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PHOSPHONIUM-CONTAINING RESINS AS BILE ACID SORBENTS: IN-VITRO BINDING STUDIES

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ABSTRACT

Three new phosphonium-containing analogs of cholestyramine (Questran) resins have been synthesized: two styrene–divinylbenzene–vinyl benzyltrimethylphosphonium chloride resins, one (Resin I) of low and one (Resin II) of high ionic content, and a diphosphonium resin (Resin III) prepared by reacting 1,2-bis(dimethylphosphino)ethane with chloromethylated styrene–divinylbenzene. The effects of variation of ionic content of the resin, hence of swellability of the backbone, of added salt in the sorbate, i.e., of increased ionic strength of the buffer on the in-vitro binding of glycocholate (GC^-), were determined. Resin I, with low ionic content (<1 mmol Cl^-/g resin), was ineffective in binding GC^- from Tris-HCl buffer (2.5 mM, pH 7.0), but the extent of binding was markedly increased when the resin was pretreated with an organic solvent (e.g., DMF), thereby causing it to swell and facilitating the diffusion of GC^- into the resin. When expressed as moles of GC^-/pendant, the isotherms for the in-vitro binding of GC^- in aqueous buffer solution by the phosphonium analogs with a sufficiently high ionic content (Resins II and III) were similar to that for cholestyramine. Increasing the ionic strength of the sorbate decreased the GC^- binding capacities of the phosphonium resins, in keeping with an ion-exchange

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mechanism. Changing the bile salt to sodium cholate did not change the binding capacity of the phosphonium resin analog of cholestyramine.

INTRODUCTION

High blood cholesterol levels have been cited as the single most important contributing factor to heart disease, the leading cause of mortality of North American adults [1–4]. The cholesterol levels can be reduced by diet, by drugs such as lovastatin, a hydroxy-betamethylglutaryl coenzyme A reductase (HMG-CoA) inhibitor, by bile-salt-sequestering resins such as cholestyramine (Questran) and colestipol (Colestid), and more drastically by bypass surgery. It has been shown that a synergistic therapy results from a combination of reductase inhibitors with bile-salt-sequestering resins [5, 6].

Bile acids are the major end-product of cholesterol metabolism in the liver. They are stored in the gallbladder until they are required to assist in the digestion of fats in the small intestine. More than 99% of the bile acids are reabsorbed from the small intestine and recirculated back to the liver (enterohepatic circulation). Compensation is made for any loss of bile acids from this essentially constant pool by the oxidation of cholesterol in the liver [7–10]. This conversion to bile acids is the primary route of the consumption of plasma cholesterol.

The currently used bile-salt-sequestering resins are nonabsorbable and nontoxic ion-exchange resins. They interrupt the enterohepatic circulation by binding of the bile salts via the carboxylate anion, thus leading to an increased fecal elimination [11]. Cholestyramine, a styrene–divinylbenzene copolymer with trimethylbenzylammonium groups [11–14], has a high in-vitro binding capacity but it does not bind bile acid anions selectively [15–16]. Hence, effective lowering of cholesterol levels requires that large quantities be ingested daily (ca. 32 g); this can lead to adverse side effects and poor patient compliance.

It has been demonstrated that compared to the ammonium-containing analogs, the resins containing phosphonium functionalities have superior catalytic activity in polymer-supported phase-transfer catalysis. In general, the reactivities and basicities of phosphonium-containing compounds are several orders of magnitude greater than those of the corresponding ammonium analogs [17–19]. Previous reports from this laboratory have considered the bile acid binding characteristics of various amino-group-containing resins [15, 20, 21]. The purpose of this study was to determine the effects of this increased basicity on the in-vitro bile-salt-binding characteristics of phosphonium-containing analogs of cholestyramine. The binding studies include a determination of the effects of systematic changes in the ionic content of the resins, hence of the swellability of the backbone, of addition of salt to the buffer, i.e., of increased ionic strength.

EXPERIMENTAL

Materials

For the syntheses described below, two chloromethylated styrene–divinylbenzenes were used: CMS-DVB-1, 1.0 meq Cl^-/g resin (Sigma) and CMS-DVB-2, 4.2 meq Cl^-/g resin (Bio-Rad, Mississauga, Ontario). Both resins were 1% crosslinked

with bead size 200–400 mesh. They were dried in vacuo at room temperature and used without further purification.

