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BINDING OF BILE ACIDS BY POLYMERIZED CYCLODEXTRIN RESINS

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ABSTRACT

Copolymers of β-cyclodextrin and epichlorohydrin were prepared by a crosslinking reaction of β-cyclodextrin with different amounts of epichlorohydrin. These copolymers were tested for their ability to bind several bile salts (including the sodium salts of cholic acid, glycocholic acid, taurocholic acid, and chenodeoxycholic acid), individually and competitively, from phosphate buffer solutions at room temperature. The polymer resins with a lower degree of crosslinking had a higher binding capacity for cholic acid. In all cases the binding of chenodeoxycholate by the polycyclodextrin resins was much more effective than that of cholate and its conjugates, which indicates the marked importance of the host cavity size relative to that of the guest molecules. In addition, there were indications that hydrophobicity plays a role in the binding of the bile salts by these resins.

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INTRODUCTION

Bile acids are amphiphilic molecules that assist in the digestion of fats by the formation of micelles and micellar aggregates. They are synthesized biologically in the liver from cholesterol. It is well documented that the sequestering of bile acid anions in the gastrointestinal tract by polymeric sorbents is effective in reducing serum cholesterol levels, hence cardiovascular diseases [1–3]. However, the binding of bile acid anions by currently used polymeric sorbents, such as cholestyramine (Questran), is based on an ion-exchange mechanism and therefore shows little discrimination in the binding [4]. As a result, other anionic species are also bound by the polymer resins, thereby depleting certain drugs [5–7] and essential nutrients, such as vitamins. Another important consequence of the indiscriminate binding in the digestive tract is a lowering of the binding capacity for the target compounds, i.e., the bile salts. Hence, there is a continuing need for new polymeric resins that sequester bile acids selectively.

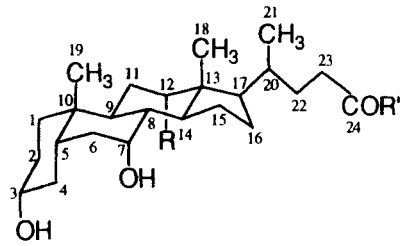
Cyclodextrins (CDs) are macrocyclic oligosaccharides that have found important applications in molecular recognition studies. These molecules take the shape of a truncated cone, with cavities of different sizes depending on the number of glucosidic units, which varies from 6 to 9 [8]. The interior of the cavities is rather hydrophobic while the exterior remains hydrophilic [8]. The cavities can be used to trap molecules of a comparable size, a property which has been exploited in chromatographic separations [8–15]. It has been reported that certain functionalized CDs and their derivatives can act as serum cholesterol level depressants [16]. Since CDs can form inclusion complexes and exhibit size specificity in the binding [8, 16–18], they were selected as candidates for studies of specific interactions with bile acids [19]. By following the proton NMR chemical shifts of the bile salt, it was demonstrated that the β- and γ-cyclodextrins can form inclusion complexes with bile salt anions [19].

Several studies have shown that, by reaction with epichlorohydrin, cyclodextrins can be crosslinked to form gel-like solids that are insoluble in water [20–22]. To further explore the unique binding properties of cyclodextrins for bile acids, we have prepared β-cyclodextrin polymers and determined the isotherms for the binding of various bile salt anions in aqueous buffer solutions. Competitive binding experiments were made to determine the selectivity of these sorbents. We report here the synthesis of the polymer resins and the results of the binding studies.

EXPERIMENTAL

Materials

β-CD (cyclohepataamylose, Sigma), epichlorohydrin (EP), sodium cholate (CA), sodium glycocholate (GCA), sodium taurocholate (TCA) and sodium chenodeoxycholate (CDCA) were purchased from Sigma. The cholestyramine resin (Questran) was washed in a Soxhlet extractor with acetone, water, and methanol consecutively for at least 24 hours each. The final product was dried in vacuum and sieved. The purity of the bile salts was verified by reverse-phased HPLC, and the products were used as received. The chemical structure of the bile acids used is shown in Fig. 1. They all possess a hydrophobic steroid skeleton to which are



Bile Acid	R	R'
Cholic Acid	OH	OH
Glycocholic Acid	OH	$NHCH_2COOH$
Taurocholic Acid	OH	$NHCH_2SO_3H$
Chenodeoxycholic Acid	H	OH

FIG. 1. The chemical structure of the bile acids used in the binding studies.

attached various hydrophilic groups, in particular hydroxyl groups and the side-chain carboxyl group.

