Anal. Chem. **2002,** *74,* 959−964

Scintillation Proximity Assay Using Molecularly Imprinted Microspheres

Lei Ye,* Ioana Surugiu, and Karsten Haupt

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

Molecularly imprinted microspheres were prepared as antibody binding mimics and used in scintillation proximity assay of a β-adrenergic antagonist, (*S*)-propranolol. By using small polymer beads, we were able to place an organic scintillator and an "antenna" component in close proximity to the imprinted binding sites. When the radioactive template bound to the polymer, radiation energy was effectively transferred, via the antenna component, to the scintillator to generate a fluorescence signal. Using molecularly imprinted microspheres instead of antibodies, we have demonstrated competitive scintillation proximity assays for (*S*)-propranolol in both organic and aqueous solvents. The experimental results were further validated by normal ligand binding analysis, where liquid scintillation counting was used for quantification.

Biomimetic materials resemble biological macromolecules in recognizing target molecules and, more importantly, in generating and transferring the binding signal. Among the most practicable approaches to biomimetic materials is molecular imprinting of synthetic polymers.[1−3] By molecular imprinting, copolymerization of functional monomer and cross-linking monomer is carried out in the presence of a molecular template, which results in a rigid polymer matrix embedding the template. Removal of the template reveals binding sites specific for the template and its closely related analogue. Although molecularly imprinted polymers (MIPs) have displayed high binding affinity and specificity mimicking natural antibodies,[4−6] there are only limited examples of imprinted polymers capable of effective signal generation, where specially designed fluorescent functional monomers were utilized to respond to the binding event with significant fluorescence intensity change.[7−9] To circumvent the synthetic difficulties, we have been interested in developing molecular imprinting methods using simple functional monomers and more general-purpose reporter molecules. When imprinted polymers were prepared in the form of microspheres,[10] the reporter could be brought into close proximity to the specific binding sites, whereby the phenomenon of resonance energy transfer[12,13] could be utilized for signal generation. In our previous study, we incorporated an organic scintillator (the fluor) into imprinted polymer microspheres containing a general functional monomer, methacrylic acid.[11] In toluene, when the imprinted polymers selectively bound the tritium-labeled template, they were able to generate a proximity scintillation signal.

In this paper, we present a molecularly imprinted scintillation polymer useful in both organic and aqueous solvents. This was achieved by studying the mechanisms of template binding and of energy transfer for signal generation. Instead of the previous aromatic solvents, an "antenna" element was covalently immobilized in our new polymer to harvest the β-emission from the bound, radioisotope-labeled template. By this design, our new imprinted scintillation polymer showed improved specificity in sensing the target analyte.

EXPERIMENTAL SECTION

Materials and Methods. Ethyl benzoyl acetate (90%), benzoyl chloride (99%), phosphorus oxychloride (99%), sodium borohydride (99%), acryloyl chloride (96%), zinc dust (<10 μm, 98%), and divinylbenzene (technical grade, 80%, mixture of isomers) were obtained from Aldrich (Dorset, U.K.). Prior to use, divinylbenzene was passed through an aluminum oxide column to remove the polymerization inhibitor. Acetic acid (glacial, 100%), sodium nitrite (99%), methacrylic acid (99%), and azobisisobutyronitrile (98%) were purchased from Merck (Darmstadt, FRG); diethyl ether (99.5%), benzene (99%), (*S*)-propranolol hydrochloride (99%), and (*R*)-propranolol hydrochloride (99%) were supplied by Fluka (Dorset, U.K.); ethanol (99.5%) was from Kemetyl AB (Haninge, SE); (*S*)-[4-^{3}H]-propranolol (specific activity 555 GBq mmol^{-1}, 66.7 μM solution in ethanol) was provided by NEN Life Science Products, Inc. (Boston, MA); scintillation liquids, Ecoscint O and Ecoscint A, were from National Diagnostics (Atlanta, GA); anhydrous acetonitrile (99.9%) used for polymer synthesis was purchased from Lab-Scan (Dublin, Ireland); other solvents were of analytical grade. (*R*)- and (*S*)-propranolol were obtained by base titration of the hydrochloride salt and extraction into methylene

* Corresponding author: (phone) + 46 46 2229560; (fax) + 46 46 2224611; (e-mail) Lei.Ye@tbiokem.lth.se.