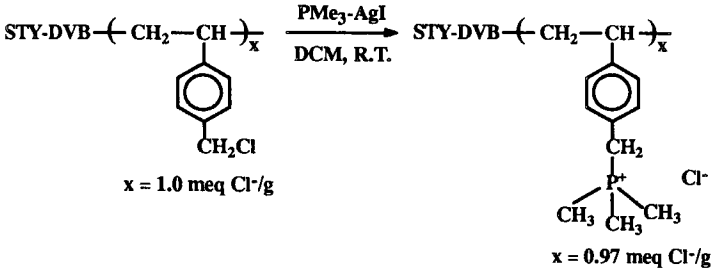
Dichloromethane (DCM, A&C) was distilled under nitrogen over P_2O_5 and stored under nitrogen at 6°C until use. Trimethylphosphine–AgI complex (0.25 g PMe_3/g complex, Aldrich), trimethyl phosphine (97%, Aldrich), 1,2-bis(dimethylphosphino)ethane (97%, Aldrich) methyl iodide (Aldrich), acetonitrile (Anachemia), methanol (Spectrograde, A&C), dimethylformamide (DMF, BDH), tetrahydrofuran (THF, A&C), and anhydrous diethyl ether (Fisher) were used without further purification.

Syntheses

The glassware was dried under nitrogen, and all transfers were carried out under a nitrogen atmosphere in a glove bag.

Resin I, Styrene–Divinylbenzene–Vinyl Benzyltrimethylphosphonium Chloride (low ionic content)

Dry DCM (25 mL) was added to dry CMS-DVB-1 (1.5 g) in a 100-mL 3-neck round bottom flask. The polymer–solvent mixture was connected to a vacuum line and degassed by freeze–thaw cycles (3×) and left under vacuum in liquid nitrogen. The PMe_3–AgI complex (5.29 g) was transferred to a 100-mL round bottom flask. A glass wool plug was placed in the neck of the flask to prevent loss by "bumping." The flask containing the complex was then connected to the vacuum line, and the PMe_3 was transferred to the flask containing the polymer–solvent mixture by heating the complex at 160°C for 1 hour. The polymer–solvent mixture was then allowed to warm to 0°C before it was removed from the vacuum line and stirred at room temperature. The reaction sequence is shown schematically in Scheme 1. After 6 days the solvent was removed in vacuo and more DCM was added. Over a period of several days the mixture was rinsed with DCM, methanol (3×), and finally with anhydrous diethyl ether (3×). The resin was then dried in vacuo at room temperature.



SCHEME 1.

Resin II, Styrene–Divinylbenzene–Vinyl Benzyltrimethylphosphonium Chloride (high ionic content)

To dry CMS-DVB-2 (7.9 g) in a 3-neck round bottom flask was added dry DCM (150 mL) along with a stirring bar. Trimethylphosphine (25 g) was cooled, transferred to the reaction flask, and stirred continuously for 8 days at room temperature. The reaction sequence is given in Scheme 2. The mixture was filtered, washed several times with DCM, water, methanol, and finally with anhydrous diethyl ether. Finally the resin was dried in vacuo at room temperature for several days.

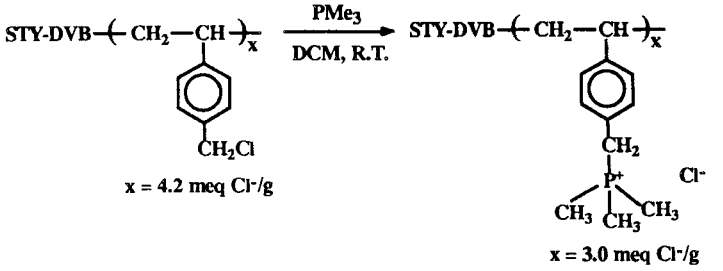
Resin III, Reaction of CMS-DVB-2 with 1,2-Bis(dimethylphosphino)ethane

To the dry CMS-DVB-2 (0.32 g) in a 5-mL round bottom flask was added 1,2-bis(dimethylphosphino)ethane (2 mL). The flask was placed into 2 Ziplock bags and shaken on a wrist-action mechanical shaker at room temperature for 43.5 hours. It was then transferred to a glove bag where the contents were filtered and washed several times with acetonitrile and anhydrous diethyl ether. Finally, it was dried on the funnel for 30 minutes.

