Pergamon

Tetrahedron Letters 42 (2001) 1839–1841

TETRAHEDRON LETTERS

Hydrophilic cholesterol-binding molecular imprinted polymers

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Received 21 December 2000; revised 5 January 2001; accepted 9 January 2001

Abstract—Novel hydrophilic molecularly imprinted polymers (MIPs) were prepared using acryloyl-containing hydrophilic monomers (including cholesteryl acrylate (CA) and acryloyl-6-amino-6-deoxy-β (or γ)-cyclodextrin), and their steroid-binding properties were analyzed in 2-PrOH by HPLC. The MIPs bound 38–50 μmol of cholesterol/g, while the corresponding nonimprinted control polymers (containing no CA) bound only 6–9 μmol cholesterol/g. The sterol-binding selectivity was illustrated with estrone, which was bound by MIPs in the range 5–36 μmol/g.

Molecularly imprinted polymers (MIPs)[1] that selectively recognize small molecules have a wide range of pharmaceutical, analytical, and biological applications.[2–4] The imprinting method is based on the template approach in which a target molecule (the template) recognizes its own recognition site by interacting with complementary functional groups of appropriate polymerizable monomers. Removal of the template yields polymers that are arranged complementary to the template's structure, resulting in selective recognition of the target molecule. Most MIPs prepared to date feature a high degree of crosslinking in the polymer network, which enables the cavities to retain their shape after template removal, giving rise to good template binding selectivity;[1] however, a high content of crosslinking agent makes it difficult to bind water-soluble compounds[5] and to remove imprinted molecules.[6] In this report we describe the preparation of water-soluble MIPs in which some of the crosslinking agent *N*,*N*′-diacryloylpiperazine (DAPA) is replaced with a hydrophilic monomer, ethyl 2-hydroxymethylacrylate (EHMA).[7] Cholesteryl acrylate (CA) template with or without CD derivatives as monomers were used to form novel hydrophilic MIPs in aqueous media via the covalent imprinting methodology. The MIPs bind selectively to sterols in 2-PrOH after the template is removed. A possible application of such polymers is to modulate the flux of cholesterol between cell membranes and lipoproteins, thus altering choles-

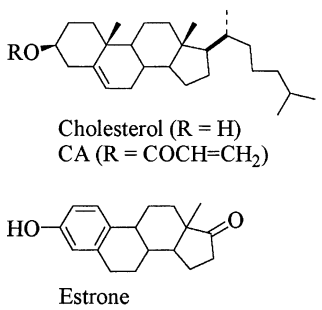
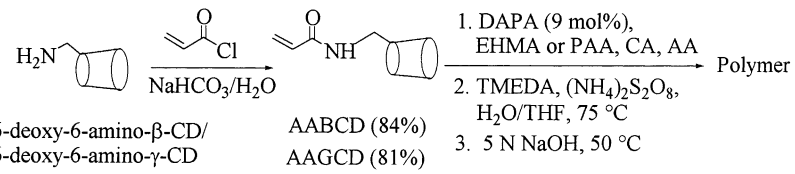


Figure 1.



Scheme 1.

Abbreviations: MIPs, molecularly imprinted polymers; CD, cyclodextrin; EHMA, ethyl 2-hydroxymethylacrylate; AA, acrylic acid; CA, cholesteryl acrylate; PAA, *N*-propylacrylamide; AABCD, acryloyl-6-amino-6-deoxy-β-CD; AAGCD, acryloyl-6-amino-6-deoxy-γ-CD; DAPA, *N*,*N*′-diacryloylpiperazine; TMEDA, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

Keywords: cholesterol; cyclodextrin.

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0040-4039/01/$ - see front matter
PII: S0040-4039(01)00045-4

Table 1. Polymer compositions[a]

Polymer	CA	AABCD	AAGCD	AA	PAA	EHMA
P1	0.20	0.54	0.00	0.00	0.00	14.00
P2	0.20	0.00	0.00	0.54	0.00	14.00
P3[b]	0.00	0.00	0.00	0.74	0.00	14.00
P4	0.20	0.00	0.54	0.00	0.00	14.00
P5	0.20	0.54	0.00	0.00	14.00	0.00
P6[b]	0.00	0.00	0.00	0.00	14.74	0.00

[a] All concentrations are in mmol. Porogens were H_2O and THF (400 and 88 mmol, respectively). Initiators were TMEDA and $(NH_4)_2S_2O_8$ (0.56 and 0.44 mmol, respectively). The crosslinking agent was DAPA (1.50 mmol).
[b] Nonimprinted polymers.