(1) Shea, K. J. *Trends Polym. Sci.* **1994**, *2*, 166−173.
(2) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812−1832.
(3) Mosbach, K.; Ramström, O. *Bio/Technology* **1996**, *14*, 163−170.
(4) Vlatakis, G.; Andersson, L. I.; Müller, R.; Mosbach, K. *Nature* **1993**, *361*, 645−647.
(5) Andersson, L. I.; Müller, R.; Vlatakis, G.; Mosbach, K. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4788−4792.
(6) Ramström, O.; Ye, L.; Mosbach, K. *Chem. Biol.* **1996**, *3*, 471−477.
(7) Turkewitsch, P.; Wandelt, B.; Darling, G. D.; Powell, W. S. *Anal. Chem.* **1998**, *70*, 2025−2030.
(8) Wang, W.; Gao, S.; Wang, B. *Org. Lett.* **1999**, *1*, 1209−1212.
(9) Matsui, J.; Higashi, M.; Takeuchi, T. *J. Am. Chem. Soc.* **2000**, *122*, 5218−5219.
(10) Ye, L.; Weiss, R.; Mosbach, K. *Macromolecules* **2000**, *33*, 8239−8245.
(11) Ye, L.; Mosbach, K. *J. Am. Chem. Soc.* **2001**, *123*, 2901−2902.
(12) Udenfriend, S.; Gerber, L.; Nelson, N. *Anal. Biochem.* **1987**, *161*, 494−500.
(13) Bosworth, N.; Towers, P. *Nature* **1989**, *341*, 167−168.

10.1021/ac015629e CCC: $22.00
Published on Web 01/26/2002

chloride. The scintillation monomer, 4-(hydroxymethyl)-2,5-diphenyloxazole acrylate was synthesized by coupling acryloyl chloride with 4-(hydroxymethyl)-2,5-diphenyloxazole[14] as described elsewhere.[11]

Polymer Syntheses. Molecularly imprinted microspheres were synthesized using a previously published method.[10] In a 150 × 25 mm borosilicate glass Pyrex culture tube, (*S*)-propranolol (137 mg, 0.529 mmol), methacrylic acid (90.4 mg, 1.05 mmol), divinylbenzene (547 mg, 4.20 mmol), 4-(hydroxymethyl)-2,5-diphenyloxazole acrylate (80.0 mg, 0.263 mmol), and azobisisobutyronitrile (3 wt % of monomer) were dissolved in anhydrous acetonitrile (40 mL). The solution was saturated with dry nitrogen. The tube was sealed, transferred to a water bath at 60 °C, and maintained for 24 h. The polymer microspheres were collected by centrifugation. (*S*)-Propranolol was removed by batch-mode solvent extraction with methanol containing 10% acetic acid (v/v), until no template could be detected from the washing solvent by spectrometric determination. Polymer microspheres were finally washed with acetone and dried in a vacuum. A nonimprinted polymer was synthesized under identical conditions except for omission of the template, (*S*)-propranolol.

Fluorescent Spectrum. Polymer microspheres (3 μg) were suspended in 3 mL of acetonitrile containing 0.5% acetic acid (v/v) in a quartz cuvette, stirred, scanned immediately with a QuantaMaster C-60/2000 spectrofluorometer, and data processed with a software Felix from Photon Technology International, Inc. (Lawrenceville, NJ). For excitation spectra, an emission wavelength at 400 nm and emission spectra an excitation wavelength at 320 nm were used.

Proximity Scintillation Counting. *Saturation Experiment.* In a series of polypropylene microcentrifuge tubes, increasing amounts of polymer microspheres were suspended in different solvents. Radiolabeled (S)-propranolol (2 pmol) was added and the volume topped with the same solvent to 500 μL. The microcentrifuge tubes were transferred into 6-mL insert counting vials and incubated at room temperature overnight. A rocking table ensured gentle mixing. After incubation, the insert vials were transferred into 20-mL standard counting vials and immediately counted for 1 min using a β-radiation counter Rackbeta 2119 (LKB Wallac, Sollentuna, Sweden).

Displacement Experiment. In a series of polypropylene microcentrifuge tubes, a fixed amount of polymer microspheres were suspended in different solvents. Increasing amounts of test compounds were added, followed by addition of the radiolabeled (*S*)-propranolol (2 pmol). The volume was topped with the same solvent to 500 μL. The remaining steps were essentially the same as that used in the saturation experiment.