Preparation of Polycyclodextrin (PCD) Resins

The polymerized β-CD were synthesized by crosslinking β-CD with EP using suspension polymerization techniques [20–22]. When the CD/EP molar ratio is low (e.g., less than 1:4), the resulting polymer tends to remain soluble in water. To obtain insoluble crosslinked polymers, different quantities of EP (4.82, 3.85, and 2.90 g) were added to an aqueous solution of β-CD (50 wt%, containing 5.68 g or 5 mmol of β-CD) to obtain approximate CD/EP ratios of 1:10, 1:8, and 1:6, respectively, and mixed by stirring at 50°C. An aqueous solution of NaOH (40%) was then added slowly to the flask, followed by stirring at the same temperature for 25 minutes. Paraffin (100 mL) was then added and stirred together for 20 hours. The reaction mixture was cooled to room temperature, and paraffin was removed by washing with ethanol. The resulting polymers were labeled as PCD1, PCD2, and PCD3, respectively. The resins were dried, and only particles of mesh size between 60 and 100 were used for the sorption studies.

Characterization of the PCD Resins

To determine the degree of crosslinking, solid-state ^{13}C-NMR spectra were recorded for the PCD resins. The NMR experiments were performed at room temperature (21 ± 1.5°C) on a Chemagnetics CMX-300 NMR spectrometer operating at 75 MHz for ^{13}C. The chemical shifts are relative to tetramethylsilane and are considered to be accurate to about ±0.1 ppm. The contact time, relaxation delay, and other parameters were adjusted for comparison of the ^{13}C peak integration.

For the semiquantitative analyses, β-CD was used as the standard. The ^{13}C-NMR signal for the methine group at position 1 of the glucose residue is at 103 ppm and is a well-defined peak for both β-CD and the PCD resins. The ^{13}C signal of the chloromethyl group of the EP residue is shown at ca. 31 ppm. The other carbons of

the glucose moieties and those of epichlorohydrin appear in the 55–90 ppm region. Based on the ratio of the integration of these two groups of NMR signals, the three PCD resins were found to have CD/EP molar ratios of 1:7.6, 1:6.2, and 1:4.0, respectively (Table 1). The amounts of unreacted residual chloromethyl groups of EP for the three resins were also estimated similarly (Table 1). All three PCD resins were found to be insoluble but swellable to a certain extent in aqueous media.

Sorption Studies

The procedures used in sorption studies were as described previously [23]. The bile salt solutions were prepared in an aqueous phosphate buffer (5 mM KH_2PO_4–NaOH, pH 7.4). The concentrations of the stock solution were ca. 3 to 4 mM, and these were diluted with buffer to the desired concentrations (0.3–3 mM). For the competitive binding studies, equimolar mixtures of selected bile salts were dissolved in the buffer to prepare the stock solution (total combined concentration of bile acids at 3–4 mM) and diluted into different fractions.

Usually 10 mg of the polymer resin was used for the binding experiments. Depending on the binding capacity, 5–20 mL of the bile salt solution was used in the experiment to ensure the accuracy of the analyses after binding. With vigorous stirring at room temperature (25°C), binding equilibrium was reached within 2 hours in all cases, as evidenced by kinetic studies. The size of the resin particles did not affect the kinetics or the capacity of the binding since all of the resins were quite hydrophilic and swelled well in the aqueous media.

A reverse-phased HPLC system (Waters 600 pump, equipped with a Waters 410 differential refractometer, Waters 700 automatic injector and C-18 column) was used for the analysis of the bile salt concentrations. The details of the HPLC analytical method have been described previously [24]. The uncertainty of the data obtained in the sorption experiments was estimated to be $\leq 5\%$, based on replicated experiments.

