is one of the strategies, that is a polymerization technique allowing monomers cross-linked while associated around a template molecule for fixing arrangements of the monomers complementary to the template molecule. Subsequent extraction of the template molecule from the self-designed polymers results in "tailor-made" formation of complementary binding sites.[4] In this study, we propose and demonstrate practical utility of MI for constructing a highly specific porphyrin-based receptor site, that is, a three-dimensional cavity on a porphyrin plane in cross-linked polymers to which a ligand is specifically bound through multiple-point interaction (Figure 1). As a model ligand, 9-ethyladenine (9EA) was selected because of its biologically importance, leading to great interests in host−guest studies.[5] The polymer syntheses were performed, using two different functional monomer species methacrylic acid (MAA) and **1**. An imprinted polymer receptor for 9EA, PPM(9EA), was prepared using both **1** and MAA. Reference imprinted polymers were also prepared using either **1** or MAA, namely PP(9EA)[6a] and PM(9EA), respectively. Corresponding nonimprint blank polymers, PPM(BL), PP(BL), and PM(BL), were prepared in the absence of 9EA using the identical monomers.

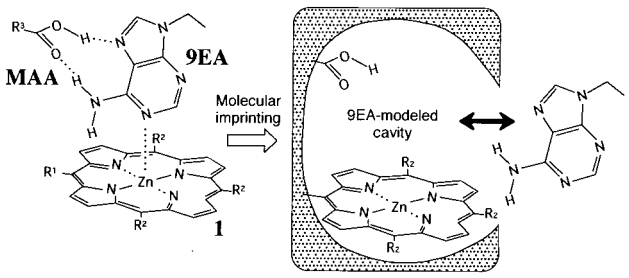

Figure 1. Schematic representation of molecular imprinting of 9-ethyladenine (9EA) using 5,10,15-tris(4-isopropylphenyl)-20-(4-methacryloyloxyphenyl)porphyrin zinc(II) complex **1** and methacrylic acid (MAA) as functional monomer. A typical recipe for PPM(9EA): into 7.75 mL of chloroform were added 9EA (50 mg, 0.31 mmol), **1** (276 mg, 0.31 mmol), MAA (53 mg, 0.62 mmol), ethylene glycol dimethacrylate (2.9 g) and 2,2′-azobis(2,4-dimethylvarelonitrile) (15.5 mg). The mixture was sonicated and heated at 45 °C for 16 h. Resultant polymer was ground, sieved, and washed with methanol−acetic acid. PM(9EA) and PP (9EA) were identically prepared using either MAA (0.62 mmol) or **1** (0.31 mmol) as functional monomer. A monomer **1** was prepared according to a procedure reported elsewhere.[6] For details, see Supporting Information 1.

The binding characteristics of the polymers were compared chromatographically using the polymers as stationary phases (Table 1). All of the imprinted polymers, PPM(9EA), PM(9EA), and PP(9EA), showed significant retention for the template species 9EA, while 9EA was eluted quickly in the blank polymers. The results suggest that each functional group randomly located in the polymers has no significant interaction for 9EA under the conditions applied and that the 9EA retentive property of the polymers was presumably induced by imprint effects, that is, complementary arrangement of functional monomers and production of 9EA-modeled cavities. The retention factor of 9EA, however, differs among the three imprint polymers. PPM(9EA) displayed a significantly larger retention factor than the other two prepared using a single functional monomer species; PM(9EA) and PP(9EA) respectively displayed 33% and 3% of the retention

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(1) (a) Falk, J. E. Porphyrins and Metalloporphyrins; Elsevier: New York, 1975. (b) Dolphin, D. The Porphyrins; Academic Press: New York, 1978.