Kinetic Binding Experiment. Polymer microspheres (0.2 mg) and radiolabeled (*S*)-propranolol (2 pmol) were incubated in 500 μL of acetonitrile containing 0.5% acetic acid (v/v) on a rocking table. At fixed intervals, the sample was taken for proximity scintillation counting to determine the fraction of bound template. After each counting, incubation was resumed until the end of the experiment.

Kinetic Dissociation Experiment. Polymer microspheres (0.2 mg) and radiolabeled (*S*)-propranolol (2 pmol) were incubated in 220 μL of acetonitrile containing 0.5% acetic acid (v/v) for 2 h, then (*S*)-propranolol (11 nmol) in 280 μL of the same solvent was added, and the incubation resumed. Proximity scintillation counting was carried out at different incubation times to estimate the fraction of radiolabeled (*S*)-propranolol bound to the polymer.

Liquid Scintillation Counting. The microcentrifuge tubes used in proximity scintillation counting were withdrawn from the insert counting vials and centrifuged at 14 000 rpm for 5 min. Supernatant (200 μL) was taken, mixed with 10 mL of scintillation liquid (Ecoscint O, or Ecoscint A if the sample contained aqueous buffer), and counted for 1 min using the same β-radiation counter. The amount of labeled (*S*)-propranolol bound to polymer microspheres was calculated by subtraction of the free fraction from the total amount added.

RESULTS AND DISCUSSION

Preparation of Molecularly Imprinted Microspheres. Molecularly imprinted microspheres are favorable in nonseparation binding analysis, because they can form relatively stable suspensions in appropriate assay solvents. It is generally accepted that, due to the polymer swelling effect, optimal template binding to an imprinted polymer can be obtained, if the experiment is carried out in the same solvent as that used for the polymer preparation. In the case of imprinted polymer microspheres, we also found that stable particle suspensions could be obtained in the solvent that was used for the imprinting reaction. In our previous paper on molecularly imprinted scintillation polymers,[11] we prepared polyacrylate-based microparticles specific for (*S*)-propranolol. Both imprinting and rebinding experiments were carried out in toluene. The main reason for using the aromatic solvent was to fulfill the requisite of effective energy transfer in the proximity scintillation assay: The radioactive decay of the bound, labeled template first transferred energy to the solvent molecule, which, in returning to its ground state, excited the incorporated organic scintillator. To generate effective resonance energy transfer, the labeled template, the aromatic solvent, and the organic scintillator had to be in close enough proximity to one another. This was made possible only when the radiolabeled template bound to the scintillation polymer. Although the β-emission from the unbound template could stimulate the ubiquitous toluene molecules, the majority of the later was too far away from the MIP, therefore unable to efficiently transfer its energy to the scintillator to generate a detectable signal. In a nonaromatic solvent, for example, in acetonitrile, although the same MIP still bound specifically to the labeled template, no scintillation signal could be detected.[15] This was due to the inefficient energy transfer from the radioactive decay directly to the scintillator.

To prepare molecularly imprinted scintillation polymers applicable in a wide range of solvents, in this paper we introduced an aromatic monomer into our MIP microspheres to mediate the resonance energy transfer (Figure 1). Covalent fixation was chosen because the monomer would become an integral part of the resulting MIP, not prone to leak when the MIP was used in real assays.

Among a large variety of aromatic monomers, we choose divinylbenzene (DVB) to prepare our new scintillation MIP based

(14) Hamerton, I.; Hay, J. N.; Jones, J. R.; Lu, S.-Y. *Chem. Mater.* **2000**, *12*, 568−572.

(15) The amount of unbound, tritium-labeled template was measured by counting the radioactivity of the sample following removal of the polymer.