RESULTS AND DISCUSSION

Binding Constants and Free Energy Changes

In this study the moles of bile acids bound per molar equivalence of cyclodextrin units never exceeded unity, at least within the concentration range studied.

TABLE 1. The CD/EP Molar Ratio and the Amount of Unreacted Chloromethyl Groups in the PCD Resins

Resin	CD/EP molar ratio		Unreacted CH_2Cl groups, %
	Feed	Final	
PCD1	1 : 10.4	1 : 7.5	47
PCD2	1 : 8.32	1 : 6.2	63
PCD3	1 : 6.26	1 : 4.0	70

Therefore, stoichiometric binding is assumed. For both individual and competitive bindings, the binding constants were calculated for the following equilibrium between the polymer resin, P, and the ligand (bile salts), L:

$$P + L \overset{K}{=} PL \tag{1}$$

The binding constant, K, is given by

$$K = [PL]/[P][L] \tag{2}$$

Therefore, the quantity of ligands bound, Q (in the unit of moles of ligands per mole equivalence of CD units on the resin) can be written as

$$Q = \frac{[PL]}{[P] + [PL]} \tag{3}$$

Using the binding constant from Eq. (2), we can rewrite Eq. (3) as

$$Q = \frac{K[L]}{1 + K[L]} \tag{4}$$

Curve fittings have shown that all the binding isotherms are in reasonable agreement with a stoichiometric binding, with the exception of the binding isotherm of PCD1 for sodium cholate, which can be better fitted with two binding constants (Fig. 1).

The change in free energy can be related to the equilibrium constant of the binding between the polymer resin and the bile salt:

$$\Delta G^\circ = -RT \ln K \tag{5}$$

where R is the gas constant and T is the temperature at equilibrium.

The binding constants and free energy changes, as obtained by the use of Eqs. 4 and 5, are listed in Table 2.

Effect of the Degree of Crosslinking

For the comparison of the degree of crosslinking, the β-CD/EP molar ratios in the PCD resins are listed in Table 1. These data are not precise indications of the degree of crosslinking since the number of free chloromethyl (CH_2Cl) groups of EP are not taken into account. As shown in Table 1, resin PCD1 seems to have fewer free CH_2Cl groups (47%) than resins PCD2 and PCD3 (63 and 70%, respectively), indicating an even higher effective degree of crosslinking for PCD1. The crosslinking reaction was difficult to control since the timing of the addition of the reactants was also critical, as were the molar ratios and concentrations of the reactants. Furthermore, these data obtained from NMR are subject to rather large experimental errors (ca. 15%). Based on the CD/EP ratios in the feed, however, the final CD/EP ratios in the copolymers seem to be reasonable estimates.

To compare their binding characteristics, all three PCD resins were tested for the binding of sodium cholate from the aqueous phosphate buffer. As shown by the isotherms in Fig. 2(A), in all three cases the amount of cholate bound per gram of resin increases continuously with increasing equilibrium concentration of the bile

TABLE 2. Equilibrium Constants and Free Energy Changes of the Binding of Bile Salts with PCD Resins in Phosphate Buffer Solutions

PCD resin	Bile salt	Type of binding	$K \times 10^{-2}$, M^{-1}	$\Delta G°$, kJ/mol
PCD1	CA	Individual	1.69	−12.7
PCD2	CA	Individual	3.82	−14.7
PCD3	CA	Individual	3.68	−14.6
	TCA		3.25	−14.3
	CDCA		26.3	−19.5
PCD3	CA	Competitive	0.321	− 8.6
	GCA		0.721	−10.6
	CDCA		30.2	−19.7
PCD3	CA	Competitive	0.948	−11.3
	CDCA		34.7	−20.2
PCD3	CA	Competitive	2.53	−13.7
	GCA		1.65	−12.7