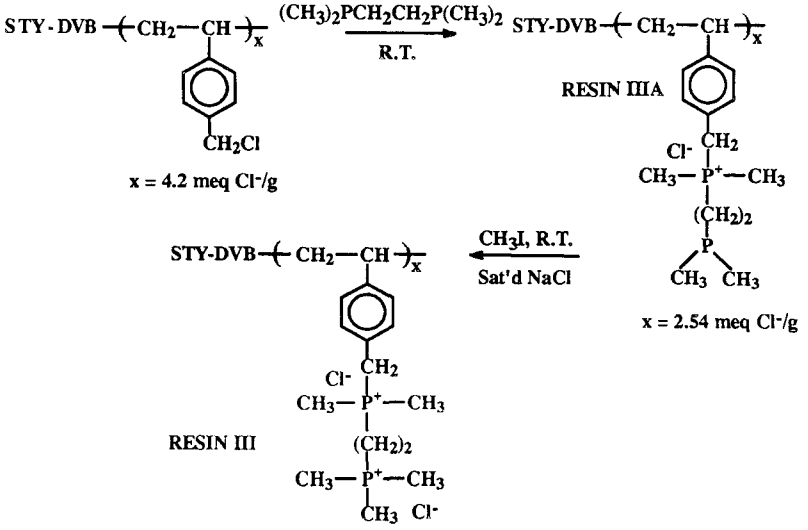
The resulting white powder (Resin IIIA, which is the monophosphonium-containing resin, see Scheme 3) was quaternized by the reaction with neat methyl iodide. The reaction mixture contained in a 5-mL round bottom flask was placed in 2 Ziplock bags and shaken in the absence of light for 12 days at room temperature. A yellow powder was collected and washed with methanol and anhydrous diethyl ether. It was then dried in vacuo at room temperature for several days. This resin was then shaken in saturated NaCl solution (ca. 4.5 M) to convert it to the chloride form. Washing was continued until no AgI precipitate was obtained upon addition of $AgNO_3$. Finally it was washed with water to remove excess chloride. It was then washed with methanol and finally with anhydrous diethyl ether. The yellow powder (Resin III, Scheme 3) was dried in vacuo at room temperature for several days.

Characterization

The FT-IR spectra obtained with an Analect AQS-18 spectrophotometer on KBr disks formed with powdered resin showed the disappearance of the peaks at 1265 and 820 cm^{-1} as a result of the reactions with phosphines. Since these peaks



SCHEME 2.



SCHEME 3.

are assigned to chloromethylated styrene groups [22], essentially complete reaction was obtained in all cases. The spectra for Resin I showed peaks at 975 and 940 cm^{-1} ($P-CH_3$ rocking modes) [22]. Similarly, new peaks at 965 ($P-CH_3$ rocking), 1299 ($P-CH_3$ symmetric deformation), and 1420 cm^{-1} ($P-CH_2$ deformations [18]) were obtained for Resin II. The IR spectra of Resins III and IIIA contained new peaks at 1451 and 1418 cm^{-1} (CH_2 rocking in $P-CH_2$), 1302 cm^{-1} ($P-CH_3$ symmetric deformation), and 962 cm^{-1} ($P-CH_3$ rocking).

The functionality of Resin I as determined in KNO_3 (0.010 M) by potentiometric titration with $AgNO_3$ (0.010 M) was 0.63 ± 0.04 mmol chloride/g resin (70% yield). The functionality of a DMF (1% yield v/w) swollen sample, determined by the same method, was 0.97 mmol chloride/g resin (ca. 100% yield). Similarly, the functionality of Resin II was determined to be 3.0 ± 0.1 mmol chloride/g resin (95% yield) and those of Resins IIIA (monophosphonium) and III (diphosphonium) were 2.54 (99.5% yield) and 4.05 mmol chloride/g resin (90% yield), respectively.

