J Incl Phenom Macrocycl Chem (2008) 61:147–151
DOI 10.1007/s10847-007-9407-z

ORIGINAL ARTICLE

Photochemical molecular imprinting of cholesterol

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Received: 12 June 2007 / Accepted: 22 December 2007 / Published online: 12 January 2008

Abstract Cinnamoylated photocrosslinkable cyclodextrin derivatives (BCC) were synthesized by the substitution of β-cyclodextrin (β-CD) with cinnamoyl chloride (CC) and crosslinked with either hexamethylenediisocyanate (HMDI) or toluenediisocyanate (TDI). Cyclodextrin rings were substituted with one or two cinnamoyl moieties, as found from mass spectrometry. The polymeric matrix with cholesterol molecular imprint was obtained on irradiation of molecular assembly formed by the cinnamoyl-functionalized β-cyclodextrin-cholesterol with light at 275 nm, absorbed exclusively by the cinnamoyl chromophores. Irradiation induced crosslinking due to the photodimerization of the cinnamoyl moieties. To determine the adsorption properties of the produced material imprinting was performed in the presence of tritiated cholesterol and the intensity of β radiation from the material was measured. The materials obtained by the adsorption of tritiated cholesterol by nonirradiated polymer were used as controls. It was found that the polymer photocrosslinked in the presence of cholesterol have shown a considerable higher adsorption capacity for cholesterol than the control materials. This confirmed successful formation of molecularly imprinted polymer (MIP) by photochemical crosslinking. The selectivity of imprinting was also confirmed using compounds of similar structures, i.e. ergosterol, dehydroergosterol, and Vitamin D.

Keywords Molecular imprinting · Cholesterol · Photodimerization · Photocrosslinking · Cyclodextrin · Chromophore

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Introduction

Molecularly imprinted polymers (MIPs) [1, 2] are being extensively investigated as selective adsorbents with wide range of potential applications. Except of their uses for separation, they become also important in the biomedical field, e.g. in clinical analysis and diagnostics [3], environmental monitoring [4], and drug delivery [5].

The synthesis of MIPs usually involves formation of complexes between functional monomer molecules and template molecules, followed by the polymerization reaction which usually requires the use of a crosslinker in order to form a polymeric matrix surrounding the template molecules. Template is then removed leaving out voids complementary in size and shape to the template molecules. The template may be attached to the molecule of monomer through easily cleavable covalent bonds or through specific interactions. The range of molecules used as templates is wide - from low molecular compounds [6–8] to proteins [9–12].

This paper describes the synthesis of a novel molecularly imprinted polymer that can recognize and bind biologically important compounds, such as cholesterol. There are several papers in literature describing results of the studies on development of the MIP for that purpose [13–22]. In a current paper the synthesis of a MIP is based on a photochemical approach. We have performed photochemical crosslinking of β-cyclodextrin derivative functionalized with a cinnamoyl chromophore. Cinnamoyl chromophores are known for their ability to undergo photodimerization when irradiated with UV light with the wavelength of about 280 nm. Thus, if the photodimerization is carried out in the presence of a template compound, a MIP is expected to be formed. The template was constructed using the functional hosts approach

proposed by Asanuma and coworkers [23]. β-cyclodextrin was used for that purpose taking advantage of its ability to form inclusion complexes with compounds whose molecules (or their parts) are small enough to fit into the cyclodextrin cavity. Thus, a molecule of β -cyclodextrin serves two purposes, being a host for the cholesterol molecule and a carrier of photoactive functionalities which on irradiation can form a polymeric matrix surrounding an imprint.

Experimental

Materials

Cinnamoyl chloride (CC, 98%), 4-dimethylaminopyridine (DMAP, 99%), cholesterol (99%), dehydroergosterol (ergosta-5.7.9(11),22-tetraen-3β-ol) (96%, HPLC), Vitamin D_3 (cholecalciferol) (HPLC grade) were purchased from Aldrich and used as received. Ergosterol (ergosta-5,7,22-trien-3β-ol, provitamin D) (>95%, Fluka) was used as received. 2-hydroxyethyl methacrylate (HEMA, Aldrich, 99%) was purified by distillation under vacuum. β-Cyclodextrin (β-CD), toluene 2,4-diisocyanate (TDI, analytical grade), hexamethylene diisocyanate (HMDI) were purchased from Fluka. Solvents: *N*,*N*′-dimethylacetamide (DMA, POCh, Gliwice, analytical grade), dimethyl sulfoxide (DMSO, POCh, Gliwice, analytical grade), and methanol (Chempur, analytical grade) were used as received. ^{3}H-labeled cholesterol was purchased from Amersham International (UK).

