

Research Article

Phthalocyanine-Based Molecularly Imprinted Polymers as Nucleoside Receptors

Luigia Longo and Giuseppe Vasapollo

Dipartimento di Ingegneria dell'Innovazione, Università del Salento, 73100 Lecce, Italy

Correspondence should be addressed to Giuseppe Vasapollo, giuseppe.vasapollo@unile.it

Received 5 September 2007; Accepted 31 October 2007

Recommended by Jannie C. Swarts

A molecularly imprinted polymer (MIP) for tri-O-acetyladenosine (TOAA), PPM(TOAA), was prepared by the combined use of methacrylic acid (MAA) and Zn(II)tetra(4'-methacryloxyphenoxy) phthalocyanine as functional monomers. This MIP exhibited a higher binding ability for TOAA compared to the MIP prepared using only MAA, PM(TOAA), in batch rebinding tests. Scatchard analysis gave a higher association constant of PPM(TOAA) for TOAA ($2.96 \times 10^4 \text{ M}^{-1}$) than that of PM(TOAA) ($1.48 \times 10^4 \text{ M}^{-1}$). The MIP prepared using only the zinc-phthalocyanine, PP(TOAA), did not show any binding capacity for TOAA. This means that the phthalocyanine in the MIP contributes to higher affinities, although it barely interacts with TOAA. Since selectivity for this kind of MIPs is more important than binding affinity, the binding of TOAA and a structurally related compound, tri-O-acetyluridine (TOAU), on the polymers was investigated. Both PPM(TOAA) and PM(TOAA) exhibited binding affinities for TOAA while they did not show any binding capacity for TOAU.

Copyright © 2008 L. Longo and G. Vasapollo. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. INTRODUCTION

Molecularly imprinted polymers (MIPs) have received much attention recently as new materials capable of molecular recognition [1]. They have been extensively utilized in solid-phase extraction [2–5], chromatography [6–8], sensing [9, 10], and catalysis [11–13]. During molecular imprinting, crosslinked polymers are formed by free-radical copolymerization of functional monomers with an excess of crosslinker around an analyte that acts as a template. After polymerization, the template is removed leaving in this way selective binding sites in the polymer network that are complementary in form and functionality to the analyte molecules [1].

The main advantages of MIPs, over conventional polymers used as separation material, are the high selectivity and affinity for the target analytes used in the imprinting procedure. Furthermore, MIPs are characterized by superior mechanical and thermal stability, as well as better inertness towards acids, bases, metal ions, and organic solvents compared to enzymes. In addition, imprinting polymerization is a very inexpensive procedure for the development of artificial receptors. In the majority of the cases, the price of an MIP

depends entirely on the price of the template used. Moreover, if the template is expensive, it is possible to recover the template and use it again. Alternatively, inexpensive template analogues can be used for the preparation of MIPs [1].

In most studies, methacrylic acid (MAA) was used as functional monomer to synthesize MIPs making the polymer preparation a simple and facile process [14–16]. MAA can form hydrogen bonds with the template molecule in porogen prior to polymerization. A more deliberate approach using synthetically designed functional monomers could enable a better control in the formation of high-affinity binding sites for each corresponding template minimizing, at the same time, the inherent nonspecific binding properties common in noncovalent imprinted polymers.

In this paper, we report for the first time the preparation of MIPs as nucleoside receptors using both methacrylic acid and a zinc-phthalocyanine peripherally substituted with methacrylic groups (Compound 1, Scheme 1) [17] as functional monomers. The receptor site is a three-dimensional cavity around the phthalocyanine plane in crosslinked polymers to which the analyte molecule could be specifically bound via coordination through the metal of the phthalocyanine and hydrogen bonding/electrostatic interaction

with MAA and the modifiers linked to the phthalocyanine (Scheme 1). Such cooperative interaction approach in MIP formation, already proposed for porphyrin derivatives [18–21], was applied here for the first time to phthalocyanine compounds.