acid. In the range of concentrations studied here, the most heavily crosslinked PCD1 resin has the lowest binding capacity. A higher degree of crosslinking obviously hinders the formation of the inclusion complexes between the hosts (β-CD cavities) and the guests (cholate anions), thereby lowering the binding capacity of the resin. Within the experimental error, resins PCD2 and PCD3 have similar binding capacities; however, the binding by resin PCD3 seems to be systematically higher, especially at higher equilibrium concentrations of the bile salt. Although a lower degree of crosslinking favors the binding, it is important to note that a further decrease in the crosslinking can result in a PCD resin that is soluble in water. Furthermore, the difference in the degrees of crosslinking of resins PCD2 and PCD3 did not lead to an important difference in the effectiveness of binding from the buffer solutions. Based on these findings, resin PCD3 was chosen for all of the subsequent binding studies.

As shown in Fig. 2(B), all three isotherms can be fitted reasonably well to Eq. (4) with only one binding constant. In the case of PCD1, however, it seems that a model with two binding constants provided a better fitting, with $K_1 = 1.09 \times 10^2$ and $K_2 = 2.40 \times 10^2\ M^{-1}$. For the other two resins, a model with two binding constants did not provide better fittings to the experimental data, K_1 being very close to the single binding constant and K_2 practically negligible.

Binding of Individual Bile Acids

Our previous NMR study showed that the binding of bile acids by CDs involves hydrophobic interactions combined with a physical entrapment of molecules of size comparable to that of the CD cavities [19]. To determine the importance of these effects on the binding by the crosslinked CD resins, separate studies were made with each of the following three bile salts: two primary bile acids of different

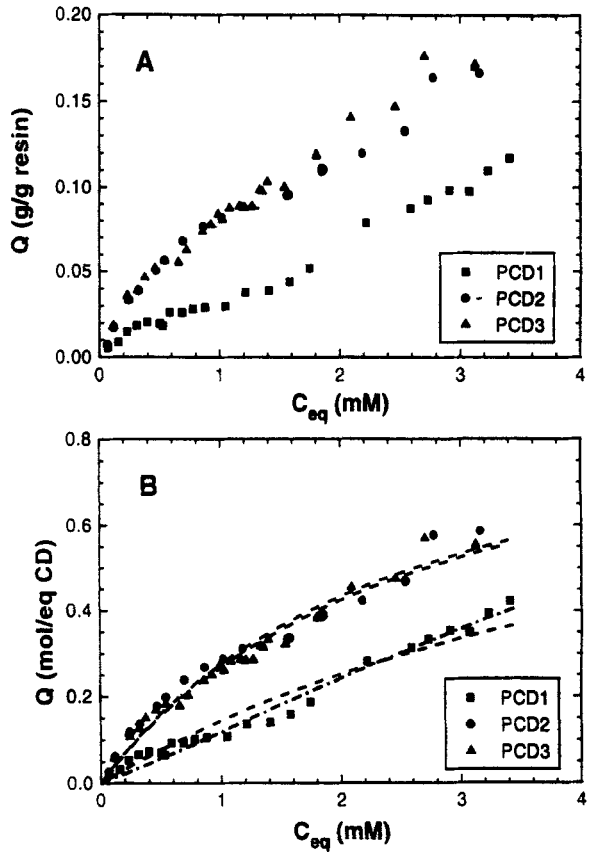


FIG. 2. Isotherms for the binding of sodium cholate in phosphate buffer solutions at 25°C by three different PCD resins of different degrees of crosslinking. The bound amount of bile salt, Q, either expressed as grams of bile salt per gram of resin (A), or moles of CD per molar equivalence of CD (B), is plotted as a function of the bile salt concentration at equilibrium (C_{eq}): ■, PCD1; ●, PCD2; ▲, PCD3. Dashed lines are fittings to Eq. (4). The dashed-dotted line for resin PCD1 is a fitting to a model with two binding constants (K_1 = 1.09×10^2 and $K_2 = 2.40 \times 10^2\ M^{-1}$).