Methods

UV/Vis spectra were recorded on HP 8452A Diode-Array spectrophotometer in the spectral range from 190 to 820 nm. Photocrosslinking of the samples was carried out using a Rayonet photoreactor equipped with 8 lamps with the maximum of emission intensity at 350 nm. The intensity o β radiation from the samples containing tritiated cholesterol was measured using α, β-Wallac 1414–003 Gurdian liquid-scintillating spectrometer controlled by PC Pentium II 300 computer. IR spectra were recorded on Bruker IFS 48 spectrometer. Mass spectra (ESI) were recorded on Finnigan MAT 95S spectrometer. Elemental analyses were carried out with Euro EA 3000 Euro Victor elemental analyzer. HPLC analyses were performed using a Waters liquid chromatograph equipped with a Waters Symmetry 5 μm 4.6 × 150 mm column and a Waters PDA 2996 Photodiode Array Detector. The eluent was methanol and the flow rate was 0.5 ml/min.

Synthesis of β-CD substituted with cinnamoyl chromophores (BCC)

To the solution of 4.74 g β-CD (4.18 mmol) in 12 ml DMA, 0.304 g (2.49 mmol) of DMAP was added. The mixture was stirred and heated to achieve a constant temperature of 33 °C. The solution of 2.1 g (12.6 mmol) of cinnamoyl chloride in 6 ml of DMA was added dropwise and the mixture was left for the next 2 h. After the reaction was completed the mixture was poured into hexane and the precipitate was thoroughly washed with the aqueous NH_3 solution until no bands characteristic of β-CD and cinnamoyl chloride could be seen in the UV spectra of the washing solution.

Crosslinking of BCC with TDI and HMDI

About 0.991 g of BCC was dissolved in 10 mL of DMSO. The solution was stirred and heated to 65 °C and then 0.76664 g (4.40 mmol) of TDI was added dropwise. The mixture was left under these conditions for the next three days. The obtained materials, BCC-TDI and BCC-HMDI were purified by washing them with the excess of methanol. The same procedure was used to crosslink BCC with HMDC using 0.981 g and 1 g (5.95 mmol) of these materials, respectively.

Imprinting of cholesterol

About 0.6 g of BCC crosslinked with TDI or HMDI was swelled in 6 mL of DMSO and irradiated for 2 h in the absence and in the presence of 0.82 mg/mL of cholesterol. The irradiation was accompanied with the change of the color of the solution from yellow to red. Then cholesterol was removed by washing with the excess of methanol.

Measurements of adsorption

To 0.02 g samples of dried imprinted and non-imprinted polymer materials (BCC crosslinked with TDI or with HMDI) 2 mL of cholesterol solution in methanol (1.11×10^{-3} mmol/mL 0.003% mol of which was ^{3}H-labeled cholesterol) was added. After two days the samples were centrifuged and about 1 mL of supernatant was taken for activity measurements.

Radioactivity measurements

The intensity of the β radiation emitted by 0.5 mL of supernatant mixed with 0.5 mL of scintillating solution was measured for 1,000 s.

Results and discussion

In order to obtain a MIP which would be capable of adsorbing cholesterol and could be imprinted by photocrosslinking a functional derivative of β-CD substituted with cinnamoyl chromophores was synthesized (Fig. 1a). These chromophores are well known for their ability to undergo photodimerization when irradiated with UV light with the wavelength of about 275 nm. That reaction results in the formation of head-to-head and head-to tail isomers of cyclobutane derivatives (Fig. 1b).

The photodimerization can be reversed by irradiation of the photodimer with the UV light with the wavelength of about 240 nm. Thus, it can be expected that a polymeric material obtained by crosslinking of β-CD substituted with cinnamoyl chromophores could be used as a matrix for photochemical reversible molecular imprinting. The material obtained may thus be potentially reused for imprinting of other template molecules. Moreover, photochemical crosslinking provides a better kinetic and spatial control over imprinting process compared to conventional imprinting. It is also important that photochemical crosslinking is a mild technique.