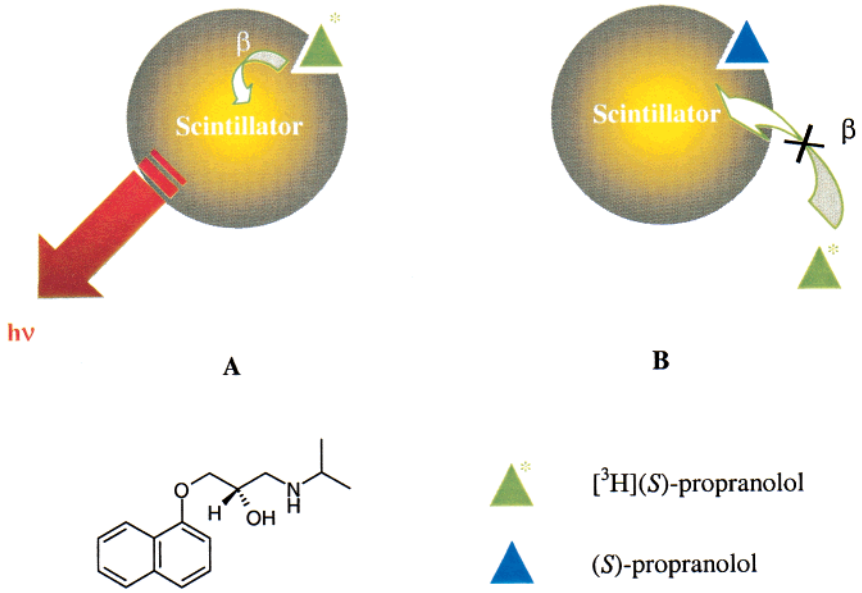

Figure 1. Schematic representation for the scintillation proximity assay of (*S*)-propranolol using imprinted microspheres. The yellow area represents the aromatic "antenna" element. (A) The bound, tritium-labeled (*S*)-propranolol triggers the scintillator to generate a fluorescent light. (B) When the tritium-labeled (*S*)-propranolol is displaced by the unlabeled (*S*)-propranolol, it is too far from the antenna and the scintillator to efficiently transfer the radiation energy; therefore, no fluorescence can be generated.

on the following considerations: (a) As a cross-linking monomer for imprinting, DVB can be used at a high molar fraction; (b) the benzene ring in DVB may provide additional $\pi-\pi$ interactions with the naphthyl moiety of (*S*)-propranolol, which will improve the MIP's binding performance in nonaromatic solvents. A normal functional monomer, methacrylic acid, was used to provide ionic and hydrogen bond interactions with the secondary amine and the hydroxyl functionalities of (*S*)-propranolol.[16] Although it is possible to immobilize the reporter element into an already prepared MIP by chemical modification (grafting),[17] in the present study we chose to incorporate, at the imprinting step, the noninterfering scintillation monomer by its copolymerization. As determined by scanning electron microscopy, the polymer microspheres prepared in acetonitrile had particle sizes of 0.6−2 μm. Identical fluorescent spectra of the imprinted and nonimprinted microspheres were recorded (Figure 2), which confirmed that equal amounts of the organic scintillator had been introduced into the imprinted and nonimprinted microspheres.

Selective Template Binding and Signal Generation. To study the selective binding of template by the imprinted microspheres, different amounts of the polymer were incubated with a fixed amount of [^{3}H](*S*)-propranolol until equilibrium was established. Proximity scintillation counting was carried out without separating the unbound labeled template. Figure 3A shows the fluorescence intensity generated by different amounts of the imprinted and nonimprinted polymers. The imprinted polymer generated a typical dose−response curve with saturation at high polymer concentration and half of the maximum proximity scintillation counts (PSCs) at a polymer concentration of ~0.2 mg mL^{-1}. Most interestingly, the nonimprinted polymer gave very low scintillation counts even at the highest polymer concentration—

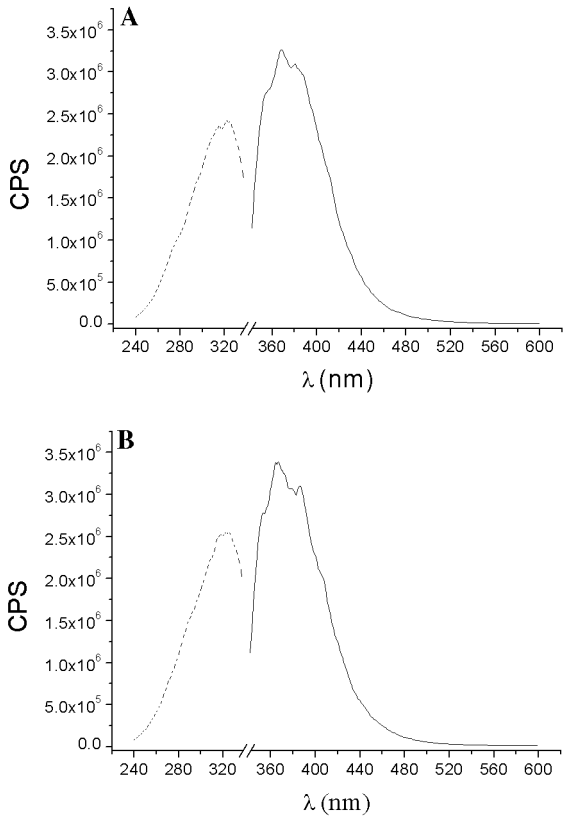

Figure 2. Excitation (dotted line) and emission spectrum (solid line) for the imprinted (A) and the nonimprinted microspheres (B). CPS, counts per second.