(2) (a) Mizutani, T.; Kurahashi, T.; Murakami, T.; Matsumi, N.; Ogoshi, H. *J. Am. Chem. Soc.* **1997**, *119*, 8991−9001. (b) Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259−271. (c) Konishi, K.; Yahara, K.; Toshishige, H.; Aida, T.; Inoue, S. *J. Am. Chem. Soc.* **1994**, *116*, 1337−1344. (d) Ogoshi, H.; Hatakeyama, H.; Kotani, J.; Kawashima, A.; Kuroda, Y. *J. Am. Chem. Soc.* **1991**, *113*, 8181−8183. (e) Aoyama, Y.; Asakawa, M.; Yamagishi, A.; Toi, H.; Ogoshi, H. *J. Am. Chem. Soc.* **1990**, *112*, 3145−3151.

(3) (a) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89. (b) Lehn, J.-M. *Supramolecular Chemistry*; VCH: Weinheim, 1995. (c) Karpishin, T. B.; Vannelli, T. A.; Glover, K. J. *J. Am. Chem. Soc.* **1997**, *119*, 9063−9064. (d) Kuroda, Y.; Kawashima, A.; Hayashi, Y.; Ogoshi, H. *J. Am. Chem. Soc.* **1997**, *119*, 4929−4933. (e) McCallien, D. W. J.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 6611−6612.

(4) (a) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812−1832. (b) Haupt, K.; Mosbach, K. *Trends Biotechnol.* **1998**, *16*, 468−475. (c) Takeuchi, T.; Haginaka, J. J. *Chromatogr. B* **1999**, *728*, 1−20.

(5) (a) Shea, K. J.; Spivak, D. A.; Sellergren, B. *J. Am. Chem. Soc.* **1993**, *115*, 3368−3369. (b) Spivak, D.; Gilmore, M. A.; Shea, K. J. *J. Am. Chem. Soc.* **1997**, *119*, 4388−4393. (c) Mathew-Krotz, J.; Shea, K. J. *J. Am. Chem. Soc.* **1996**, *118*, 8154−8155. (d) Williams, K.; Askew, B.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1090. (e) Zimmerman, S. C.; Wu, W. *J. Am. Chem. Soc.* **1989**, *111*, 8054−8055. (f) Zimmerman, S. C.; Zeng, Z. *J. Org. Chem.* **1990**, *55*, 4789−479. (g) Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055.

(6) (a) Matsui, J.; Tachibana, Y.; Takeuchi, T. *Anal. Commun.* **1998**, *35*, 225−227. (b) Imahori, H.; Yamada, K.; Yoshizawa, E.; Hagiwara, K.; Okada, T.; Sakata, Y. J. *Porphyrins Phthalocyanines* **1997**, *1*, 55−66. (c) Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem.* **1987**, *52*, 827−836.

(7) (a) Yamamura, H. I.; Enna, S. J.; Kuhar, M. J. N*eurotransmitter Receptor Binding*; Raven Press: New York, 1985; Chapter 3.

(8) For Scatchard plots of PP(9EA) and PM(9EA), see Supporting Information 2.

(9) Shea has reported on a 9EA-imprinted polymer using MAA as functional monomer, showing a binding constant as 7.5×10^4 M^{-1} in chloroform, (see ref 5a). Although the recipe is not identical, the comparable value can be a proof of our Scatchard analysis for assessing the affinity of PM(9EA).

(10) Comparison of PPM(9EA) and PP(9EA) was also made by UV−vis absorption spectra. See Supporting Information 3.

(11) It is currently unknown how **1** and MAA interact with 9EA in the pre-polymerization mixture, although the interaction of 9EA with a zinc porphyrin and butyric acid has been independently studied; Ogoshi and Kuroda suggested that 9EA coordinates to a zinc porphyrin at its N1 with $K_a = 5300$ (see ref 2d), and Lancelot reported that butyric acid forms double or single hydrogen bond(s) at (N1 + amino-proton), (N7 + amino-proton) and N3 with $K_a = \sim 150$ [Lancelot, G. *J. Am. Chem. Soc.* **1977**, *99*, 7037−7042.]. Therefore, it would be reasonable to consider that **1** bound to N1, and MAA bound to (N7 + amino-proton), as illustrated in Figure 1, or to N3 in our case.