hydrophobicities, cholate and chenodeoxycholate, and the taurine conjugate of cholate. The isotherms for the sorption of the three bile salts by PCD3 are shown in Fig. 3. Clearly, the binding of chenodeoxycholate by PCD3 is much more effective than that of the other two bile salts, as shown also by the relatively large differences in the binding constants and free energy changes (Table 2). Since chenodeoxycholate is more hydrophobic than the others, it appears at first glance that the hydrophobicity of the bile salt anions has an important role. Yet, cholate anion is more hydrophobic than its taurine conjugate, but no significant difference in the binding is observed. Hence, the hydrophobicity is not the only determining factor. For this pair of bile acids, the difference in hydrophobicity is due to the difference of the bile acid side chain but not the steroid skeleton. As indicated in our previous studies by NMR titration and molecular modeling [19], polar hydroxyl groups at positions

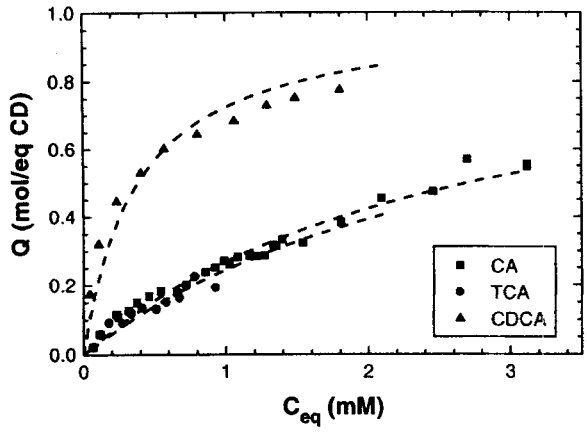


FIG. 3. Isotherms for the binding of individual bile salts in phosphate buffer solutions at 25°C by PCD3 resin: ■, cholate; ▲, chenodeoxycholate; ●, taurocholate.

7 and 12 on the steroid skeleton have a marked effect on the complexation with β-CD. The bulkier 12-OH group (compared with H) located on the steroid skeleton can interfere with the penetration of the bile acid anion into the CD cavity. Thus, the size of the guest molecule may account for the large difference in binding between chenodeoxycholate and the cholates. Moreover, the hydrophilic nature of the 12-OH group does not favor the entry into a hydrophobic cavity either.

Competitive Binding of Bile Acids by PCD3

To determine the binding selectivity of the PCD3 resin, studies were made with an equimolar mixture of three bile salts having the same total bile acid concentration (4 mM). To shorten the HPLC analysis time, taurocholate was replaced by glycocholate, which is also more hydrophilic than cholate but less hydrophilic than the taurine conjugate. The isotherms for the competitive binding are shown in Fig. 4. Again, the binding of chenodeoxycholate is favored, confirming the marked effect of the size of the guest molecule. The difference between the binding of cholate and of glycocholate is very small even though the former is more hydrophobic.

To simplify the interpretation of this phenomenon, a further competitive binding study was made with a sorbate solution containing an equimolar amount of only two bile salts. As shown in Fig. 5, the data follow the same trend with the extent of the binding of chenodeoxycholate being much higher than that for cholate. The effects of hydrophobicity were examined by analyzing the competitive binding behavior of resin PCD3 in sorbate solutions containing only cholate and its glycine conjugate (Fig. 6). Despite the experimental uncertainty, the binding of cholate by resin PCD3 is systematically higher than that for glycocholate. This difference becomes more obvious with increasing equilibrium concentration of the bile salts, which shows that the hydrophobicity still plays a role in the binding. Our previous study suggested that the bile acid anions enter the β-CD cavity via the smaller side chain with the carboxylic acid group [19]. The glycine conjugate of cholate has a