Substitution of β-CD with CC was confirmed with IR spectroscopy (data not shown). As found from mass spectrometry the product of the reaction of β-CD with CC is a mixture of β-CD substituted with one or two cinnamoyl chromophores at the molar ratio of about 3/2. The triply substituted derivative was present only in trace amounts.

The ability of the cinnamoyl chromophores attached to β-CD to undergo photodimerization was then confirmed.

It was observed that irradiation of BCC in methanol solution with the light absorbed by the cinnamoyl chromophores resulted in a decrease of the absorption band at 275 nm which is indicative of the photodimerization reaction (Fig. 2) [24].

Based on the UV spectra of irradiated BCC the degree of photocrosslinking (DC) as a function of the irradiation time was estimated using a formula:

$$DC = (A_0 - A(t))/A_0) \times 100\,\%$$

where A_0 and A are the absorbances of the sample at 275 nm before and after irradiation for various periods of time, t, respectively (Fig. 3).

It was found that the photodimerization process was fast for the first 40 min of irradiation, while after that period the photoreaction slowed down for more concentrated BCC solutions or even stopped for diluted solutions. On the other hand, a degree of photocrosslinking (in the time interval studied) was higher for more diluted solutions which could be probably explained considering the inner filter effect. That suggestion is supported by the fact that for more concentrated solutions the reaction still proceeds at longer irradiation time although at a slow rate. The degree of photocrosslinking ranged from 19% to 26% after 4 h of irradiation.

Because of low degree of substitution of BCC with photocrosslinkable cinnamoyl chromophores production of highly crosslinked polymeric matrix by photodimerization of BCC in the presence of a template molecule would be rather ineffective. Therefore, BCC was pre-crosslinked chemically using TDI or HMDI to form a hydrogel,

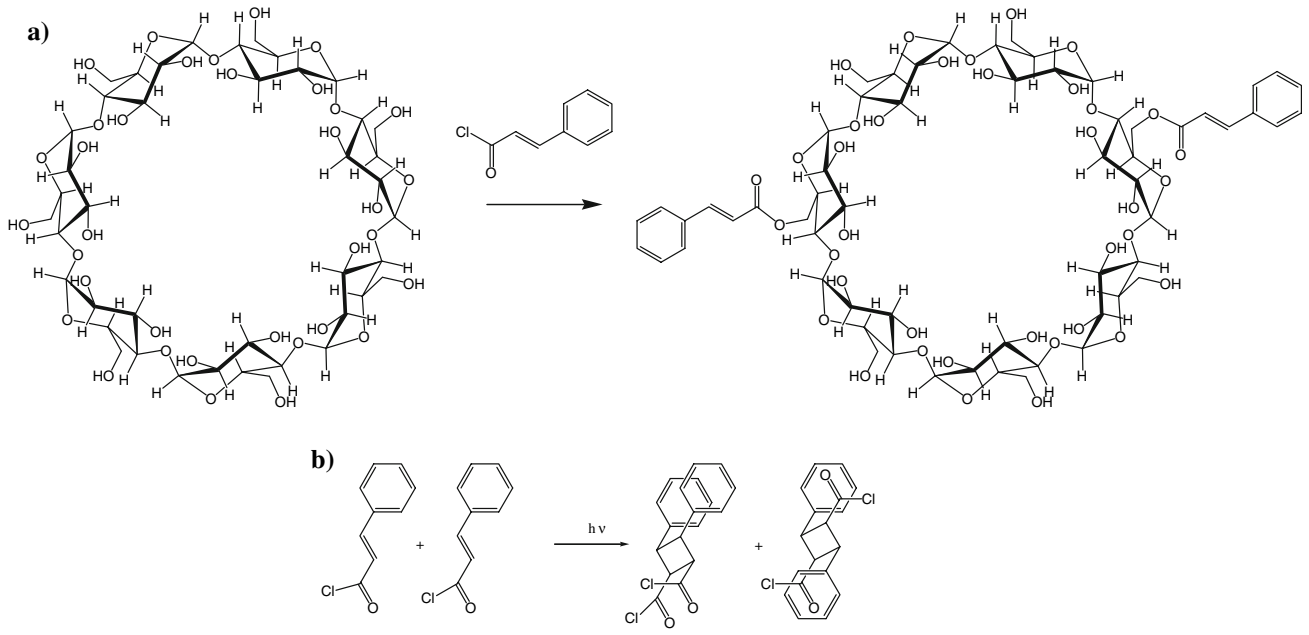

Fig. 1 (**a**) Derivatization of β-CD with cinnamoyl chromophores, (**b**) photodimerization of cinnamoyl chromophores