An organic soluble nucleoside derivative, tri-O-acetyl-adenosine (TOAA), was utilized in this work as template in the preparation of phthalocyanine-based MIPs. The development of synthetic receptors that recognize nucleotide bases and their derivatives is an important area in chemistry today. The literature provides many examples of artificial receptors for each of the common nucleoside bases [15, 16, 22–25]. Recently, these receptors have provided insights into DNA-DNA and protein-DNA interactions, and applications are envisioned in the fields of biosensors, drug therapy, separation science, and genetic engineering. TOAA was selected as model template in a previous work [17] where the binding affinity and selectivity of the zinc-phthalocyanine 1 towards different nucleosides were evaluated by UV-vis titration experiments. Binding experiments showed that zinc-phthalocyanine 1 binds TOAA most strongly, giving a binding constant K_a of 1.35×10^4 , 500 times that of 1 to tri-O-acetyluridine (TOAU), showing in this way a high selectivity [17]. The presence of the 2-aminopyridine moiety in the structure of TOAA could be probably responsible for the good binding characteristics of this nucleoside derivative with compounds functionalized with methacrylic groups [15].

An imprinted polymer receptor for TOAA, namely, PPM(TOAA), was prepared using both 1 and MAA as functional monomers. Imprinted polymers were also prepared using either MAA or 1, called PM(TOAA) and PP(TOAA), respectively, and used as references. Corresponding unimprinted blank polymers, PPM(BL), PM(BL), and PP(BL), were prepared using the same monomers in the absence of TOAA. Batch rebinding studies were conducted by UV-vis spectroscopy and the binding characteristics of the MIPs were examined by Scatchard analysis. In order to verify the selectivity of the MIPs, the binding of TOAA and its structurally related compound, TOAU, on the all prepared polymers was investigated.

2. EXPERIMENTAL SECTION

2.1. Materials and methods

Ethylene glycol dimethacrylate (EGDMA), 2',3',5'-tri-O-acetyl-adenosine (TOAA), 2',3',5'-tri-O-acetyluridine (TOAU), and acetic acid were purchased from Sigma-Aldrich (Steinheim, Germany). α - α' -Azobisisobutyronitrile (AIBN) and methacrylic acid (MAA) were supplied from Fluka (Steinheim, Germany). Analytical grade dichloromethane and methanol were purchased from J. T. Baker (Deventer, Holland). Zn(II) tetra(4'-methacryloyloxyphenoxy)phthalocyanine (1) was prepared on the basis of a published method [17]. UV-Vis spectra were obtained with a Cary 100 Scan UV-vis spectrophotometer.

2.2. Polymer preparation

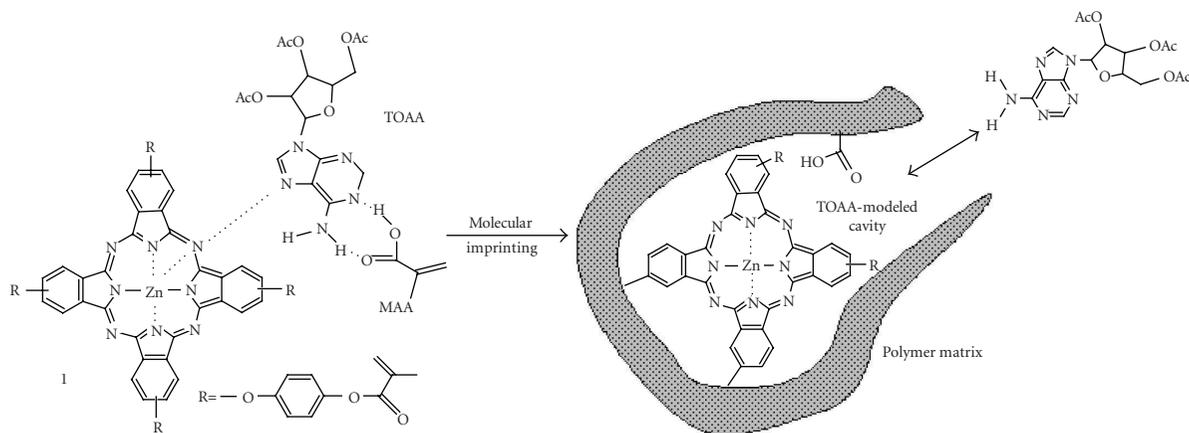
The preparation of PPM(TOAA) was carried out as follows: to a solution of TOAA (0,0468 mmol, as template) in dichloromethane (262 μ L) were added phthalocyanine 1 (0.0117 mmol, as the first functional monomer) and MAA (0.1758 mmol, as the second functional monomer) in a glass tube. After adding of EGDMA (0.9360 mmol, as cross-linker) and AIBN (0.0112 mmol, as initiator), the mixture was sonicated for 5 minutes flushing with nitrogen gas and then polymerized by heating at 60°C for 16 hours. The resultant polymer was crushed and sieved. The template molecules were removed by washing the polymer first with methanol/acetic acid (7/3 v/v) and then with methanol until no template molecules were detected from the recovered solutions with a UV-vis spectrophotometer. Drying under vacuum afforded particles which were used for rebinding studies. PM(TOAA) and PP(TOAA) were identically prepared using either MAA (0.1758 mmol) or 1 (0.0117 mmol) as functional monomer, respectively. The corresponding blank polymers, PPM(BL), PM(BL), and PP(BL), were prepared in the same manner in the absence of TOAA.