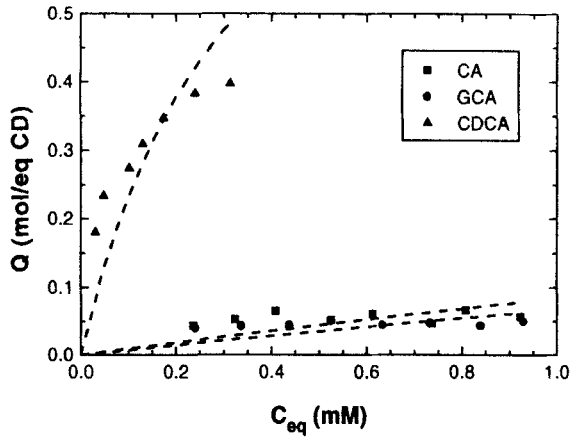


FIG. 4. Isotherms for the competitive binding of three bile salts in phosphate buffer solutions at 25°C by PCD3 resin: ■, cholate; ▲, chenodeoxycholate; ●, glycocholate.

longer side chain, which in principle should allow a better penetration into the β-CD cavity and, hence, a more stable inclusion complex. However, the more hydrophilic nature of this side chain does not favor the entry of the group into a hydrophobic cavity. As a result, no significant difference between cholate and its glycine or taurine conjugates was observed.

When Figs. 4 and 5 are compared with Fig. 2, it is clear that the binding for cholate at identical equilibrium concentrations is less effective when chenodeoxycholate is also present, showing a clear selectivity of the PCD3 resin for chenodeoxycholate. In all cases for chenodeoxycholate the binding constants are higher and the apparent free energy changes are more significant (Table 2).

In all the isotherms for the binding of bile salts by the PCD resins presented above, the amount of bile salt bound per mole of CD residues on the resin was

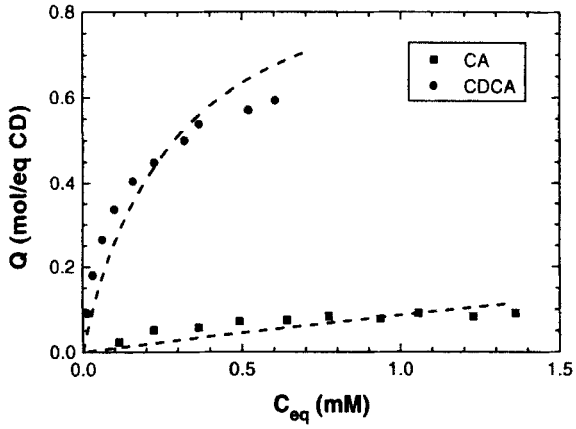


FIG. 5. Isotherms for the competitive binding of two bile salts in phosphate buffer solutions at 25°C by PCD3 resin: ■, cholate; ▲, chenodeoxycholate.

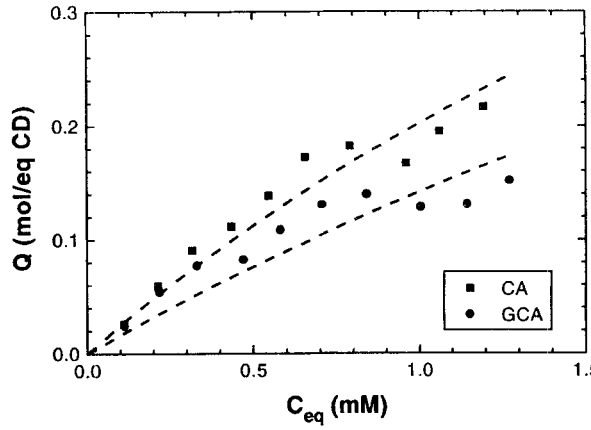


FIG. 6. Isotherms for the competitive binding of cholate and glycocholate in phosphate buffer solutions at 25°C by PCD3 resin: ■, cholate; ●, glycocholate.

plotted as a function of the equilibrium concentration of the individual bile salt. In order to assess the binding efficiency of the cyclodextrin cavities in the polymer resin, we have taken data for the competitive binding of chenodeoxycholate by resin PCD3 from Figs. 4 and 5 and replotted both as a function of its individual concentration at equilibrium (Fig. 7A) and as a function of the total equilibrium concentration of all the bile salts (Fig. 7B). When the binding capacity approaches a plateau, as shown in the figure, 60–70% molar equivalent of the CD cavities are occupied by chenodeoxycholate and the competing cholate occupies another 10% of the cavities. It is possible that some of the cavities are not accessible for the binding because of the heavy crosslinking. These estimates indicate that as much as 80% of the cavities can be occupied when the equilibrium concentrations of bile salts are sufficiently high, which shows a reasonably good binding efficiency of the resin for bile salts.