presumably a result of very low nonspecific template binding. To confirm that the favorable optical response was due to specific template binding to the imprinted polymer, we quantified the free [^{3}H](*S*)-propranolol in the same samples following removal of the

(16) Andersson, L. I. *Anal. Chem.* **1996**, *68*, 111−117.
(17) Ye, L.; Cormack, P. A. G.; Mosbach, K. *Anal. Chim. Acta* **2001**, *435*, 187−196.

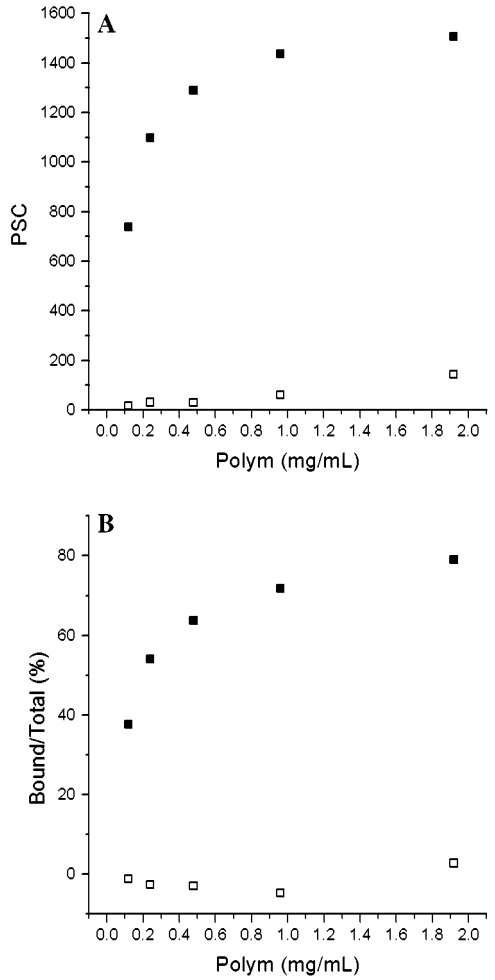

Figure 3. Titration of [^{3}H](*S*)-propranolol (4 nM) with the imprinted (filled square) and the nonimprinted polymer (open square) in acetonitrile containing 0.5% (v/v) acetic acid. (A) Proximity scintillation counting without separation. (B) Bound percentage calculated from liquid scintillation counting of the supernatant following removal of the polymer microspheres. Data are mean values of triplicate determinations.

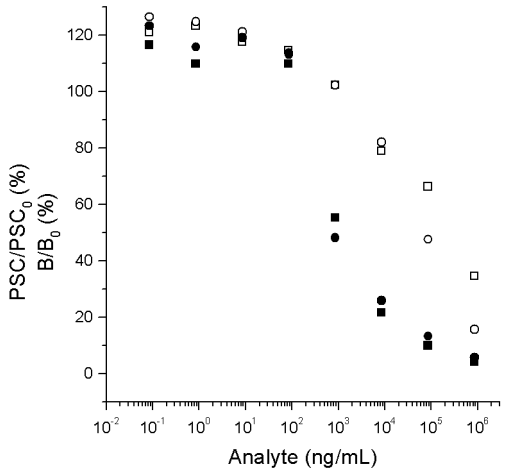

Figure 4. Displacement of [^{3}H](*S*)-propranolol binding to the imprinted polymer (0.1 mg) by (*S*)-propranolol (filled symbol) and (*R*)-propranolol (open symbol) evaluated by proximity scintillation counting (square) and liquid scintillation counting (circle). PSC and PSC_0 were proximity scintillation counts in the presence and absence of the competing analyte; *B* and B_0, the percentage of bound [^{3}H](*S*)-propranolol in the presence and absence of the competing analyte, were calculated from liquid scintillation counting. Data are mean values of triplicate determinations.