2.3. Batch rebinding experiments and Scatchard analysis

The polymer (20 mg) was added to a dichloromethane solution (3.5 mL) of TOAA of known concentrations (2.0 – 8.0×10^5 M) in vials. The resulting suspension was shaken for 16 hours at room temperature, then the polymer was rapidly removed by filtration and the resulting solution was analyzed by UV-vis spectrophotometer at 258 nm. The amount of TOAA bound to the polymer, B , was calculated by subtraction of the concentration of free TOAA, $[TOAA]$, from the initial TOAA concentration. $[TOAA]$ was determined as an average value of three measurements. Scatchard analysis was provided by the Scatchard equation, $B/[TOAA] = (B_{max} - B) K_a$, where K_a is the association constant and B_{max} is the apparent maximum number of binding sites. Therefore, K_a and B_{max} of the polymer were determined from the slope and the intercept, respectively, by plotting of $B/[TOAA]$ versus B . Batch rebinding experiments and Scatchard analysis were performed in a similar manner for PPM(TOAA), PM(TOAA), PP(TOAA), and the corresponding blank polymers. The rebinding tests were also carried out incubating the polymers with TOAU in order to verify their binding selectivity.

3. RESULTS AND DISCUSSION

TOAA-imprinted and unimprinted polymers were obtained by the above method. The binding behavior of the prepared MIPs was evaluated by batch rebinding tests and the binding data were processed with Scatchard equation in order to estimate the binding properties of the polymers. Figure 1 shows the Scatchard plot for PPM(TOAA). As can be seen, it is a single straight line, which indicates that there exists one kind of binding sites populated in the MIP. The Scatchard plot is linear also in the case of PM(TOAA). Similar Scatchard plots



SCHEME 1: Schematic representation of the molecular imprinting of TOAA using both 1 and MAA as functional monomers.

TABLE 1: Association constant (K_a) and maximum number of binding sites (B_{\max}) for PPM(TOAA) and PM(TOAA).

Polymer	K_a (M^{-1})	B_{\max} ($\mu M g^{-1}$)
PPM(TOAA)	$(2.96 \pm 0.5) \times 10^4$	6.53
PM(TOAA)	$(1.48 \pm 0.6) \times 10^4$	1.66

were obtained in a study of Yan et al. [26] with malachite green-imprinted polymers. This fact is very interesting since a nonlinear profile was commonly observed in the Scatchard assessment of MIPs indicating the presence of binding sites that exhibit various affinities to the ligand [18, 20, 21]. Table 1 shows the values of K_a and B_{\max} for PPM(TOAA) and PM(TOAA). As shown, the MIP prepared with both functional monomers 1 and MAA, PPM(TOAA), exhibited higher binding affinities for TOAA compared to PM(TOAA), prepared solely with MAA. PP(TOAA), prepared only with 1, did not show any binding capacity for TOAA. This means that phthalocyanine 1 contributes to higher binding affinities, although 1 itself barely interacts with TOAA. The effects of the use of both functional monomers strongly suggest that the imprint that allows the simultaneous multipoint interactions of the template with the carboxylic residues of MAA and the Zn(II) ion of 1 shows a higher binding ability for TOAA than that allowing only the individual template/functional monomer interactions. The corresponding blank polymers, PPM(BL), PM(BL), and PP(BL), showed no binding affinities for TOAA confirming that the selectivity was due to the imprinting of the polymer matrix and not to the intrinsic affinity of the template to the functional monomers alone.