Sorption Experiments

The techniques used for the derivation of the binding isotherms were essentially as described previously [23]. The bile acid binding experiments were carried out using bile salts (sodium glycocholate or sodium cholate, Sigma) dissolved in Tris-HCl buffer (1.5–5.0 mM, pH 7.0), distilled deionized water, and aqueous NaCl (2.5–5.0 mM). Equal volumes (5 mL, Resin I; 20 mL, Resin I + DMF; 50 mL, Resins II and III) of known concentrations of bile salt (0.020–1.03 mM) were added to the dried resins (5–10 mg). The mixtures were shaken at room temperature (18–26°C) on a mechanical wrist-action shaker for 2 hours (24 hours for Resin I), a period of time determined from kinetic experiments to be sufficient for equilibration

to have occurred. The mixtures were filtered, and the filtrate was analyzed by reverse-phase HPLC, as described previously [24].

RESULTS AND DISCUSSION

Resin I, Low Ionic Content

Figure 1 presents isotherms that compare the binding of glycocholate (GC^-) from aqueous solutions of sodium glycocholate (NaGC) in Tris-HCl buffer (2.5 mM, pH 7.0) by Resin I with that of cholestyramine under similar conditions [16]. (The solid lines represent a visual best fit to the data.) Clearly, Resin I is a relatively ineffective sequestering agent for GC^-; it binds <0.1 mol of GC^-/mol ionic group on the resin at an equilibrium concentrations (C_{eq}) of NaGC of 0.2 mM. This corresponds to 16 $\pm$ 2 mg NaGC/g resin. This ineffective binding of GC^- is largely due to the failure of the aqueous solution to wet Resin I. This was verified by the addition of a small amount of organic solvent to the resin (0.1 mL THF or DMF to ca. 5–11 mg resin) prior to addition of the GC^- solution. This caused the resin to swell, and it remained in a swollen state even after addition of the aqueous GC^- solution. With the addition of DMF, the binding of GC^- by Resin I increased from 0.1 to ca. 0.8 mol NaGC/mol ionic group (corresponding to 1510 mg GC^-/g resin) at values of $C_{eq} \geq 0.2$ mM (Fig. 1). A similar addition of DMF to cholestyramine did not result in a significant change in the binding (Fig. 1).

The increase in binding capacity at "plateau" values of C_{eq} that accompanied the swelling of the resin with organic solvent depended somewhat on the volume of

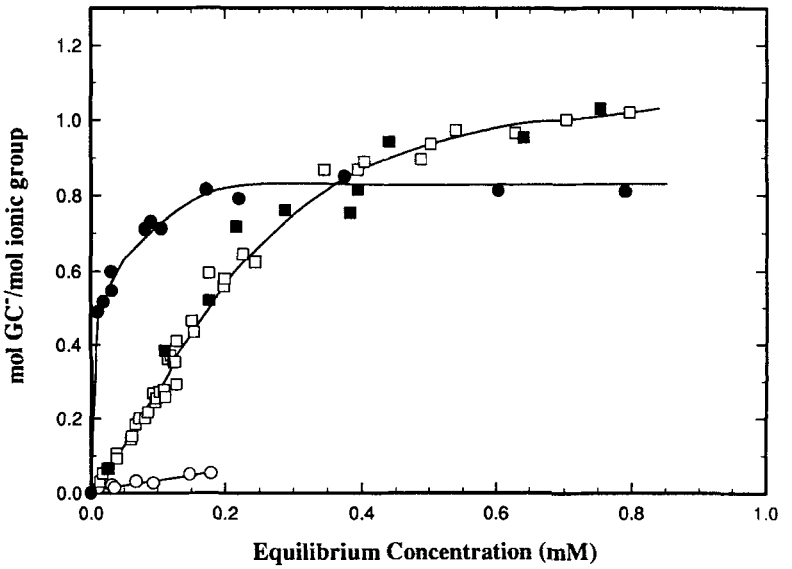


FIG. 1. Isotherms for the sorption of GC^- in 2.5 mM Tris buffer (pH 7.0) by Resin I (○), Resin I pretreated with DMF (●), cholestyramine (□) and cholestyramine pretreated with DMF (■).

solvent (DMF or THF) added (Fig. 2). For example, optimal binding for DMF occurred at a ratio of 0.01 mL/mg resin, so that this amount was used as the additive. In one set of experiments, DMF was added to Resin I and the resin was allowed to swell. When the solvent was removed from the resin, in vacuo, it did not bind any GC^-. Apparently the effect of DMF is to make additional sites accessible to GC^-. This was confirmed by the 35% increase in chloride content that was obtained when the functionality of the resin was determined *after* the addition of 0.01 mL DMF/mg resin. Thus, more sites were available for sorption, indicating that diffusion effects are important.

