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Journal of Chromatography A, 938 (2001) 131–135

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Preparation of sterol-imprinted polymers with the use of 2-(methacryloyloxy)ethyl phosphate

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Abstract

Steroid-selective polymers were prepared by the molecular imprinting technique, using 2-(methacryloyloxy)ethyl phosphate as functional monomer. The retentivity and selectivity of the obtained imprinted polymers were evaluated by liquid chromatography. The cholesterol-imprinted polymer showed higher affinity for cholesterol than that for cholesterol derivatives. The selectivity of the imprinted polymer was superior to the imprinted polymer prepared with the conventional functional monomer, 2-(trifluoromethyl)acrylic acid. Estradiol was also imprinted and gave similar results, demonstrating that 2-(methacryloyloxy)ethyl phosphate would be suitable for imprinted polymers of cholesterol and related compounds.

Keywords: Molecular imprinting; Sterols; Methacryloyloxyethyl phosphate; Phosphates; Cholesterol

1. Introduction

Molecular imprinting is a polymerization technique for the tailor-made preparation of synthetic polymer-receptors for given molecules [1–4]. The principles of the molecular imprinting technique can be summarized as follows: (1) complexation of a target molecule (template) and monomers bearing functional groups capable of interacting with the target molecule (functional monomers), (2) polymerization of the complex in the presence of a crosslinking agent, (3) removal of the target molecules from the polymer matrix. The functional groups of the resulting binding sites should be arranged at suitable positions to interact with the template molecule.

It is known that phosphate groups participate in molecular interaction in organisms. DNA–protein binding [5] and protein–protein binding [6,7] are typical interactions in which the phosphate groups of protein or DNA participate. Also, cholesterol interacts with phosphate groups of phospholipids in biomembranes [8]. By employing the binding ability of phosphate in molecular imprinting, polymers that have excellent molecular recognition ability could be prepared. In this study, a functional monomer, 2-(methacryloyloxy) ethyl phosphate, was employed and a cholesterol-imprinted polymer was prepared. Estradiol was also used as the target molecule, having a steroid structure and hydroxyl groups similar to cholesterol, and the affinity of the imprinted polymers was evaluated by liquid chromatography.

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)01384-X

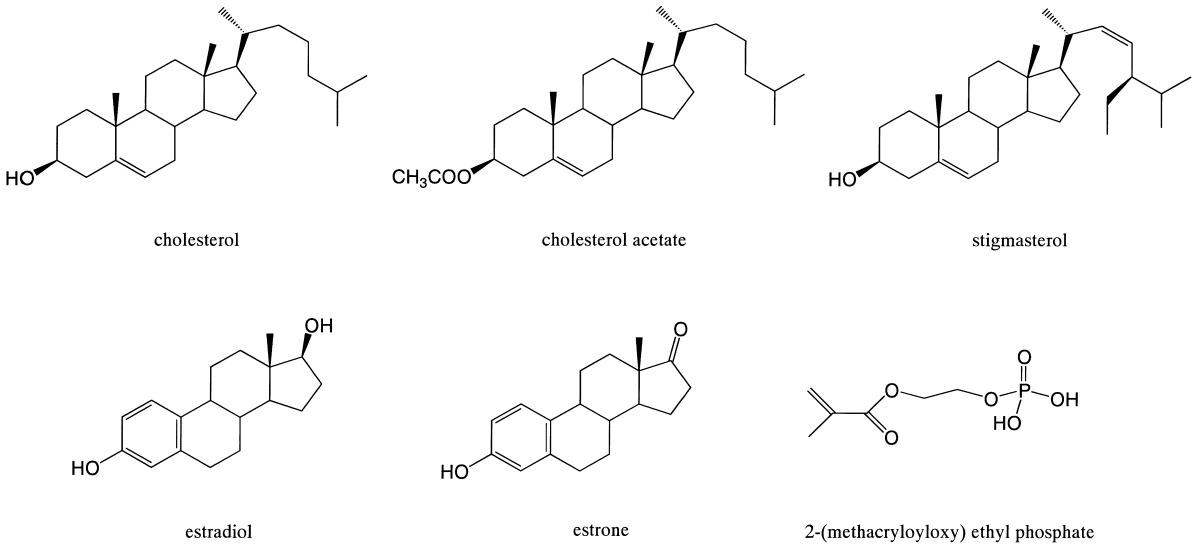

Fig. 1. Structures of compounds and functional monomer used in this study.