As for the selectivity (Figure 2), both PPM(TOAA) and PM(TOAA) exhibited binding affinities for TOAA while they did not show any binding capacity for TOAU. As expected, also PP(TOAA) did not show any binding capacity for TOAU. The corresponding blank polymers showed no binding affinity for TOAU too. If we examine the chemical structure of TOAA and TOAU, it is clear that TOAA has more chances than TOAU to form hydrogen bonds with the MIPs because

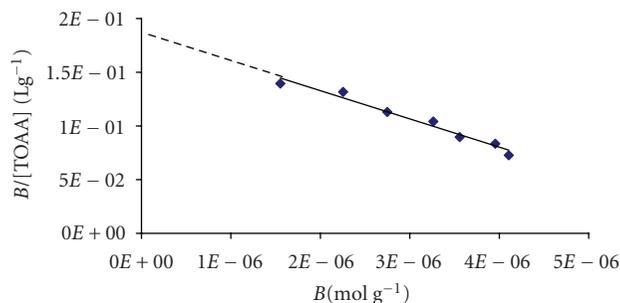


FIGURE 1: Scatchard plot for PPM(TOAA).

of more functional groups that could form hydrogen bonds with the polymers. Consequently, the polymers PPM(TOAA) and PM(TOAA) showed a good binding affinity for TOAA and no binding capacity for TOAU. This is not surprising since the inability of carboxylic acid receptors to bind uracil derivatives appears to be rather general as evidenced in the literature [15, 27, 28].

The higher binding affinity of PPM(TOAA) for TOAA in comparison with PM(TOAA) could be explained considering the possibility of coordination of the Zn(II) ion of 1 with the nitrogen atoms of the 2-aminopyridine moiety of TOAA. Similar multipoint interactions have already been proposed by Takeuchi et al. [18] in a study on the preparation of MIPs for cinchonidine by the combined use of MAA and a vinyl-substituted zinc(II) porphyrin as functional monomers. The coordination of the Zn(II) ion with the nitrogen atoms of TOAA could be explained considering that the zinc(II) ion is the acid with moderate hardness and the nitrogen atom of the 2-aminopyridine moiety is the base with moderate softness [18]. The absence of this kind of nitrogen atom in the chemical structure of TOAU could explain the inability of PPM(TOAA) to bind TOAU. On the other hand, the inability of PP(TOAA) to bind TOAA suggests that the coordination of the Zn(II) ion of the phthalocyanine with the nucleoside is not sufficient alone to assure the recognition and subsequent complementary binding between the receptor and the

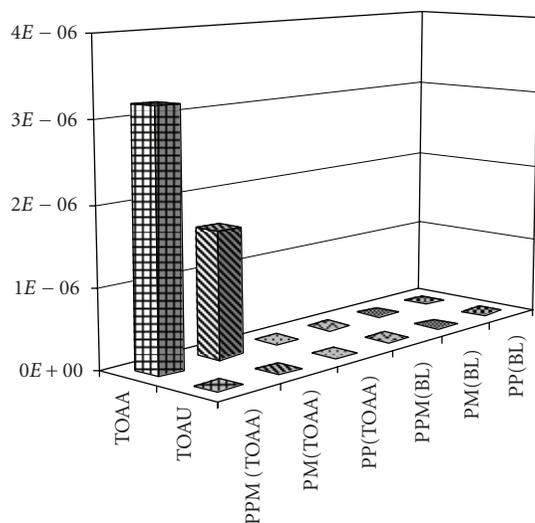


FIGURE 2: Binding selectivity test of TOAA and TOAU on the MIPs.

nucleoside molecule, confirming in this way that the analyte molecule is specifically bound to the polymer through multipoint interactions.

