Cholesterol Recognition and Binding by Cyclodextrin Dimers

Ronald Breslow* and Biliang Zhang

Department of Chemistry, Columbia University
New York, New York 10027

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Cyclodextrin dimers in which the two units are linked by various spacers have shown strong binding of ditopic substrates such as bis-(*p*-nitrophenyl) phosphate and other substrates carrying two phenyl groups or substituted phenyl groups.[1−4] However, such cyclodextrin dimers should also be very effective at binding long hydrophobic molecules such as sterols. For example, cholesterol (**6**) is ca. 15 Å long, including the side chain, while a β-cyclodextrin ring is ca. 7.8 Å high (Scheme 1). Thus a cyclodextrin dimer with a very short linker between the two rings might well be able to bind a cholesterol molecule cooperatively into the two cyclodextrin rings. We have found that this is the case.

We constructed dimer **1** in 44% yield by heating 6-iodo-6-deoxycycloheptaamylose with sodium sulfide.[5] The compound had the expected ^{1}H-NMR spectrum and the correct FAB-MS spectrum with $M + 1 = 2268$. It was a good ligand (Table 1) for BNS **4**, and particularly for the bis(adamantylethyl) phosphate **5**.

The binding constant of cholesterol to **1** was determined by measuring the increased solubility of cholesterol in water containing various concentrations of **1**. The solutions of **1** in water were stirred with an excess of solid cholesterol for 24 h, and the filtrate was then analyzed for cholesterol by a fluorimetric method[6] or an enzymatic method.[7] As the data in Table 1 show, results from the two analytical methods were in reasonable agreement.

The solubility technique for determining binding constants[8] is reliable if the host is partially saturated by the guest, as we found (Figure 1) in this case. From the slope and intercept (s_0) of this plot, the standard treatment (eq 1)[8] gives a formation constant for the complex (the linearity of the plot in Figure 1 indicates a 1:1 complex) that is 200−300 times greater than that reported[9] for cholesterol with monomeric β-cyclodextrin.

$$K_a = \text{slope}/s_0(1 - \text{slope}) \qquad (1)$$

Cholesterol has been removed from dairy products as its complex with β-cyclodextrin.[10] Also, a polymer of β-cyclodextrin has been used commercially to sequester cholesterol and other hydrophobic materials.[11] Thus we examined the binding of cholesterol to a soluble polymer **3** of β-cyclodextrin, made by cross-linking it with epichlorohydrin.[12] One could imagine that in such a polymer one might observe cooperative binding

Scheme 1

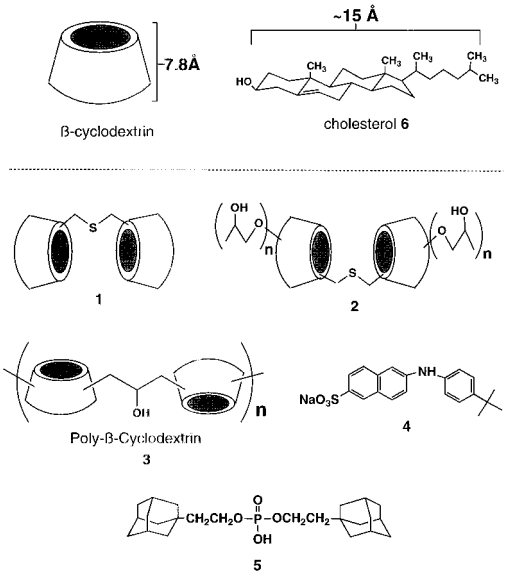

Table 1. Binding Constants of Host−Guest Complexes in Water at 25 °C

host	guest	K_a (M^{-1})
dimer **1**	BNS **4**	$(7.40 \pm 0.10) \times 10^5$ [a]
dimer **1**	BNS **4**	$(6.37 \pm 0.25) \times 10^5$ [b]
dimer **1**	phosphate **5**	$(1.14 \pm 0.20) \times 10^7$ [b]
dimer **1**	cholesterol **6**	$(3.30 \pm 0.80) \times 10^6$ [c]
dimer **1**	cholesterol **6**	$(5.54 \pm 0.76) \times 10^6$ [d]
hydroxypropyl dimer **2**	cholesterol **6**	$(1.47 \pm 0.62) \times 10^5$ [c]
polymer **3**	BNS **4**	$(1.63 \pm 0.15) \times 10^5$ [b]
polymer **3**	cholesterol **6**	$(5.07 \pm 0.58) \times 10^4$ [d]
β-cyclodextrin	cholesterol **6**	1.7×10^4 [e]
hydroxypropylated β-cyclodextrin	cholesterol **6**	1.9×10^4 [e]