2. Experimental

2.1. Materials

Cholesterol, cholesterol acetate, stigmasterol, estradiol, ethyleneglycol dimethacrylate (EGDMA), and 2,2′-azobis(2,4-dimethylvaleronitrile) were purchased from Wako. 2-(Methacryloyloxy)ethyl phosphate (MEP) was purchased from Aldrich. 2-(Trifluoromethyl) acrylic acid (TFMAA) was purchased from Tokyo Chemical Industry. EGDMA and other reagents were distilled prior to use. The structures of compounds and functional monomer are shown in Fig. 1.

2.2. Polymer preparations

The imprinted polymers were prepared as shown in Table 1. The reaction mixtures were degassed by sonication, and thermally polymerized at 50°C for 12 h and followed by heating at 80°C for 3 h under nitrogen atmosphere. Each polymer was ground and sieved to yield a particle size of 32–63 μm. The polymer particles were slurry-packed into a stainless steel column (150 mm×4.6 mm I.D.), and the template molecule was washed out with chloroform–acetic acid (4:1, v/v). Blank polymers were prepared in the same manner without adding the template molecules.

Table 1
Preparation of molecularly imprinted polymers

Polymer	Template (mmol)	MEP (mmol)	TFMAA (mmol)	EGDMA (mmol)	Solvent 1 (ml)	Solvent 2 (ml)
P_{MEP}(cholesterol)	1.5	3.0	0	30	6.0	0
P_{MEP}(none)	0	3.0	0	30	6.0	0
P_{TFMAA}(cholesterol)	1.5	0	3.0	30	6.0	0
P_{MEP}(β-estradiol)	1.0	2.0	0	20	0	4.5
P_{MEP}(none)	0	2.0	0	20	0	4.5

MEP, 2-(Methacryloyloxy)ethyl phosphate; TFMAA, 2-(trifluoromethyl)acrylic acid; EGDMA, ethyleneglycol dimethacrylate. Solvent 1 was toluene–chloroform (7:1, v/v) and solvent 2 was chloroform–1,4-dioxane (2:1, v/v). 2,2′-Azobis(2,4-dimethylvaleronitrile)(0.24 mmol) as an initiator was added to each reaction mixture.

2.3. Chromatography

The affinity of each polymer was evaluated by measuring retentivity by a Gilson HPLC system (pump, Model 305; auto sample injector, Model 234) and the detection was carried out with an evaporative light scattering detector (SEDERE, Model SEDEX 55). An eluent was hexane as the chief ingredient at a flow-rate of 1.0 ml/min at room temperature. The sample concentration was 10 m*M*, and the sample size for injection was 20 μl. The capacity factor was calculated from the equation $k=(t_R-t_0)/t_0$, where t_R is a retention time of the samples and t_0 is time to elute a void marker, toluene.

3. Results and discussion

3.1. Evaluation of the affinity in cholesterol-imprinted polymer prepared with MEP as functional monomer

The cholesterol-imprinted polymer [P_{MEP}(cholesterol)] was prepared with MEP as a functional monomer, and affinity and selectivity of the polymers were evaluated by liquid chromatography. Table 2 shows the capacity factors for steroids in P_{MEP}(cholesterol). The polymer showed affinities for cholesterol and stigmasterol, but no affinity for cholesterol acetate. Since cholesterol acetate has no hydroxyl groups, the phosphate groups derived from functional monomer, MEP, would be concerned in binding to hydroxyl group of cholesterol and stigmasterol. Though cholesterol and stigmasterol have a similar structure where the difference is in the side chain at the C-17 carbon (Fig. 1), the cholesterol-imprinted polymer showed higher affinity for cholesterol than that for stigmasterol. The separation factor (α) for cholesterol vs. stigmasterol in the imprinted polymer also improved in comparison with the blank polymer [P_{MEP}(none)] prepared without adding the template molecule. Therefore, P_{MEP}(cholesterol) could distinguish the structure of the side chain of cholesterol. By the imprint effect, phosphate group derived from MEP would be arranged at the stable position for interaction with cholesterol and the binding site that is fitting to cholesterol would be constructed.

It has been known that methacrylic acid is an excellent functional monomer in molecular imprinting [9], and it is also reported that TFMAA is a further excellent functional monomer because of its strong hydrogen bonding ability as a hydrogen donor [10]. Therefore, we compared the binding ability of MEP-based polymers with TFMAA-based polymers. The cholesterol-imprinted polymer prepared with MEP showed lower affinity for cholesterol and its derivatives than the cholesterol-imprinted polymer prepared with TFMAA, but the selectivity for cholesterol in P_{MEP}(cholesterol) was superior (Table 3). The carboxyl group of TFMAA could strongly interact with cholesterol, and the cholesterol-imprinted polymer prepared with TFMAA [P_{TFMAA}(cholesterol)] would show the high affinity for cholesterol. Since too strong binding ability could lead to non-specific binding, P_{TFMAA}(cholesterol) would not show the high selectivity for cholesterol. Therefore, MEP, used in this study, could be demon-