Table 2. Properties of polymers[a]

Polymer	Water solubility (g/100 mL)[b]	Cholesterol bound (μmol/g of MIP)[c]		Estrone bound (μmol/g of MIP)[c]
		Initial cholesterol concentration		Initial estrone concentration
		0.5 mg/mL	1.0 mg/mL	1.0 mg/mL
P1 (CA + AABCD + EHMA)	5.0	24 ± 4	43 ± 5	24 ± 2
P2 (CA + AA + EHMA)	5.0	15 ± 1	38 ± 3	5 ± 5
P3 (AA + EHMA)	5.0	2 ± 3	6 ± 3	2 ± 5
P4 (CA + AAGCD + EHMA)	5.0	30 ± 2	50 ± 2	36 ± 8
P5 (CA + AABCD + PAA)	1.3	27 ± 5	41 ± 3	25 ± 11
P6 (PAA)	0.6	5 ± 3	9 ± 2	13 ± 4

[a] Templates were removed by treating with 5N NaOH at 5°C for 7 days.
[b] Data are the average of two measurements.
[c] The results shown are the mean of three independent measurements. The amounts of the bound ligands per g of polymer were calculated according to $A \times C \times 10^3/(2 \times MW)$ (μmol/g). Absorption A was calculated according to $A = (C-y)/C \times 100\%$. C is the initial ligand concentration (mg/mL). y was obtained from $y = bx$, the standard curve equation ($b = 0.1931$ for cholesterol, $b = 0.1475$ for estrone), x is ligand peak area.[13]

terol transport and absorption. Many other synthetic receptors that encapsulate cholesterol and other apolar compounds, such as double-decker cyclophanes, would have limited biological utility because of their very low aqueous solubility.[8]

The syntheses of monomers CA (Fig. 1)[9] and PAA[10] are straightforward. EHMA was prepared as described previously.[7] AABCD[11] and AAGCD[12] were prepared as outlined in Scheme 1. The polymers were prepared by (1) formation of monomeric complexes between CA and acryloyl-CD and (2) polymerization using a low degree of crosslinking agent and 1:1 THF/H_2O as porogens. A low degree (9.2 mol%) of crosslinking agent (DAPA) was used to prepare polymers **P1**–**P6** (Table 1). The content of crosslinking agents employed in previous studies of cholesterol-imprinted polymers was much higher, e.g. 83 mol% crosslinking agent was used to prepare molecularly imprinted CDs[4] and 95 mol% was used in other covalently imprinted polymers.[2] Polymer **P1** was prepared using the covalent imprinting technique with CA as the template and AABCD as one of the monomers. Polymer **P2** was also prepared using the covalent imprinting technique, but AABCD was replaced by AA. Polymer **P3**, in which AA was used instead of CA and/or AABCD, was prepared as a control for **P1** and **P2** (Table 1). Polymer **P5**, in which monomer PAA was used instead of monomer EHMA, was prepared to compare with the non-PAA imprinted **P1**. Polymer **P6**, in which monomer PAA was used instead of monomers AA and EHMA, was synthesized without the template, and is to be compared with the nonimprinted polymer **P3**. Polymer **P4**, in which monomer AAGCD was used instead of monomer AABCD, was synthesized like **P1** (Table 1).

Before the template was removed **P1**, **P2**, **P4**, and **P5** were practically insoluble in water. Cleavage of the template enhanced the water solubility of polymers **P1**, **P2**, and **P4** (5.0 g/100 mL, Table 2). The water solubility of **P5** was lower (1.3 g/100 mL), perhaps because of the introduction of the amide-containing monomer PAA instead of the ester-containing monomer EHMA. During template hydrolysis, the esters are converted to free acids, but the amide is stable. The free acids and hydroxymethyl groups present in the matrix of **P1**–**P4** apparently contribute to the water solubility properties of the polymers.

The binding data of the MIPs to cholesterol and estrone (see structures, Fig. 1) are summarized in Table 2. In MIPs **P1**, **P2**, **P4**, and **P5**, 38–50 μmol cholesterol was bound per gram of polymer. The corresponding nonimprinted control polymers (**P3** and **P6**) contained only 6–9 μmol cholesterol bound per gram of polymer. The MIPs bound estrone in the range 5–36 μmol/g of

polymer. The presence of CD, which is known to bind to steroids,[14] may account for the wide range of bound estrone; the non-CD containing MIPs (such as **P2**) bound less estrone (5 μmol/g). Decreasing the initial cholesterol concentration by one-half (0.5 mg/mL) resulted in a decrease in cholesterol bound in MIPs by about one-half (Table 2). **P4**, which contains a γ-CD[15] residue, bound slightly more cholesterol (50 μmol/g) than **P1**, which contains a β-CD residue (Table 2). The larger cavity monomer is apparently more favorable for binding cholesterol in the polymer matrix. MIP **P2**, which does not contain any CD residues, took up less cholesterol than MIPs **P1** and **P4**, indicating that CD residues allow MIPs to take up cholesterol more efficiently.

To examine whether the amide functionality in the polymers affects the template binding, PAA was introduced into **P5** and **P6**. The amount of bound cholesterol in **P5** (41 μmol/g) was the same as that in the corresponding non-PAA imprinted polymer **P1** (43 μmol/g) (Table 2). Nonimprinted **P6** bound slightly more cholesterol than nonimprinted control **P3**. These data indicate that the amide functionality did not affect the template binding in MIPs.

Acknowledgements

This work was supported by NIH Grant HL-16660.

