Polymers Recognizing Biomolecules Based on a Combination of Molecular Imprinting and Proximity Scintillation: A New Sensor Concept

Lei Ye and Klaus Mosbach*

Pure and Applied Biochemistry, Chemical Center Lund University, Box 124, 221 00 Lund, Sweden

Received December 18, 2000

Modern drug discovery and demand for quick diagnostics require development of highly sensitive and efficient analytical methods. Sensitive techniques such as radiometric and fluorescent measurements have been widely applied to detect trace amounts of analytes. In many cases, for example in clinical samples and combinatorial libraries, a target analyte coexists with a large number of interfering compounds. To detect/quantify the target, a separation step is often required in which a selective binding material is employed to fish out the target analyte during the analyses. Biological antibodies, receptors, enzymes, and single-strand DNA segments have been utilized as the binding materials since they are able to recognize corresponding antigens, agonists/antagonists, substrates/inhibitors, and complementary nucleotide sequences with high specificity. It is possible to eliminate the separation step, thereby greatly increasing the sample throughput by integrating the biological binding materials with appropriate signal transduction systems, for example as in Scintillation Proximity Assays (SPA).[1]

To substitute biological binding materials in general, synthetic host molecules have been studied very intensively in supramolecular chemistry,[2] and more recently, in molecular imprinting of cross-linked polymers.[3] As a synthetic approach based on template-assisted assembly of a polymeric host, molecular imprinting generates binding "cavities" that are complementary to the original template in both shape and functionality. Molecularly imprinted polymers (MIPs) have high binding affinity and specificity, and they are stable and relatively easy to prepare. In addition to separation and more routine analytical applications involving affinity adsorbents,[4] they have been used as recognition elements in various sensors.[5] In most cases, an imprinted polymer is put in physical contact with a transducer. The physicochemical response (change in mass, resistance, capacitance, refractive index, etc.) from binding a target analyte is translated into a sensor signal. This simple method, however, often leads to MIP sensors showing relatively low sensitivity and specificity. In a more sophisticated manner, a fluorescent functional monomer is incorporated as a "reporter" into the MIP's specific "cavity".[6] The fluorescence intensity changes when a target analyte binds to the cavity. In principle this sensor design should deliver superior specificity, since only specific binding generates a sensor signal. However, for different target analytes, new reporter monomers need to be synthesized.

In this communication we describe a molecularly imprinted polymer incorporating a "universal" reporter (Figure 1). Generation of the binding signal is based on the principle of proximity energy transfer, e.g. proximity scintillation. Due to the small size of the MIP particles, the signal-producing element (scintillation fluor) is located in close proximity to the MIP's binding "cavity". The scintillation fluor virtually does not affect template re-binding, therefore it can be used for both covalent and noncovalent imprinting. In this study we use the general functional monomer, methacrylic acid (MAA), for noncovalent imprinting of an antagonist, (*S*)-propranolol (**2**). A scintillation monomer (**1**) is covalently incorporated into our MIP microparticles during the imprinting reaction. Rather than being confined within the binding cavity, the fluor is randomly distributed throughout the polymer matrix. The scintillation monomer, 4-hydroxymethyl-2,5-diphenyloxazole acrylate (**1b**), is synthesized by coupling **1a**[7] with acryloyl chloride. In toluene its scintillation efficiency is approximately 62% of the commercial fluor, 2,5-diphenyloxazole (PPO). MIPs incorporating different amounts of **1** are synthesized by using a previously reported precipitation method (Table 1),[8] in which trimethylolproprane trimethacrylate (TRIM) is used as the cross-linking monomer. Spectrofluorometric and elemental analyses of the obtained polymers confirm that the imprinted polymer (IP) and nonimprinted polymer (NP) contain approximately equal amount of **1**.

Despite the difference in toluene fraction used for polymer synthesis, all the polymer microparticles obtained have an average diameter of 0.6−1 μm. These are nonporous, and have a surface area of approximate 7 $m^2\ g^{-1}$. A relatively stable suspension of polymer microparticles is readily obtained in toluene due to their small size. The polymers bearing scintillation fluor **1** displayed excitation and emission spectra very similar to that of the scintillation monomer **1b** (virtually identical maximum excitation and emission wavelength). This confirmed that incorporation into a polymer matrix does not affect the fluor's scintillation efficacy.

To evaluate the MIPs' response to the specific binding of the tritium-labeled template, increasing amounts of the imprinted and nonimprinted polymer are incubated in toluene (500 μL, containing 0.5% acetic acid (v/v)) with a fixed amount of [H^3](*S*)-propranolol. Samples are counted without removing the unbound [H^3](*S*)-propranolol (proximity scintillation counting).[9] In both cases the imprinted polymers deliver much higher counts than the nonimprinted polymers, which is attributed to the specific binding of the labeled template with the imprinted polymers—a fact confirmed by quantifying the free fraction with liquid scintillation counting.[10] When 0.2 mg of polymer is used, the two imprinted polymers generate approximately half of the maximum

* To whom correspondence should be addressed. Phone: +46-46-2229560. Fax: +46-46-2224611. E-mail: Klaus.Mosbach@tbiokem.lth.se.