Table 2
Capacity factors of cholesterol and its derivatives in cholesterol-imprinted polymer

Polymer	Capacity factor			$\alpha_{1/3}$
	Cholesterol (*1*)	Cholesterol acetate (*2*)	Stigmasterol (*3*)	
P_{MEP}(cholesterol)	15.8	1.2	8.2	1.93
P_{MEP}(none)	6.4	1.2	4.4	1.45

MEP, 2-(methacryloyloxy)ethyl phosphate. The eluent was *n*-hexane and the flow-rate was 1.0 ml/min. The detection was carried out with an evaporative light scattering detector. The separation factor (α) was calculated by dividing the capacity factor for cholesterol by that for stigmasterol.

Table 3
Capacity factors of cholesterol and its derivatives in cholesterol-imprinted polymers

Polymer	Functional monomer	Capacity factor			$\alpha_{1/3}$
		Cholesterol (*1*)	Cholesterol acetate (*2*)	Stigmasterol (*3*)	
P_{MEP}(cholesterol)	MEP	6.2	0.6	3.2	1.94
P_{TFMAA}(cholesterol)	TFMAA	11.3	1.0	6.3	1.79

MEP, 2-(methacryloyloxy)ethyl phosphate; TFMAA, 2-(trifluoromethyl)acrylic acid. The eluent was *n*-hexane–chloroform (98:2, v/v) and the flow-rate was 1.0 ml/min. The detection was carried out with an evaporative light scattering detector. The separation factor (α) was calculated by dividing the capacity factor for cholesterol by that for stigmasterol.

strated to be a useful functional monomer for steroid imprinting having hydroxyl groups.

3.2. Evaluation of the affinity in estradiol-imprinted polymer prepared with MEP as functional monomer

It is demonstrated that the functional monomer, MEP, was useful for cholesterol imprinting. In order to verify the imprint effect by using this functional monomer, estradiol was used as the template molecule and estradiol-imprinted polymer [P_{MEP}(estradiol)] was also prepared with MEP. In the preparation of P_{MEP}(estradiol), the chloroform–dioxane mixture was used instead of the toluene–chloroform mixture because of a low solubility of estradiol.

Estradiol is a steroid hormone with two hydroxyl groups (Fig. 1). P_{MEP}(estradiol) showed higher affinity for estradiol than that for other reference compounds (Table 4). The imprint factor (I_f), which was calculated by dividing the capacity factor for the test compound in the imprinted polymer by that in the blank polymer, for estradiol was 1.36, while those for cholesterol, cholesterol acetate and estrone were 0.75, 1.0 and 0.97, respectively. The difference in structure of steroid and no existence of hydroxyl group would cause the low affinity and imprint factor of cholesterol and estrone. The aromatic hydroxyl group of estradiol that has a rigid molecular structure would strongly interact with the phosphate group of the functional monomer, and also the existence of another hydroxyl group would affect the higher affinity for estradiol in the imprinted polymer. The highest imprint factor would be brought from the template effect and suitable binding sites for estradiol could be constructed by the estradiol imprinting.

Cholesterol is the attractive target molecule in clinical field, therefore, it has been sometimes used as the target molecule in molecular imprinting [11]: Whitcombe et al. reported the cholesterol-imprinted polymer using cholesteryl(4-vinyl)phenyl carbonate as the template molecule [12] and Komiyama et al. also using cyclodextrin as the functional monomer [13]. Both imprinted polymers demonstrated a good selectivity for cholesterol. The cholesterol-imprinted polymer prepared with MEP could be synthesised more easily than the imprinted polymer reported before because of no esterification step in preparation and inexpensive acquirement of MEP. By using the

Table 4
Capacity factors of cholesterol and its derivatives in cholesterol-imprinted polymers

Polymer	Capacity factor [Imprint effect]			
	β-Estradiol	Cholesterol	Cholesterol acetate	Estrone
P_{MEP}(β-estradiol)	15 [1.36]	0.3 [0.75]	0.1 [1.0]	3.3 [0.97]
P_{MEP}(none)	11	0.4	0.1	3.4

The eluent was *n*-hexane–chloroform (1:1, v/v) and the flow-rate was 1.0 ml/min. The detection was carried out with an evaporative light scattering detector. The imprint factor was calculated by dividing the capacity factor for test compound in the imprinted polymer by that in blank polymer.

interaction of the phosphate group, it would be applicable for imprinted polymers that recognise steroids having hydroxyl groups, generally.

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