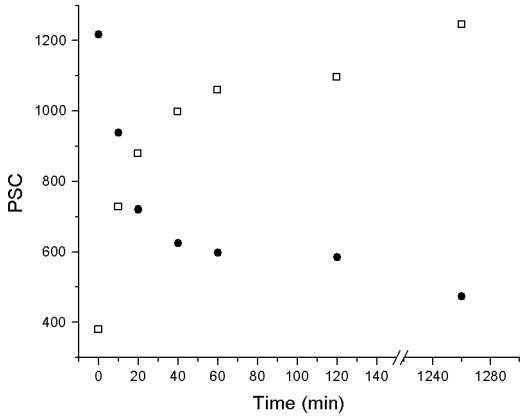

Figure 5. In situ monitoring for the binding (open square) and dissociation (filled circle) of [^{3}H](*S*)-propranolol. Proximity scintillation counts were recorded for the imprinted microspheres. In the dissociation experiment, (*S*)-propranolol was added to displace the [^{3}H]-(*S*)-propranolol previously bound to the MIP.

microspheres, using liquid scintillation counting applied to the supernatants[18] (Figure 3B). Comparison of panels A and B of Figure 3 indicated the following: (a) Proximity scintillation signal was generated in proportion to the labeled template that bound to the MIP's specific cavities.[19] (b) The unbound template was too far away from the organic scintillator incorporated in the polymer microspheres and, therefore, unable to trigger fluorescence generation.

To investigate chiral selectivity of the imprinted polymer, we used (*S*)- and (*R*)-propranolol as competitors to inhibit the binding of [^{3}H](*S*)-propranolol to the MIP. The competition curves obtained by proximity scintillation counting and liquid scintillation counting overlapped satisfactorily (Figure 4). In acetonitrile containing 0.5% acetic acid, The IC_{50} value of (*R*)-propranolol was at least 50 times higher than that of (*S*)-propranolol, which gave the MIP an estimated cross-reactivity to the *R* isomer of less than 2%.[20] The scintillation microspheres displayed chiral selectivity remarkably similar to that obtained previously for an imprinted bulk polymer, even though the latter was characterized in a less polar solvent (toluene containing 0.5% (v/v) acetic acid).[16] Using the homologous competitive experiment data in Figure 4, a gross estimation of binding parameters for the template ((*S*)-propranolol) with a one-site model gave a dissociation constant (K_D) of 7.9×10^{-7} M and a site population (B_{max}) of 4.4 μmol g^{-1} polymer for

(18) Liquid scintillation counting has been used to quantify unbound radioligand in several MIP-based drug assays. For examples, see refs 4−6 and 10.

(19) The efficiency of the proximity scintillation counting was constant over the whole binding range, although significantly lower than that of the liquid scintillation counting. In liquid scintillation counting, a secondary scintillator is always added in the commercial counting liquid to shift fluorescence emission to longer wavelength, where the photomultiplier tube becomes the most sensitive. Incorporation of a secondary scintillator into the imprinted microspheres, or a more appropriate instrumentation, is expected to significantly improve the counting efficiency for the proximity scintillation counting.

(20) The IC_{50} value is the analyte concentration required to inhibit the binding of [^{3}H](*S*)-propranolol by 50%. The cross-reactivity of the analyte is calculated as the ratio of IC_{50}(template)/IC_{50}(analyte).

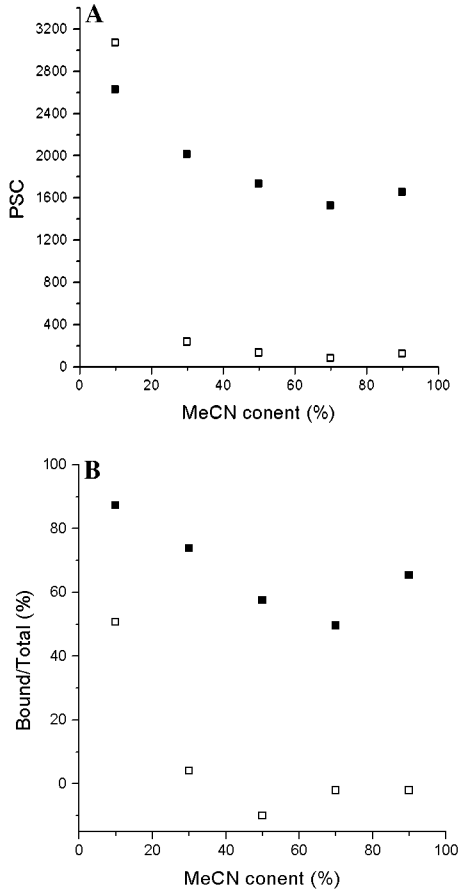

Figure 6. Binding of [^{3}H](*S*)-propranolol by 0.5 mg of the imprinted (solid square) and the nonimprinted polymer (open square) in 500 μL of citrate buffer (25 mM, pH 6.0) containing different amounts of acetonitrile. Samples were analyzed by proximity scintillation counting (A) and liquid scintillation counting (B).

