Molecularly imprinted polymer of β-cyclodextrin for the efficient recognition of cholesterol

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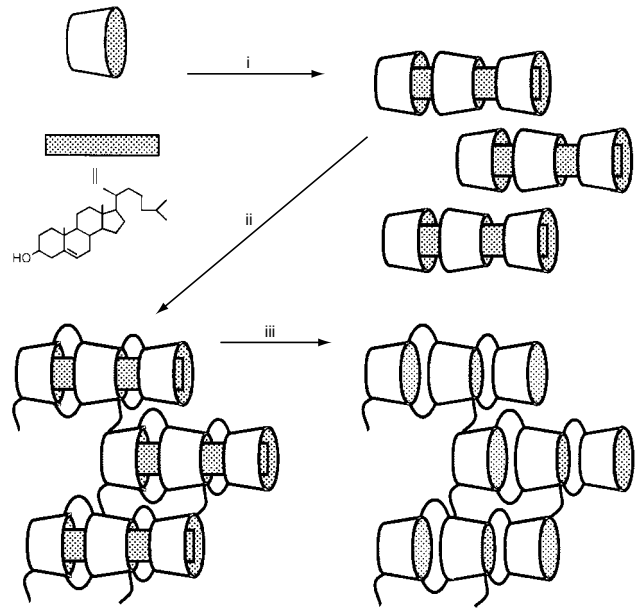
Polymeric receptors for cholesterol have been prepared by crosslinking β-cyclodextrin with diisocyanates in dimethyl sulfoxide in the presence of cholesterol as template.

To date, a number of organic receptors which recognize small guest molecules have been synthesized.[1–3] Highly selective guest-binding was successfully achieved by placing appropriate functional residues at the required sites. Furthermore, a molecular imprinting method was developed to prepare tailor-made artificial receptors.[4–10] In this technique, the adducts between a guest and functionalized monomer(s) are polymerized, and hence the positions and orientations of the functional residues of the monomers in the adducts, which fit the physicochemical properties of the guest, are immobilized in the polymers. The studies on the recognition of small guest molecules have been so fruitful that the current interest is gradually focusing on receptors for still larger guests, such as steroids, oligopeptides, oligosaccharides, *etc*. Whitcombe *et al.* prepared a receptor for cholesterol by polymerizing a cholesterol-attached monomer, followed by the removal of the steroid residue.[11] Another promising strategy for the preparation of these receptors is to build up several host molecules, each of which binds a part of the large guest molecule, so that the resultant assembly of the hosts as a whole recognizes the guest.[12] However, the methodology for the construction of these ordered assemblies has not yet been established.

Recently, Breslow and Zhang[13] showed that cholesterol can be bound by a receptor in which two molecules of β-cyclodextrin (β-CyD: a cyclic oligomer of seven glucose units)[14–16] are connected to each other by an appropriate linker. These two β-CyD residues are precisely placed to bind cholesterol, which is too large to be accommodated in the cavity of a single β-CyD residue (free β-CyD forms a 3 : 1 complex with cholesterol).[17] Here we prepare polymeric receptors for cholesterol by crosslinking β-CyD molecules. The mutual orientations of the β-CyD residues are regulated by using the molecular imprinting technique. The crucial importance of the choice of both the crosslinking agent and the solvent for the templating is evidenced.

The templated β-CyD polymers were prepared as depicted in Scheme 1.† In dimethyl sulfoxide (DMSO), the β-CyD–cholesterol complex was treated with either hexamethylene diisocyanate (HMDI) or toluene 2,4-diisocyanate (TDI). After the crosslinking reaction, the cholesterol (used as the template) was removed from the polymer by treatment with acetone, THF and hot EtOH, and this was confirmed from ^{13}C-CP/MAS NMR spectroscopy. These polymers were then dried and used for the adsorption experiments.

The templated β-CyD polymer, prepared using HMDI as the crosslinking agent (abbreviated as HMDI-Imp), efficiently adsorbed cholesterol, which was present in a water–THF mixture (5 : 6 v/v).‡ The adsorption reaction was completed within 5 min. By this simple procedure, 15% of the cholesterol was removed from the liquid phase (Table 1). The amount of adsorbed cholesterol was proportional to the amount of the polymer. Stigmasterol, a derivative of cholesterol, was also adsorbed by the polymeric receptor in a similar amount (10%). In contrast, non-templated β-CyD polymer (synthesized in the absence of the cholesterol template: HMDI-Non) did not adsorb cholesterol to a measurable extent. Thus, the 'templating' is essential to prepare the polymeric receptor which notably binds cholesterol. The templated polymer TDI-Imp showed an even greater adsorbing activity (70% of the cholesterol was adsorbed). This was probably due to the molecular rigidity of TDI being able to regulate the positions of β-CyD residues more precisely.