4. CONCLUSION

A highly specific and selective TOAA-imprinted polymer was prepared by the combination of MAA and a zinc-phthalocyanine substituted with methacrylic groups as functional monomers. The effects of the simultaneous use of the two functional monomers suggest the effective cooperation of the phthalocyanine-based and carboxylic residues rather than independent operation for retaining of TOAA.

Considering these promising results for easily constructed and highly selective MIPs for nucleosides, new investigations are now being directed towards the development of MIP-based sensors arrays for nucleoside discriminations.

ACKNOWLEDGMENT

This work was supported by PRIN 2004-MIUR (No. 20040340210_03).

REFERENCES

- [1] S. A. Piletsky and A. P. F. Turner, "New materials based on imprinted polymers and their applications in optical sensors," in *Optical Biosensors: Present and Future*, F. S. Ligler and C. A. Rowe Taitt, Eds., pp. 397–425, Elsevier, Amsterdam, The Netherlands, 2002.
- [2] P. Dzygiel, E. O'Donnell, D. Fraier, C. Chassaing, and P. A. G. Cormack, "Evaluation of water-compatible molecularly imprinted polymers as solid-phase extraction sorbents for the selective extraction of sildenafil and its desmethyl metabolite from plasma samples," *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, vol. 853, no. 1-2, pp. 346–353, 2007.
- [3] F. G. Tamayo, E. Turiel, and A. Martín-Esteban, "Molecularly imprinted polymers for solid-phase extraction and solid-phase microextraction: recent developments and future trends," *Journal of Chromatography, A*, vol. 1152, no. 1-2, pp. 32–40, 2007.
- [4] C. He, Y. Long, J. Pan, K. Li, and F. Liu, "Application of molecularly imprinted polymers to solid-phase extraction of analytes from real samples," *Journal of Biochemical and Biophysical Methods*, vol. 70, no. 2, pp. 133–150, 2007.
- [5] J. Haginaka, "Molecularly imprinted polymers for solid-phase extraction," *Analytical and Bioanalytical Chemistry*, vol. 379, no. 3, pp. 332–334, 2004.
- [6] Y. C. Lin, H. H. Pan, C. C. Hwang, and W. C. Lee, "Side chain functionality dominated the chromatography of N-protected amino acid on molecularly imprinted polymer," *Journal of Applied Polymer Science*, vol. 105, no. 6, pp. 3519–3524, 2007.
- [7] S. Wei and B. Mizaikoff, "Recent advances on noncovalent molecular imprints for affinity separations," *Journal of Separation Science*, vol. 30, no. 11, pp. 1794–1805, 2007.
- [8] Y. Luo, L. Liu, L. Li, and Q. Deng, "Chiral resolution of racemic 4-phenyl(benzyl)-2-oxazolidone by use of molecularly imprinted polymers," *Chromatographia*, vol. 65, no. 11-12, pp. 675–679, 2007.
- [9] K. Hattori, Y. Yoshimi, and K. Sakai, "Chemical sensors using molecularly imprinted polymers as molecular recognition elements," *Chemical Sensors*, vol. 22, pp. 110–115, 2006.
- [10] K. Haupt and K. Mosbach, "Molecularly imprinted polymers and their use in biomimetic sensors," *Chemical Reviews*, vol. 100, no. 7, pp. 2495–2504, 2000.
- [11] A. Visnjovski, R. Schomäcker, E. Yilmaz, and O. Brüggemann, "Catalysis of a Diels-Alder cycloaddition with differently fabricated molecularly imprinted polymers," *Catalysis Communications*, vol. 6, no. 9, pp. 601–606, 2005.
- [12] R. Kalim, R. Schomäcker, S. Yüce, and O. Brüggemann, "Catalysis of a β -elimination applying membranes with incorporated molecularly imprinted polymer particles," *Polymer Bulletin*, vol. 55, no. 4, pp. 287–297, 2005.
- [13] Z. Chen, Z. Hua, J. Wang, Y. Guan, M. Zhao, and Y. Li, "Molecularly imprinted soluble nanogels as a peroxidase-like catalyst in the oxidation reaction of homovanillic acid under aqueous conditions," *Applied Catalysis, A: General*, vol. 328, no. 2, pp. 252–258, 2007.
- [14] M. J. Syu, J. H. Deng, and Y. M. Nian, "Towards bilirubin imprinted poly(methacrylic acid-co-ethylene glycol dimethacrylate) for the specific binding of α -bilirubin," *Analytica Chimica Acta*, vol. 504, no. 1, pp. 167–177, 2004.
- [15] D. A. Spivak and K. J. Shea, "Binding of nucleotide bases by imprinted polymers," *Macromolecules*, vol. 31, no. 7, pp. 2160–2165, 1998.
- [16] D. Spivak, M. A. Gilmore, and K. J. Shea, "Evaluation of binding and origins of specificity of 9-ethyladenine imprinted polymers," *Journal of the American Chemical Society*, vol. 119, no. 19, pp. 4388–4393, 1997.
- [17] L. Longo, G. Vasapollo, A. Scardino, R. A. Picca, and C. Malitesta, "Synthesis of a new substituted zinc phthalocyanine as functional monomer in the preparation of MIPs," *Journal of Porphyrins and Phthalocyanines*, vol. 10, no. 8, pp. 1061–1065, 2006.
- [18] T. Takeuchi, T. Mukawa, J. Matsui, M. Higashi, and K. D. Shimizu, "Molecularly imprinted polymers with metalloporphyrin-based molecular recognition sites coassembled with methacrylic acid," *Analytical Chemistry*, vol. 73, no. 16, pp. 3869–3874, 2001.
- [19] J.-D. Lee, N. T. Greene, G. T. Rushton, K. D. Shimizu, and J.-I. Hong, "Carbohydrate recognition by porphyrin-based