Effect of Ionic Content

For similar crosslinking densities, increasing the ionic group content should lead to a resin which is swellable in aqueous solution. This is reflected by the isotherm for the binding of GC^- in Tris-HCl (2.5 mM, pH 7.0) by Resin II (high ionic content) plotted in Fig. 3. On a per equivalent basis, a 3.4-fold increase in binding capacity was obtained by increasing the concentration of ionic groups 3-fold, from 0.97 mmol/g for Resin I to 3.0 mmol/g for Resin II. In general the isotherm for the binding of GC^- by Resin II is similar to that for the binding by cholestyramine (shown as the dashed line in Fig. 3) but at low values of C_{eq} the amount of GC^- bound by Resin II is somewhat greater than that of cholestyramine, in keeping with stronger binding. It is of note that the amount of GC^- bound by Resin II is also similar, on a weight basis (weight of NaGC/g resin), to that bound by Resin I with added DMF.

Since the binding of GC^- by these resins involves ion-exchange, increasing the ionic content further by attaching pendants containing diphosphonium rather than

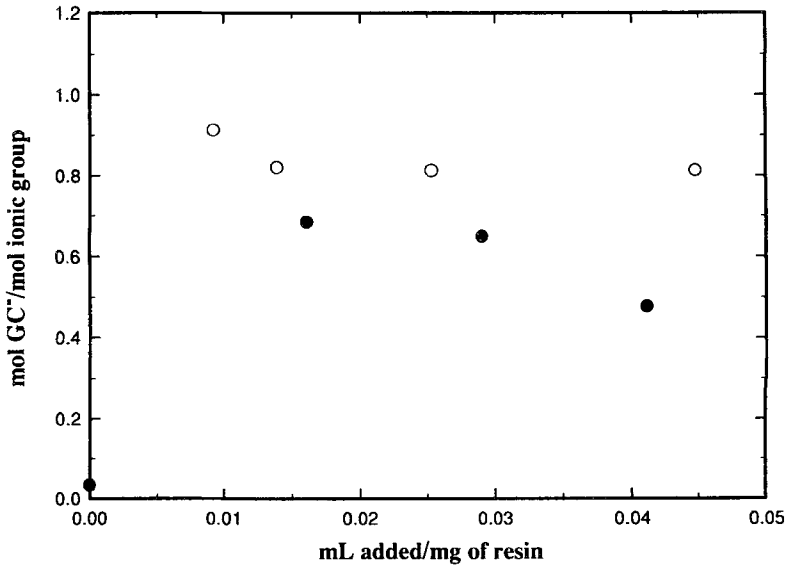


FIG. 2. Effect of added organic solvent on the sorption of GC^- in 2.5 mM Tris buffer (pH 7.0) by Resin I (○, DMF; ●, THF).

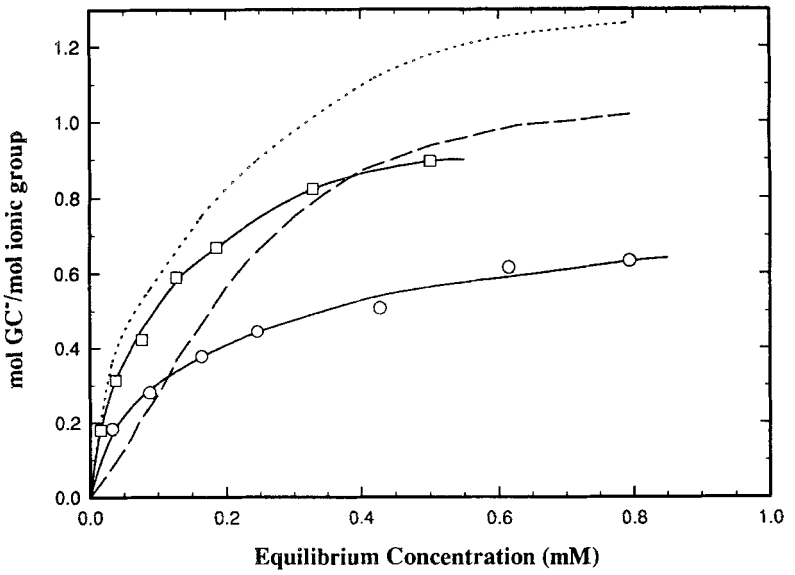


FIG. 3. Isotherms for the sorption of GC^- in 2.5 mM Tris buffer (pH 7.0) by Resin II (□) and Resin III (○). The dashed line shows the corresponding isotherm for the sorption by cholestyramine (taken from Fig. 1), and the dotted line represents the sorption by Resin III when expressed in terms of moles of GC^-/mol of *pendant*).