[a] By fluorimetric titration in 50 mM phosphate buffer, pH 7.0; average of more than three independent runs. [b] By calorimetric titration in 20 mM HEPES buffer, pH 7.0; average of more than three independent runs. [c] By the solubility method, using an enzymatic determination of cholesterol. [d] By the solubility method, using a fluorimetric determination of cholesterol. [e] From ref 9.

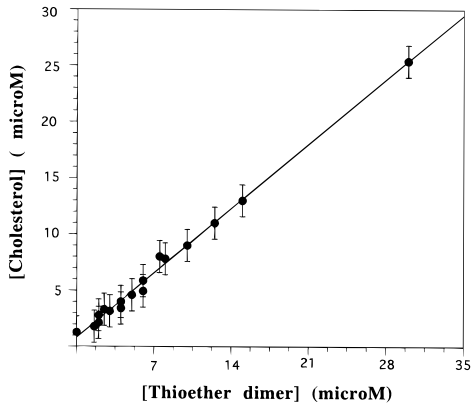

Figure 1. Solubility of cholesterol, determined by the enzymatic method, in water at 25 °C containing various amounts of the thioether cyclodextrin dimer **1**. The straight line indicates a 1:1 complex, and the slope and intercept indicate the binding constant listed in Table 1.

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(5) The thioether dimer **1** was purified by reverse-phase chromatography by eluting with a gradient from water to 80% methanol−water. The desired product was collected in 70% methanol−water fractions. The purity was determined by TLC, $R_f = 0.1$ (silica, 5/7/7/4 H_2O/CH_3COOEt/i-PrOH/NH_4-OH).
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(7) Using the Sigma Chemical Company's diagnostic kit for the quantitative enzymatic determination of total cholesterol by the combined action of cholesterol oxidase and peroxidase with *p*-hydroxybenzene sulfonate leuco dye. This method and the fluorimetric method (ref 6) were fully calibrated before use.
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by two β-cyclodextrin rings, as in our dimer. However, by the solubility test we find (Table 1) that the affinity of the polymer for cholesterol is only three times that of simple β-cyclodextrin and thus ca. 100 times less than that of our dimer **1**.

Reaction of β-cyclodextrin with propylene oxide under basic conditions produces a material with attached hydroxypropyl groups that is even more soluble than is β-cyclodextrin and is used for drug delivery.[13] It has been reported[9] that the binding constant of cholesterol to hydroxypropylated β-cyclodextrin (Table 1) is slightly higher than that to simple β-cyclodextrin. Thus we examined the effect of hydroxypropylation on the affinity of our dimer **1** for cholesterol, again by the solubility method. From NMR analysis, ca. five hydroxypropyl groups were attached to each β-cyclodextrin ring in our modified dimer **2**. As the data in Table 1 show, hydroxypropylation of **1** to produce **2** decreases its affinity for cholesterol by 20−40-fold, although hydroxypropylation did increase the water solubility of the dimer. Apparently the hydroxypropyl groups interfere with the ability of the two cyclodextrin rings to align correctly for cooperative binding.

Since cyclodextrin dimers such as **1** are easily prepared and can have superior binding properties as in this case, they seem attractive candidates for applications in molecular detection, in drug delivery, and perhaps in therapy. They certainly represent attractive ligands for steroids such as cholesterol. Some other synthetic cholesterol ligands have been reported elsewhere,[14−16] but its easy accessibility makes **1** particularly attractive.

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(12) The water-soluble polymer was prepared by adding 80 mmol of epichlorhydrin dropwise to a solution of 10 mmol of β-cyclodextrin with 7.5 g of NaOH in 25 mL of water at 70−80 °C. After cooling to room temperature over 3 h, the solution was neutralized with conc. HCl, diluted to 200 mL, and desalted with Amberlite MB-3 resin. This is a standard method (ref 13); with a larger proportion of epichlorhydrin, the insoluble polymers can be prepared.

(13) Reference 11, p 59.

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