- molecularly imprinted polymers,” *Organic Letters*, vol. 7, no. 6, pp. 963–966, 2005.
- [20] J. Matsui, M. Higashi, and T. Takeuchi, “Molecularly imprinted polymer as 9-ethyladenine receptor having a porphyrin-based recognition center,” *Journal of the American Chemical Society*, vol. 122, no. 21, pp. 5218–5219, 2000.
- [21] A. Tong, H. Dong, and L. Li, “Molecular imprinting-based fluorescent chemosensor for histamine using zinc(II)-protoporphyrin as a functional monomer,” *Analytica Chimica Acta*, vol. 466, no. 1, pp. 31–37, 2002.
- [22] K. J. Shea, D. A. Spivak, and B. Sellergren, “Polymer complements to nucleotide bases. Selective binding of adenine derivatives to imprinted polymers,” *Journal of the American Chemical Society*, vol. 115, no. 8, pp. 3368–3369, 1993.
- [23] K. Yano, K. Tanabe, T. Takeuchi, J. Matsui, K. Ikebukuro, and I. Karube, “Molecularly imprinted polymers which mimic multiple hydrogen bonds between nucleotide bases,” *Analytica Chimica Acta*, vol. 363, no. 2-3, pp. 111–117, 1998.
- [24] H. Tsunemori, K. Araki, K. Uezu, M. Goto, and S. Furusaki, “Surface imprinting polymers for the recognition of nucleotides,” *Bioseparation*, vol. 10, no. 6, pp. 315–321, 2001.
- [25] D. A. Spivak and K. J. Shea, “Investigation into the scope and limitations of molecular imprinting with DNA molecules,” *Analytica Chimica Acta*, vol. 435, no. 1, pp. 65–74, 2001.
- [26] S. Yan, Z. Gao, Y. Fang, Y. Cheng, H. Zhou, and H. Wang, “Characterization and quality assessment of binding properties of malachite green molecularly imprinted polymers prepared by precipitation polymerization in acetonitrile,” *Dyes and Pigments*, vol. 74, no. 3, pp. 572–577, 2007.
- [27] S. C. Zimmerman, W. Wu, and Z. Zeng, “Complexation of nucleotide bases by molecular tweezers with active site carboxylic acids: effects of microenvironment,” *Journal of the American Chemical Society*, vol. 113, no. 1, pp. 196–201, 1991.
- [28] G. Lancelot, “Hydrogen bonding between nucleic acid bases and carboxylic acids,” *Journal of the American Chemical Society*, vol. 99, no. 21, pp. 7037–7042, 1977.