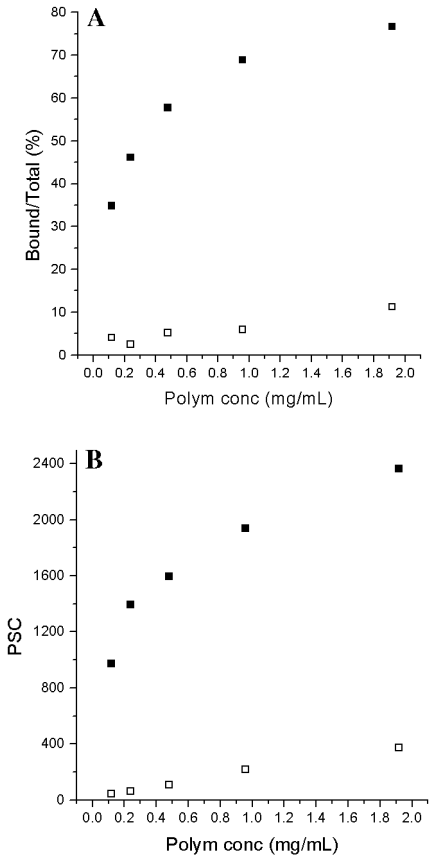

Figure 7. Titration of [^{3}H](*S*)-propranolol (4 nM) with the imprinted (filled square) and the nonimprinted polymer (open square) in citrate buffer (25 mM, pH 6.0) containing 50% acetonitrile (v/v). (A) Bound percentage calculated from liquid scintillation counting of the supernatant following removal of the polymer microspheres. (B) Proximity scintillation counting without separation. Data are mean values of triplicate determinations.

the imprinted polymer. We noticed that the MIP bound more radioligand in the presence of low concentration of both (*S*)- and (*R*)-propranolol than in the absence of the competing ligands (both B/B_0 and PSC/PSC_0 were higher than 100%), similar to what had been described in earlier competitive assays using imprinted polymers.[10,21,22] It is possible that addition of the competing ligands reduced nonspecific adsorption of the radioligand to the tubes, which resulted in higher *B* and PSC counts, and was especially striking in the low-concentration range of the competing ligands.

In Situ Monitoring of the Binding Event. In our previous study, we found that the small particle size of imprinted microspheres made analyte transfer to the binding sites significantly faster.[23] Using proximity scintillation counting, we were able to monitor the process of template binding and dissociation in real time. Figure 5 shows that after a 2-h incubation, both template binding and dissociation reached more than 85% of the equilibrium status.[24] In principle, kinetics of template binding to the imprinted microspheres can be investigated; however, at present, the binding site heterogeneity made it too complicated to calculate the real association (k_{on}) and dissociation constant (k_{off}).

Template Recognition in Aqueous Solution. Several examples have shown that molecularly imprinted polymers prepared in organic solvents could selectively rebind the template molecules from aqueous solutions.[5,16] To make the hydrophobic MIPs wettable in water, it is customary to add a certain amount of surfactant or water-miscible organic solvent. In our study, we found that addition of 0.1% Triton X-100 surfactant made the polymer microspheres easy to suspend in buffer. However, only low template binding (<20%) could be achieved even with the highest polymer concentration (2 mg mL^{-1}). This in turn resulted in very low proximity scintillation counts. Instead of Triton X-100, acetonitrile was found to restore the MIP's selective binding, which we attribute to the swelling effect of the polymer in the solvent mixture. Figure 6 shows the selective binding of [^{3}H](*S*)-propranolol in citrate buffer mixed with different amounts of acetonitrile. At low acetonitrile content (<10%), the labeled template bound equally to the two polymers, mainly via nonselective hydrophobic interactions. When acetonitrile content was higher than 30%, specific binding of the template to the imprinted polymer was reestablished, whereas binding to the nonimprinted polymer became very low. In later experiments, we used citrate buffer (25 mM, pH 6.0) containing 50% acetonitrile (v/v) as the binding solvent.

In citrate buffer/acetonitrile (50:50, v/v), a saturation study generated binding curves similar to those obtained in acetonitrile.