(12) Efforts have been made to understand the molecular imprinting mechanism: (a) Katz, A.; Davis M. E. Macromolecules 1999, 32, 4113−4121. (b) Sellergren, B.; Lepisto, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853−5860.

(13) Wang, J. H. *J. Am. Chem. Soc.* **1958**, *80*, 3168.

10.1021/ja000064+ CCC: $19.00
Published on Web 05/12/2000

Table 1: Retention Property of the Imprinted and Blank Polymers[a]

polymer	retention factor			
	9-EA	adenine	4-AP	2-AP
PPM(9EA)	28.9	0.0	2.23	0.79
PP(9EA)	0.85	2.25	0.52	0.04
PM(9EA)	9.57	0.0	1.41	0.48
PPM(BL)	0.14	0.58	0.39	0.28
PP(BL)	0.15	0.47	0.86	0.15
PM(BL)	0.09	0.19	0.37	0.14

[a] A mixture of dichloromethane, methanol and acetic acid (97:2:1, v/v/v) was used as the eluent at a flow rate of 0.5 mL min^{-1}. The column size was 100 mm × 4.6 mm i.d. and the sample size was 20 μL (1.0 mM). The elution was monitored by UV absorption at 260 nm. 4-Aminopyridine (4AP) and 2-aminopyridine (2AP) were tested because of their structural analogy to 9EA.

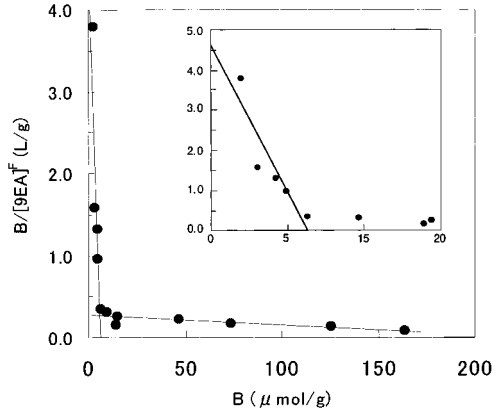

Figure 2. Scatchard plot of PPM(9EA). The polymer (3.5 mg) was incubated with 9EA (0−2.0 mM) in dichloromethane (3.5 mL). After centrifugation, supernatants were analyzed by reversed-phase HPLC (column: Supelco LB-8-DB, eluent: dichloromethane) to quantify 9EA bound to the polymer. Bound/free values are plotted versus the amount of 9EA bound. From the plot, the binding constant and the number of binding sites were estimated to be 7.5×10^5 M^{-1} (SD. 9.7×10^4) and 6.2 μmol/g (SD. 0.39), respectively.

factor value for 9EA compared to that marked by PPM(9EA). The drastic effects of the simultaneous use of two functional monomers strongly suggest the effective cooperation of the porphyrin-based and carboxylic residues rather than independent operation for retaining 9EA. Noteworthy improvement of selectivity was also marked by the supplemental use of MAA, compared to the previous imprinting system using **1** only:[6a] while PP(9EA) did not discriminate 9EA and its precursor adenine, PPM(9EA) exhibited the longest retention of 9EA and no significant retention of adenine, again suggesting the presence of cooperative arrangements of **1** and MAA for forming binding site complementary to 9EA.

Affinity of the imprinted polymers was assessed by Scatchard analysis (Figure 2).[7] A nonlinear profile was displayed, which is commonly observed in the Scatchard assessment of molecularly imprinted polymers and is suggestive for the presence of binding site exhibiting various affinities to the ligand.[5a,7] The assessment was therefore conducted, paying particular attention to a partially linear section observed at a range of 2.5−70 μM where relatively high-affinity binding sites of each polymer can be estimated. As a result, PPM(9EA) marked a larger binding constant (7.5×10^5 M^{-1}, SD. 9.7×10^4) than PP(9EA) (3.8×10^4 M^{-1}, SD. 6.0×10^3) and PM(9EA) (1.36×10^5 M^{-1}, SD. 5.2×10^3).[8] This leads us to a conclusion that **1** and MAA are cooperatively arranged by the single template molecule to form the high-affinity binding site, because independent arrangements of the two functional monomers can only contribute to the total number of binding sites. It is also notable that the binding constant estimated for PPM(9EA) was higher compared to artificial 9EA receptors previously reported.[5,9]