monophosphonium groups should double the binding capacity. As illustrated by the isotherms in Fig. 3, for Resin III with the diphosphonium-containing pendants the binding capacity *per ionic group*, i.e., per chloride, is ca. 50% that of Resin II which has the monophosphonium-containing pendants. At C_{eq} = 0.5 mM, Resin III binds ca. 0.5 mol GC^-/mol ionic group as compared to a binding of ca. 0.9 for Resin II. On a per pendant basis, i.e., when each pendant is considered as containing two quaternary phosphine groups (shown as the dotted line in Fig. 3), the molar binding capacity of Resin III is only slightly greater than that of Resin II. Thus, little advantage in the binding capacity is realized from doubling the phosphonium content per pendant. When considered on a weight basis, the binding isotherms of Resins II and III are similar, i.e., virtually no gain in binding capacity is realized by doubling the ionic content of the pendants. It may be that the longer pendants interfere in the binding of additional bile salts.

Effect of Increasing Ionic Strength

Since ionic interactions are important, increasing the ionic strength of the sorbate should reduce the binding capacity. This was tested in additional sorption studies with GC^- in Tris-HCl buffer (2.5 mM, pH 7.0) to which was added NaCl (2.5 mM). The addition of the sodium chloride reduced the binding capacities of Resin II (Fig. 4) and cholestyramine (Fig. 5) by ca. 0.1 mol GC^-/mol ionic group at "plateau" values of C_{eq}, i.e., >0.7 mM. Sorption with GC^- in 5.0 mM Tris-HCl gave similar binding capacities (Figs. 4 and 5), as might be expected if the chloride ion is the main competing species. The Tris ion does not appear to greatly affect

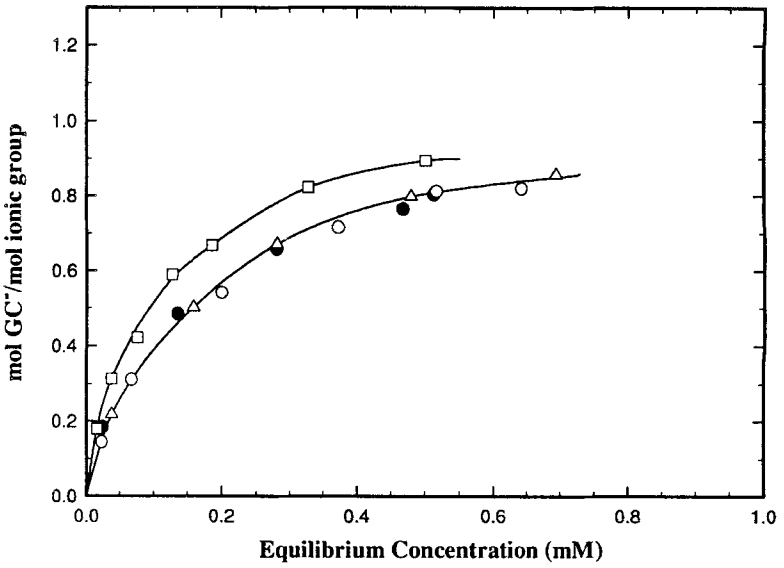


FIG. 4. Isotherms showing the effect of sorbate composition on the sorption of GC^- in 2.5 mM Tris buffer (pH 7.0) by Resin II. (□, 2.5 mM Tris; ○, 2.5 mM Tris and 2.5 mM NaCl; ●, 5.0 mM Tris; △, 5.0 mM NaCl).