It can be seen in Fig. 7(A) that the binding capacity of the resin for chenodeoxycholate is somewhat higher when fewer bile salt species are present. Figure 7(B) shows that the binding capacity for a given bile acid depends on the total equilibrium concentration of all the bile salts present in the system. This indicates that the competition among the guest molecules is also a process driven by entropy, at least to a certain extent. Similar observations can also be made with the binding of cholate.

We have observed that, in the competitive binding studies, curve fitting with Eq. (4) showed sometimes significant deviations for the experimental data points (Figs. 5–7). If we choose to use the total equilibrium concentration of all the bile salts present, however, we have found that the fitting is very good (Fig. 7B). Binding constants can be calculated for the isotherms in Fig. 7(B). In the competitive binding with cholate and glycocholate, $K_{CDCA} = 3.78 \times 10^2\,M^{-1}$, and in the competitive binding with cholate alone, $K_{CDCA} = 8.05 \times 10^2\,M^{-1}$.

In both the individual and the competitive binding studies of bile acids, the difference between chenodeoxycholate and cholates (including the conjugates) is quite clear. These results are consistent with the NMR titration studies [19]. Based

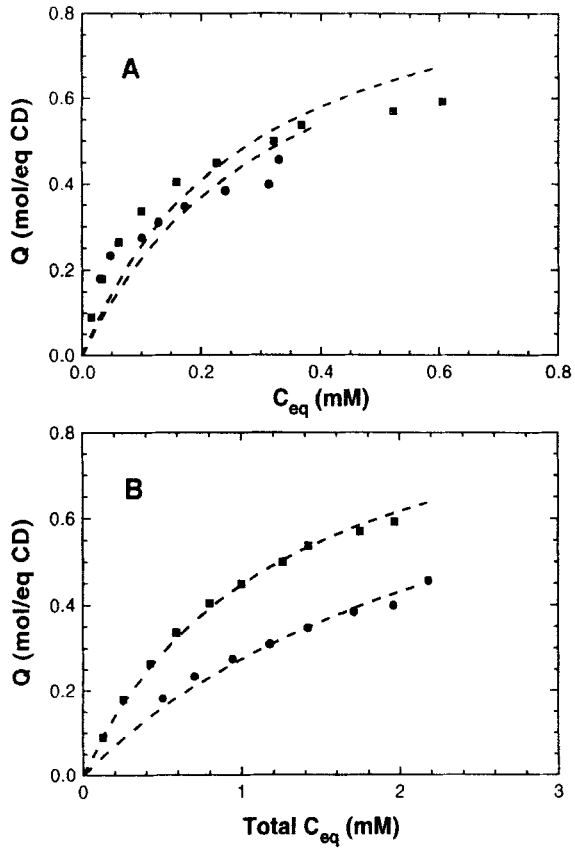


FIG. 7. Isotherms for the binding of chenodeoxycholate in phosphate buffer solutions at 25°C by PCD3 resin. The binding capacity for chenodeoxycholate is plotted (A) as a function of the equilibrium concentration of chenodeoxycholate, and (B) as a function of the total equilibrium concentration of all the bile salts present: ■, competitive binding data with cholate; ●, competitive binding data with cholate and glycocholate.

on these results, it is reasonable to expect the binding characteristics of the conjugates of chenodeoxycholate (such as glycochenodeoxycholate) would be similar to those of its nonconjugated form. Furthermore, it has been demonstrated in the separation of bile acids by reverse-phased HPLC that the addition of soluble CDs to the mobile phase [12–15] results in a marked dependence of the capacity factors on the presence of a 12-OH group on the steroid skeleton [14]. The results of this study agree well with that observation.