(1) (a) Bosworth, N.; Towers, P. *Nature* **1989**, *341*, 167−168. (b) Udenfriend, S.; Gerber, L.; Nelson, N. *Anal. Biochem*. **1987**, *161*, 494−500.

(2) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89−112.

(3) (a) Mosbach, K.; Ramström, O. *Bio/Technology* **1996**, *14*, 163−170. (b) Wulff, G. *Angew. Chem., Int. Ed. Engl*. **1995**, *34*, 1812−1832. (c) Shea, K. J. *Trends Polym. Sci*. **1994**, *2*, 166−173. (d) Wulff, G.; Gross, T.; Schönfeldt, R. *Angew. Chem., Int. Ed. Engl*. **1997**, *36*, 1962−1964. (e) Lübke, C.; Lübke, M.; Whitcombe, M. J.; Vulfson, E. N. *Macromolecules* **2000**, *33*, 5098−5105. (f) Alexander, C.; Smith, C. R.; Whitcombe, M. J.; Vulfson, E. N. *J. Am. Chem. Soc*. **1999**, *121*, 6640−6651.

(4) (a) Kempe, M.; Mosbach, K. *J. Chromatogr. A* **1994**, *664*, 276−279. (b) Sellergren, B. *Anal. Chem*. **1994**, *66*, 1578−1582. (c) Andersson, L. I. *Anal. Chem*. **1996**, *68*, 111−117. (d) Ye, L.; Ramström, O.; Mosbach, K. *Anal. Chem*. **1998**, *70*, 2789−2795.

(5) (a) Haupt, K.; Mosbach, K. *Chem. Rev*. **2000**, *100*, 2495−2504. (b) Kröger, S.; Turner, A. P. F.; Mosbach, K.; Haupt, K. *Anal. Chem*. **1999**, *71*, 3698−3702. (c) Malitesta, C.; Losito, I.; Zambonin, P. G. *Anal. Chem*. **1999**, *71*, 1366−1370. (d) Lin, J.-M.; Yamada M. *Anal. Chem*. **2000**, *72*, 1148−1155.

(6) (a) Turkewitsch, P.; Wandelt, B.; Darling, G. D.; Powell, W. S. *Anal. Chem*. **1998**, *70*, 2025−2030. (b) Wang, W.; Gao, S.; Wang, B. *Org. Lett*. **1999**, *1*, 1209−1212. (c) Matsui, J.; Higashi, M.; Takeuchi, T. *J. Am. Chem. Soc*. **2000**, *122*, 5218−5219.

(7) (a) Clapham, B.; Richards, A. J.; Wood, M. L.; Sutherland, A. J. *Tetrahedron Lett*. **1997**, *38*, 9061−9064. (b) Hamerton, I.; Hay, J. N.; Jones, J. R.; Lu, S.-Y. *Chem. Mater*. **2000**, *12*, 568−572.

(8) (a) Ye, L.; Cormack, P. A. G.; Mosbach, K. *Anal. Commun*. **1999**, *36*, 35−38. (b) Ye, L.; Weiss, R.; Mosbach, K. *Macromolecules* **2000**, *33*, 8239−8245.

(9) Samples are preincubated overnight to ensure equilibrium binding, although a short incubation time (2 h) is sufficient. A standard liquid scintillation counter (β-radiation counter) is used.

(10) Samples are centrifuged following the proximity scintillation counting. A fraction of supernatant is mixed with a commercial scintillation liquid and counted with a β-radiation counter.