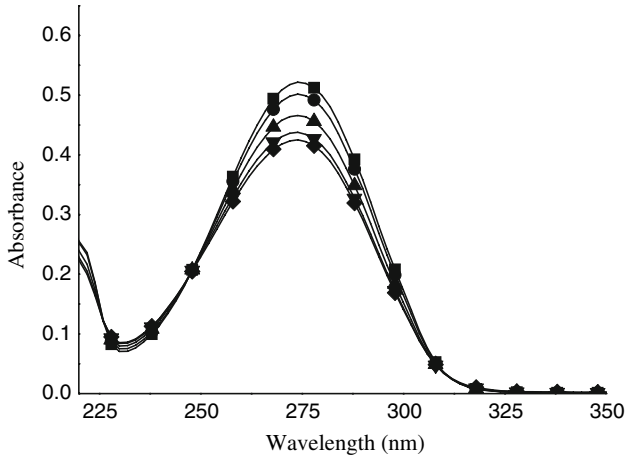

Fig. 2 UV spectra of BCC solution in methanol (0.0086 mg/mL) nonirradiated (■) and irradiated with UV light (λ_{max}=350 nm) for 2 (●), 7 (▲), 17 (▼), and 40 min(◆)

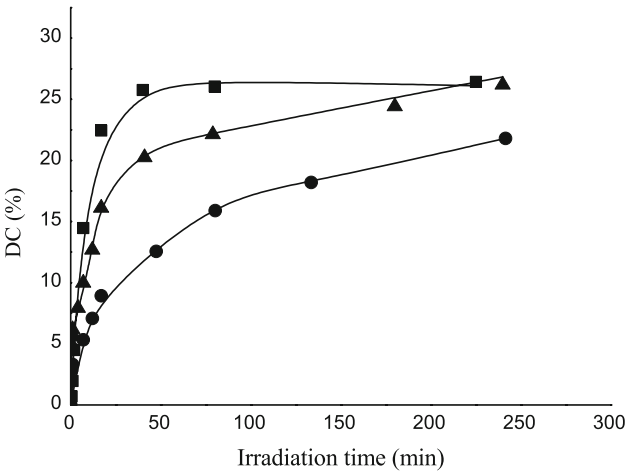

Fig. 3 Degree of photocrosslinking of BCC at (■) 0.032, (●) 0.506, and (▲) 1.343 mg/ml irradiated with UV light (λ_{max}=350 nm)

following the crosslinking method by Asanuma et al. [25]. The occurrence of crosslinking reaction was confirmed by IR spectra and elemental analysis (data not shown). That hydrogel was then used to obtain a molecularly imprinted polymer by photocrosslinking with UV light in the presence of cholesterol which was selected as a model template. The concentration of cinnamoyl chromophores was large enough compared to that of cholesterol so that the irradiating light was absorbed almost exclusively cinnamoyl chromophores.

The efficiency of molecular imprinting was checked using ^{3}H-labeled cholesterol. The polymeric matrices were immersed in the solutions of the radioactive cholesterol in methanol. As control materials the non-photocrosslinked matrices were used. The adsorption of cholesterol was measured as a decrease of the radioactivity of the cholesterol solution after filtering out of the imprinted polymer. Materials obtained from BCC crosslinked with two different diisocyanate crosslinkers were studied. The results are given in Table 1.

These results clearly show that photochemical imprinting was successful for materials obtained from photocrosslinked BCC since the photoimprinted polymeric materials are capable of adsorbing about twice as much of cholesterol as the non-imprinted materials under our experimental conditions. Also, the influence of the length of a diisocyanate crosslinker used can be observed. The non-imprinted material crosslinked with HMDI has a greater adsorption capacity for cholesterol than the one obtained with TDI, in spite of a slightly higher crosslinking density of the former. This may be explained with a greater length of HMDI molecules than that of TDI molecules. Therefore, the matrix obtained with crosslinking with HMDI is expected to have larger pores necessary for the adsorption of bulky cholesterol molecules.