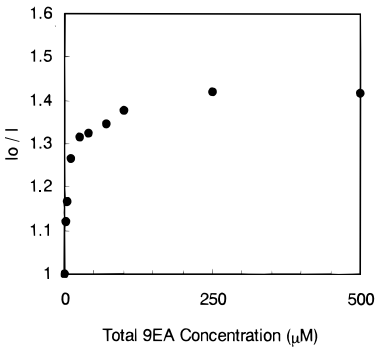

Figure 3. Fluorescence intensity at emission maximum around 605 nm (ex: 423 nm) of PPM(9EA) suspension (2.0 mg) incubated with 9EA in dichloromethane (2.0 mL). Dichloromethane was chosen due to suitability for the metal coordination and hydrogen bond formation expected between the polymers and 9EA, and the density close to that of the polymer tested for stable suspension.

Fluorescence spectra of the polymers are helpful for inquiring behaviors of porphyrin-based binding sites, while the chromatographic and batch binding tests evaluate average characteristics of all the binding sites. As shown in Figure 3, PPM(9EA) exhibited the quenching of fluorescent emission when it was incubated with 9EA. The extinction was more sensitive at a lower concentration range and became saturating at a higher concentration range. The nonlinear profile suggests that the quenching is due to the binding of 9EA to the porphyrin-based recognition site center, and more importantly, confirms that the porphyrin residues are engaged in high affinity binding sites observed in the Scatchard plot at the low concentration range. Fluorescent extinction at a low concentration range, where the high affinity binding sites are expected to be dominant, was further investigated by comparing the selectivity of porphyrin-based binding sites formed in PPM(9EA) and PP(9EA). A structurally 9EA analogue, 4-Aminopyridine (4AP), was used as a reference ligand, which was retained comparably by PP(9EA) and less retained in PPM(9EA) compared to 9EA in the chromatographic tests. With a good consistency with the chromatographic results, PPM(9EA) displayed a superior selectivity for 9EA to PP(9EA) in fluorescent extinction sensitivity; PPM(9EA) and PP(9EA) displayed 1.13 and 1.05, respectively, as selectivity factor defined as $(I_0/I_{9EA})/(I_0/I_{4AP})$ where I_0 is fluorescence intensity without any ligand, and I_{9EA} and I_{4AP} are those with a corresponding ligand at 25 μM. The results show that porphyrin-based binding sites formed in PPM(9EA) take up a ligand on the basis of a different mechanism from that of PP(9EA), probably with cooperative assistance of methacrylic acid residues.[10] Structure of the binding sites is currently unknown,[11] which can hardly be understood because of difficulty of spectroscopic studies of cross-linked, undissolved polymers and heterogeneity of binding sites formed.[12]

MI demonstrated here thus appeared effective for constructing a porphyrin-based binding site with three-dimensional, ligand-complementary modification by carboxylic residue(s). Assessing binding characteristics of the imprinted polymer by the three independent approaches, it was suggested that MI using the metalloporphyrin monomer **1** is useful for constructing a binding site recognizing a ligand via multi-point interaction and signaling second messages as fluorescence. Because porphyrins immobilized in polymers can be important biological models as demonstrated by Wang,[13] the findings of this study encourage MI using plural functional monomer species for synthesizing porphyrin-based artificial receptors with highly important, biological meanings.

Supporting Information Available: Experimental details for the preparation of **1** (PDF), Scatchard plots of PP(9EA) and PM(9EA) (PDF), and UV−vis absorption spectra for PPM(9EA) and PP(9EA) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA000064+