10.1021/ja005896m CCC: $20.00
Published on Web 03/06/2001

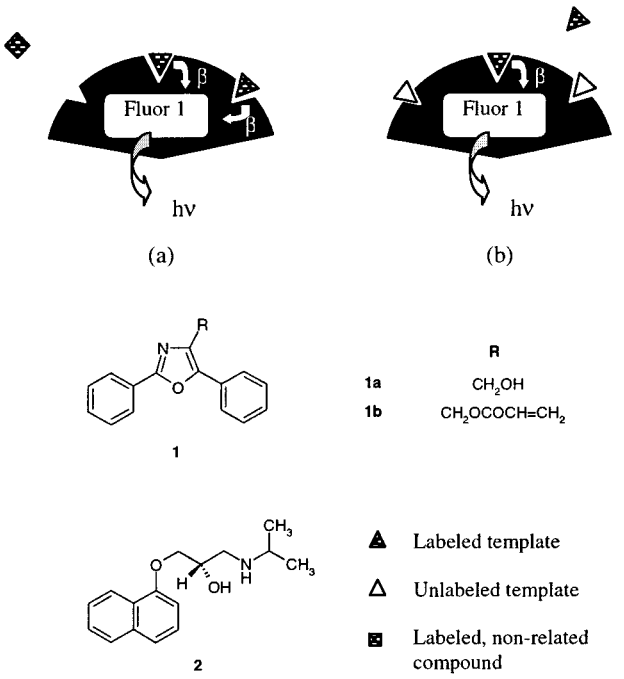

Figure 1. Schematic representation of chemical sensing with an imprinted polymer. Scintillation fluor (**1**) incorporated by its copolymerization into the polymer converts β-radiation from the bound tritium-labeled template into a fluorescent signal. The unbound labeled compound is too far away from **1** for effective energy transfer, therefore no fluorescent light is generated. The MIP is useful in two aspects: (a) in direct quantification of the template that coexists with other compounds in a radioisotope-labeled sample and (b) in competitive assay of the template where the labeled template is used as a tracer, whose binding is inhibited by the unlabeled template. In the present study, (*S*)-propranolol (**2**) is used as a model template for demonstration.

Table 1. Syntheses of Imprinted and Nonimprinted Polymers[a]

		monomer		
polymer	**2** (mmol)	MAA (mmol)	**1b** (mmol)	TRIM (mmol)
IP **1**	0.7	1.39	0.69	1.39
NP **1**		1.39	0.69	1.39
IP **2**	0.6	0.78	0.16	0.78
NP **2**		0.78	0.16	0.78

[a] The reaction components and the initiator, azobisisobutyronitrile (2 wt % of monomer), are dissolved in anhydrous toluene (40 mL). The solution is saturated with nitrogen and heated at 60 °C for 16 h. Discrete polymer microparticles are obtained after a brief treatment in an ultrasonic water bath followed by centrifugation. The template is removed by repetitively washing the polymers with methanol−acetic acid (90:10, v/v) and acetone. The polymers are dried in a vacuum.

fluorescence counts, which are two times higher than the nonimprinted polymers. The MIPs bearing scintillation fluor specifically bind their templates. More importantly, the specific binding event is translated into a scintillation signal.

In a competitive mode, binding of tritium-labeled (*S*)-propranolol to the imprinted polymer is inhibited by increasing the amount of the unlabeled template, which results in decreased proximity scintillation counts (PSC) (Figure 2). With the non-imprinted polymer, however, the nonlabeled template does not change the PSC significantly.

When less scintillation monomer is incorporated, the obtained polymer IP **2** generates PSC change at even lower analyte

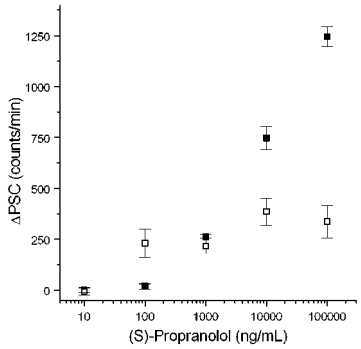

Figure 2. Competitive assay of (*S*)-propranolol with polymer IP **1** (solid square) and NP **1** (open square). [H^3](*S*)-propranolol (2 pmol) and polymer microparticles (0.2 mg) are incubated in toluene (500 μL, containing 0.5% acetic acid (v/v)) with an increasing amount of (*S*)-propranolol prior to proximity scintillation counting. ΔPSC is the change in proximity scintillation counts caused by the unlabeled analyte competing for the binding sites. Data are mean values of triplicate measurements.

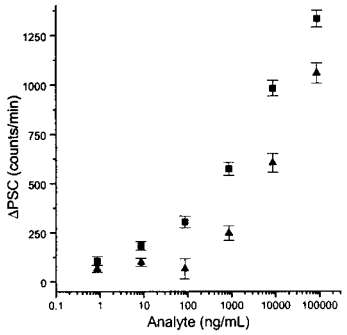

Figure 3. Competitive assay of (*S*)-propranolol (solid square) and (*R*)-propranolol (solid triangle) with IP **2**. [H^3](*S*)-propranolol (2 pmol) and the polymer (0.1 mg) are incubated in toluene (500 μL, containing 0.5% acetic acid (v/v)) with an increasing amount of analytes prior to proximity scintillation counting.

concentration, e.g. this chemo sensor displays a lower detection limit (Figure 3). The sensor is chiral selective, since the antipode of the template, (*R*)-propranolol, causes a much smaller PSC change. This chiral discrimination can be further improved if required, although more important in this communication is the chiral specificity as such.

The present example has demonstrated a new sensor concept using molecular imprinting and proximity scintillation. We have used a photomultiplier tube (PMT) for signal quantification. When combined with arrays of PMTs or imaging systems, for example with a CCD camera, imprinted scintillation polymers can be deposited in microtiter plate wells and used for high throughput screening purposes. MIP sensors based on other proximity energy transfer systems are also envisioned.

Acknowledgment. This work is in part supported by the Swedish Research Council for Engineering Sciences (TFR). The authors thank Dr. Kenneth Wärnmark, Organic Chemistry I, Lund University for assistance in NMR measurement.

Supporting Information Available: Synthesis and ^{1}H NMR data for **1b** and fluorescent spectra for **1b** and polymer IP **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA005896M