CONCLUDING REMARKS

We have shown that crosslinked cyclodextrins can be used effectively in the binding of bile salts. Inclusion complexes between the CD cavities and the guest molecules were formed during the binding. The degree of crosslinking must be

optimized since, when it becomes excessive, it can hinder the formation of such complexes, which decreases the binding capacity. In both the individual and competitive binding studies, chenodeoxycholate was more favored in the binding than cholate and its conjugates, which is an indication of the importance of the cross-sectional size of the guest molecules and their hydrophobicity. The size effect appeared to be more pronounced than that of hydrophobic interactions. The cavity size can be altered by the use of cyclodextrin of larger macrocyles (such as γ-CD). Furthermore, competitive bindings between bile acids and other molecular species (such as drugs) should be interesting for these resins. The introduction of positively-charged functional groups in such resins may further increase the binding affinity and capacity for bile acids. Such functionalization of cyclodextrins is already well documented in the literature [8].

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REFERENCES

[1] D. H. Blankenhorn, *Can. J. Cardiol., 5*, 206 (1989).
[2] G. Brown, J. J. Albers, L. D. Fisher, S. M. Schaefer, J.-T. Lin, C. Kaplan, X.-Q. Zhao, B. D. Bisson, V. F. Fitzpatrick, and H. T. Dodge, *New Engl. J. Med., 323*, 1289 (1990).
[3] J. Loscalzo, *Ibid., 323*, 1337 (1990).
[4] R. Kos, J. L. White, S. L. Hem, and M. T. Borin, *Pharm. Res., 8*, 238 (1991).
[5] T. Y. Ti, H. G. Giles, and E. M. Sellers, *CMA J., 119*, 607 (1978).
[6] C. Johansson, U. Adamsson, U. Stierner, and T. Lindsten, *Acta med. Scand., 204*, 509 (1978).
[7] S. M. Harmon and C. F. Seifert, *Ann. Intern. Med., 115*, 658 (1991).
[8] J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht, 1988.
[9] H. J. Issaq, J. H. McConnell, D. E. Weiss, D. G. Williams, and J. E. Saavedra, *J. Liq. Chromatogr., 9*, 1783 (1986).
[10] D. W. Armstrong, X. Yang, S. M. Han, and R. A. Menges, *Anal. Chem., 59*, 2594 (1987).
[11] J. Snopek, E. Smolkova-Keulemansova, I. Jelinek, J. Dohnal, J. Klinot, and E. Klinotova, *J. Chromatogr., 450*, 373 (1988).
[12] K. Shimada, T. Masue, K. Toyoda, M. Takani, and T. Nambara, *J. Liq. Chromatogr., 11*, 1475 (1988).
[13] K. Shimada, T. Oe, C. Kanno, and T. Nambara, *Anal. Sci., 4*, 377 (1988).
[14] K. Shimada, Y. Komine, and T. Oe, *J. Liq. Chromatogr., 12*, 491 (1989).

[15] K. Shimada, H. Yoshida, and Y. Komine, *Ibid., 14*, 605 (1991).
[16] M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, New York, NY, 1978.
[17] W. Saenger, *Angew. Chem. Int. Engl. Ed., 19*, 344 (1980).
[18] H. Bender, *Adv. Biotechnol. Processes, 6*, 31 (1986).
[19] Z. J. Tan, X. X. Zhu, and G. R. Brown, *Langmuir, 10*, 1034 (1994).
[20] J. Solms and R. H. Egli, *Helv. Chim. Acta, 48*, 1225 (1965).
[21] Institute of Fermentation Resesarch, *Polycyclodextrin Beads*, Japan Kokai, 83,171,404.
[22] W. Xu, J. N. Demas, B. A. Degraff, and M. Whaley, *J. Phys. Chem., 97*, 6546 (1993).
[23] X. X. Zhu, G. R. Brown, and L. E. St-Pierre, *J. Pharm. Sci., 81*, 65 (1992).
[24] X. X. Zhu and G. R. Brown, *Anal. Lett., 23*, 2011 (1990).

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