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9. Monomer CA was prepared by reaction of cholesterol with acryloyl chloride in pyridine/CH_2Cl_2 (rt, 30 min); 60% yield, white powder; R_f 0.40 (hexane/EtOAc 50:1); ^{1}H NMR ($CDCl_3$) δ 6.37 (d, 1H, $J=17.2$ Hz), 6.06 (dd, 1H, $J=10.4$, $J=17.2$ Hz), 5.79 (d, 1H, $J=10.4$ Hz), 5.37 (d, 1H, $J=4.4$ Hz), 4.70 (m, 1H), 2.34 (d, 2H, $J=8.0$ Hz), 0.90–2.10 (m, 26H); 1.01 (s, 3H), 0.89 (d, 3H, $J=6.4$ Hz), 0.84 (d, 6H, $J=6.4$ Hz), 0.66 (s, 3H); ^{13}C NMR δ 165.6, 139.6, 130.3, 129.0, 122.7, 74.1, 56.7, 56.1, 50.0, 42.3, 39.7, 39.5, 38.1, 35.8, 31.9, 31.8, 28.2, 28.0, 27.7, 24.3, 23.8, 22.8, 22.6, 21.0, 18.7, 11.8; MS calcd for $C_{30}H_{48}O_2$ $(M-H)^+$ m/z 440.71, found 440.30.
10. Monomer PAA was synthesized in 88% yield as a light-yellow liquid by reaction of n-$PrNH_2$ with acryloyl chloride in CH_2Cl_2 (rt, 30 min); R_f 0.15 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 6.29 (d, 1H, $J=16.8$ Hz), 6.13 (ddd, 1H, $J=2.8$, 10.4, 16.8 Hz), 5.87 (br s, 1H); 5.64 (d, 1H, $J=10.4$ Hz), 3.29 (q, 2H, $J=6.6$ Hz), 1.53–1.62 (m, 2H), 0.95 (t, 3H, $J=7.2$ Hz); ^{13}C NMR δ 165.9, 131.2, 126.4, 41.6, 23.0, 21.3, 14.4, 11.6; MS calcd for $C_6H_{11}NO$ (M^+) m/z 113.16, found 113.10.
11. Monomer AABCD was obtained as a white powder in 84% yield by reaction of 6-deoxy-6-amino-β-CD[16] with acryloyl chloride and $NaHCO_3$ in H_2O, followed by passage through a Sephadex G-25 column (eluted with H_2O) and precipitation from MeOH; R_f 0.39 (n-PrOH/H_2O/EtOAc/conc. NH_4OH 5:3:1:1); ^{1}H NMR (D_2O) δ 6.17 (d, 1H, $J=10.2$ Hz), 6.07 (d, 1H, $J=17.1$ Hz), 5.66 (d, 1H, $J=10.2$ Hz), 4.95 (br s, 7H), 3.13–3.85 (m, ~42H); ^{13}C NMR (D_2O) δ 169.37, 130.79, 128.52, 102.78, 102.41, 84.28, 82.01, 73.96, 72.95, 72.67, 72.54, 71.06, 61.15, 60.61, 49.80. Electrospray MS calcd for $C_{45}H_{74}NO_{35}$ $(M+H)^+$ m/z 1188.4, found 1188.2.
12. Monomer AAGCD was prepared from 6-deoxy-6-amino-γ-CD[17] in a similar fashion as AABCD; 81% yield, white powder; R_f 0.38 (n-PrOH/H_2O/EtOAc/conc. NH_4OH 5:3:1:1); ^{1}H NMR (D_2O) δ 6.17 (d, 1H, $J=10.3$ Hz), 6.09 (d, 1H, $J=17.1$ Hz), 5.66 (d, 1H, $J=10.1$ Hz), 4.98 (br s, 8H), 3.32–3.92 (m, 48H); ^{13}C NMR (D_2O) δ 167.02, 128.21, 126.22, 100.11, 99.72, 80.88, 78.88, 78.68, 71.36, 70.74, 70.18, 70.00, 68.49, 58.65, 58.24, 47.33. Electrospray MS calcd for $C_{51}H_{84}NO_{40}$ $(M+H)^+$ m/z 1350.5, found 1351.0.
13. For example, the peak area of cholesterol in the **P1** sample (for initial cholesterol concentration of 1.0 mg/mL) was 3.443; therefore, $y=0.1931\times3.443=0.665$; $A=[(1.0-0.665)/1.0]\times100\%=33.5\%$. The samples were filtered through a 0.45 μm Teflon® Cameo filter to remove the polymers prior to HPLC analysis. Cholesterol was analyzed on a BioCad Sprint Chromatography System, C18 (Hewlett Packard) column (4.6×100 mm), mobile phase 100% 2-PrOH, flow rate 0.8 mL/min, Sedex Model 55 evaporative light-scattering detector. Estrone was measured on a HP Model 1050 HPLC, Prodigy (Phenomenex) silica column (4.6×150 mm), mobile phase 100% 2-PrOH, flow rate 0.8 mL/min, detection at 254 nm.
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