Scheme 1 *Reagents and conditions*: i, hexane–H_2O; ii, diisocyanate in DMSO; iii, acetone, H_2O, THF, EtOH

All the adsorbed cholesterol was easily removed from the polymeric receptors by treating the polymer–cholesterol adducts with boiling EtOH. On the next run, both the rate of cholesterol adsorption and the adsorbing capacity were identical to those in the foregoing adsorption. Thus, the adsorption described here is totally reversible. The potential applicability

Table 1 Adsorption of cholesterol from the water–THF mixture by crosslinked β-CyD polymers

Crosslinking agent	Adsorbing activity[a]		Templating effect[b]
	Templated	Non-templated	
HMDI	0.15	< 0.01	> 15
	(0.05)[c]	(0.05)[c]	(1.0)[c]
TDI	0.70	0.19	3.7
Epichlorohydrin	< 0.01	< 0.01	—

[a] The ratio of the decrease in cholesterol concentration in the liquid phase to the initial concentration, under the adsorption conditions presented in footnote ‡. [b] The ratio of the adsorbing activity of the templated polymer to that of non-templated polymer. [c] Phenol is used as the guest, in place of cholesterol.

of these adsorbents to practical removal of cholesterol has been indicated.[18]

It is noteworthy that the β-CyD polymers, prepared by crosslinking with epichlorohydrin,[19]§ did not adsorb cholesterol (Table 1; a similar result was reported by Breslow and Zhang).[13] The templated polymer synthesized from the β-CyD–cholesterol complex (in place of free β-CyD) was also inactive. The use of DMSO is advantageous for the 'templating', since the β-CyD inclusion complexes are satisfactorily formed in this solvent.[14,15,20]¶ The formation of well-defined complexes under mild conditions is presumably responsible for the selectivity. The crosslinking of β-CyD by epichlorohydrin is achieved under highly alkaline conditions, where the β-CyD–cholesterol complexes mostly dissociate.

These arguments are further supported by the fact that the adsorbing activity of the HMDI-Imp on phenol was identical with the value of the HMDI-Non (see the numbers in parentheses in Table 1).[14,15]‖ The present finding indicates that artificial polymeric receptors for varieties of large guest molecules can be prepared by using the molecular imprinting technique for the immobilizaton of β-CyD.

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Footnotes and References

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† The 3 : 1 β-CyD–cholesterol complex was prepared according to ref. 17, and was reacted with the diisocyanate agents in dry DMSO at 65 °C for a few hours ([diisocyanate]/[β-CyD] = 4.2). All the reactants (β-CyD, cholesterol and the diisocyanates) were satisfactorily soluble in DMSO. The polymer, obtained by pouring the mixture into acetone, was ground with a mortar and pestle, and then sufficiently washed with hot water, THF and hot EtOH to remove the cholesterol and free β-CyD completely. The ratios of the diisocyanate residue to β-CyD in the polymers were 3.4 for both HMDI and TDI, as estimated by elemental analysis. Non-templated β-CyD polymers were similarly prepared in the absence of the cholesterol template.

‡ The adsorption experiment was carried out by incubating the polymeric receptor (0.5 mmol of β-CyD residue) in 11 ml water–THF (5 : 6 v/v) containing 0.05 mmol of cholesterol. After being magnetically stirred at 25 °C, the mixture was centrifuged and the liquid phase was analysed by gas chromatography. The water–THF mixture was used as the solvent, since the solubility of cholesterol in water is too small and the addition of THF was required to provide homogeneous solutions.

§ The epichlorohydrin-immobilized β-CyD was prepared by reacting β-CyD (25 g) with the crosslinking agent (20 g) at 50 °C in 50 wt% of aqueous NaOH solution, according to ref. 19. The ratio of the crosslinking residue to β-CyD residue in the polymer was 3.6.

¶ The complex formation between β-CyD and cholesterol in DMSO was confirmed by ^{1}H NMR spectroscopy using 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]propionic acid as the internal standard. When β-CyD (30 mM) was added to a DMSO solution of cholesterol (2 mM) at 25 °C, the 18 methyl protons of cholesterol, for example, showed upfield shift (0.046 ppm) with respect to the value in the absence of β-CyD. The remarkable 'templating' efficiency is partially associated with the fact that, due to a proximity effect, the β-CyD residues in the cholesterol complex are crosslinked by the diisocyanates more quickly than are free β-CyD molecules in the solutions.

‖ In order to shed light on the role of the cavity of β-CyD in the present cholesterol binding, dextran was crosslinked by HMDI under the conditions employed for the immobilization of β-CyD. The resultant dextran polymer did not adsorb phenol at all. The possibility that phenol is simply adsorbed to either the outside of β-CyD or the crosslinking residue is unlikely. The complex formation between phenol and the templated polymer was further confirmed by solid-state ^{13}C-NMR: C1 of β-CyD showed a downfield shift of 0.4 ppm on the complex formation, whereas the shift of C4 was marginal. The C2, C3 and C5 carbon atoms could not be sufficiently resolved under the conditions.

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