(21) Ansell, R. J.; Mosbach, K. *Analyst* **1998**, *123*, 1611−1616.
(22) Surugiu, I.; Ye, L.; Yilmaz, E.; Dzgoev, A.; Danielsson, B.; Mosbach, K.; Haupt, K. *Analyst* **2000**, *125*, 13−16.
(23) Ye, L.; Cormack, P. A. G.; Mosbach, K. *Anal. Commun.* **1999**, *36*, 35−38.
(24) With the conventional MIP, 80 and 90% of equilibrium binding was reached within 3 and 5 h of incubation according to ref 4.

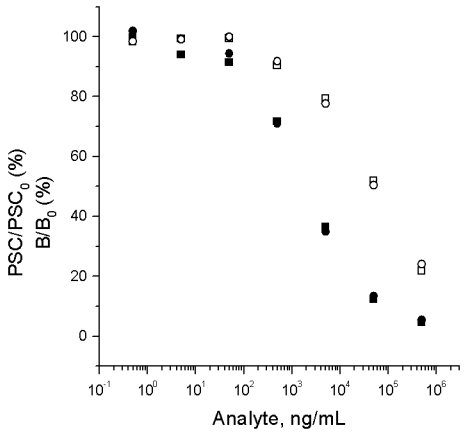

Figure 8. Displacement of [^{3}H](*S*)-propranolol binding to the imprinted polymer (0.1 mg) by (*S*)-propranolol (filled symbol) and (*R*)-propranolol (open symbol) evaluated by proximity scintillation counting (square) and liquid scintillation counting (circle). Solvent: citrate buffer (25 mM, pH 6.0) containing 50% acetonitrile (v/v). PSC and PSC_0 were proximity scintillation counts in the presence and absence of the competing analyte; *B* and B_0, the percentage of bound [^{3}H](*S*)-propranolol in the presence and absence of the competing analyte, were calculated from liquid scintillation counting. Data are mean values of triplicate determinations.

Nonspecific binding, as reflected by the nonimprinted polymer, was very low (Figure 7A). At a polymer concentration of ~0.2 mg mL^{-1}, the imprinted polymer provided half of the maximum binding, which gave about half of the maximum proximity scintillation counts (Figure 7B). In the competitive binding experiment, a MIP concentration of 0.2 mg mL^{-1} was used. Unlabeled (*S*)-propranolol restrained binding of the labeled template, which resulted in a decreased scintillation signal (PSC) (Figure 8). In contrary, the *R* enantiomer could not effectively displace the labeled template from the imprinted polymer. In the present aqueous solvent, the imprinted polymer bound the chiral template with a high specificity; e.g., the *R* enantiomer gave a cross-reactivity of less than 2%. When used in an aqueous environment, our imprinted scintillation microspheres displayed chiral selectivity remarkably higher than that of the previously reported bulk MIP.[16,20] This improvement may be explained by the more defined attractive $\pi-\pi$ interactions between the aromatic moiety of our imprinted polymer and the naphthyl group of the template. Using the homologous competitive binding data and a one-site model, we have estimated that the imprinted polymer had a dissociation constant (K_D) of 5.5×10^{-6} M and a site population (B_{max}) of 38 μmol g^{-1} polymer for (*S*)-propranolol.

CONCLUSION

In this study, we prepared molecularly imprinted scintillation polymers in a microbead format. The imprinted microspheres displayed chiral recognition for the template molecule, a β-adrenergic antagonist, (*S*)-propranolol. By introducing an aromatic cross-linking monomer as a radiation-harvesting element into the imprinted microspheres, we have successfully translated the specific binding event on the MIP into an optical signal, more importantly, in both organic and aqueous solvents. The same approach may be used to prepare MIPs in other configurations such as thin films and membranes, which are useful for simultaneous assay of multiple samples. Application of the molecularly imprinted microspheres for the determination of drugs in biological fluids, either directly or after their extraction, will be further investigated. In addition to molecularly imprinted polymers, combined use of the aromatic monomers and organic scintillators should give polymer beads that may be used to immobilize biological antibodies in other scintillation proximity assays.

ACKNOWLEDGMENT

This work was in part supported by the Swedish Foundation for Strategic Research (SSF) program "Biomimetic Materials Science" and the Swedish Research Council for Engineering Sciences (TFR).

Received for review September 20, 2001. Accepted October 31, 2001.

AC015629E