Next we have checked how selectively the obtained polymeric material adsorbs imprinted molecules. For that purpose, we have used a series of cholesterol-related compounds, i.e. ergosterol, dehydroergosterol, and Vitamin D_3 with the degree of structural similarity to cholesterol decreasing in that sequence (Fig. 4). We have checked the adsorption capability of BCC-TDI towards cholesterol and these compounds. The process was followed by the HPLC technique. The results obtained are given in Table 2.

The results obtained clearly show that the cholesterol-imprinted polymer adsorbs cholesterol most strongly and that adsorption of ergosterol, structurally very similar to cholesterol, is quite close to that of cholesterol, while adsorption of Vitamin D_3, which has three rings instead of four, is very low, adsorption of dehydroergosterol being the intermediate case.

We believe that the imprinting effect can be improved, e.g. by using β-cyclodextrin with greater degree of substitution. This is the subject of our current studies and the results will be published shortly.

Table 1 The comparison of the capability of the synthesized imprinted materials to adsorb cholesterol

Matrix	Decrease of cholesterol concentration [mmol/L] for 1 g of the MIP	
	Non-photocrosslinked	Photocrosslinked
BCC-HMDI	0.013	0.029
BCC-TDI	0.008	0.015

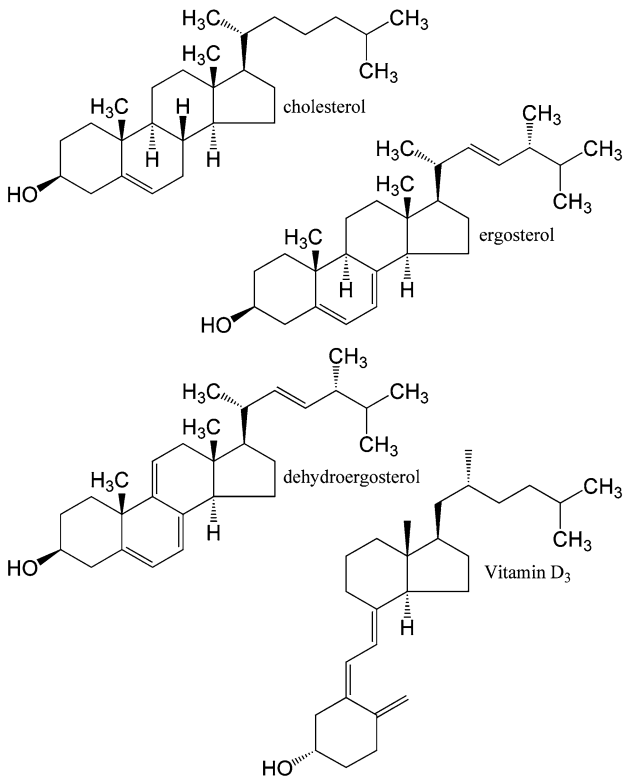

Fig. 4 Structures of studied cholesterol-related compounds

Table 2 Selectivity of cholesterol-imprinted BCC-TDI towards various cholesterol-related compounds

Compound	Relative decrease of concentration/1 g of polymer (%)
Cholesterol	27
Ergosterol	22
Dehydroergosterol	12
Vitamin D_3	1.6

Conclusions

It was found that molecularly imprinted polymers can be obtained photochemically. Photocrosslinked polymeric matrix obtained from β-CD moieties substituted with photodimerizable cinnamoyl chromophores was found to adsorb twice as much of cholesterol compared to non-photocrosslinked material under the experimental conditions. Application of HMDI as a crosslinking agent resulted in a higher adsorption capacity compared to TDI, most likely, due to greater size of pores in the matrix obtained. The degree of selectivity of cholesterol-imprinted MIP obtained increases with increasing structural difference between cholesterol and given compound.

Acknowledgements The authors are grateful to Prof. Jerzy Mietelski and Dr Paweł Gaca from Institute of Nuclear Physics, Polish Academy of Sciences in Kraków, Poland, for their help in the measurements of β radiation.

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