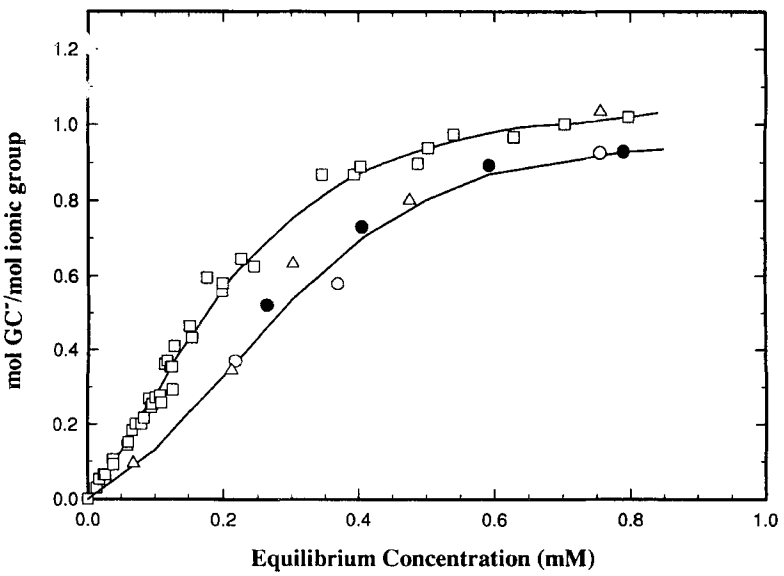


FIG. 5. Isotherms showing the effect of sorbate composition on the sorption of GC^- in 2.5 mM Tris buffer (pH 7.0) by cholestyramine. (□, 2.5 mM Tris; ○, 2.5 mM Tris and 2.5 mM NaCl; ●, 5.0 mM Tris; △, 5.0 mM NaCl).

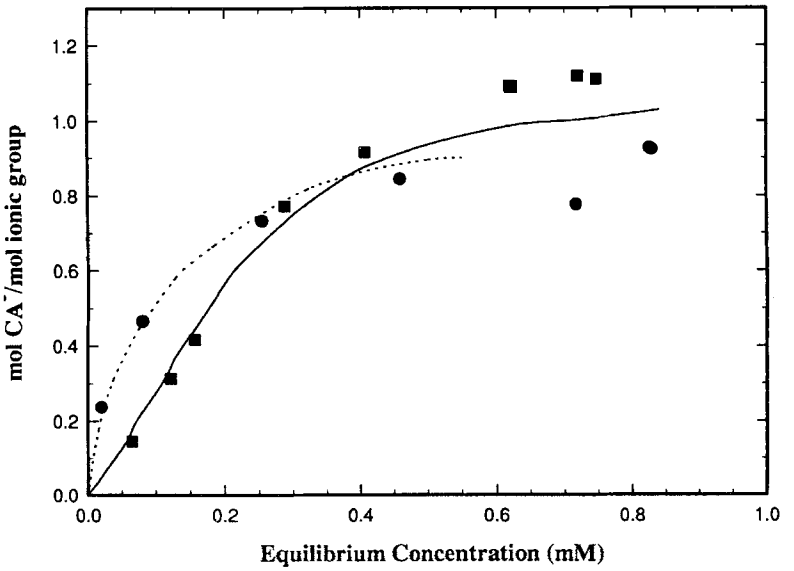


FIG. 6. Isotherms for the sorption of cholate in 2.5 mM Tris buffer (pH 7.0) by Resin II (●) and by cholestyramine (■). The solid line shows the corresponding isotherm for the sorption of GC^- by cholestyramine and the dotted line that for Resin II.

the binding capacities. This is supported by the fact that for both Resin II and cholestyramine the binding isotherms remained unchanged when the sorbate was changed to GC^- in 5.0 mM NaCl (Figs. 4 and 5). Although the binding by both resins is affected by the presence of chloride ions, as expected if ion-exchange is the principal mechanism of interaction, the sorption by cholestyramine is somewhat more sensitive to the presence of chloride ions, especially at low values of GC^-.

Effect of Changing the Bile Salt

It has been postulated that cholestyramine not only exhibits ion-exchange binding interactions but also demonstrates hydrophobic interactions [14]. However, as shown in Fig. 6, the binding of cholate ions from sodium cholate is similar to that of glycocholate which is somewhat more hydrophilic [25, 26]. Unlike cholestyramine, however, the binding capacities of Resin II for glycocholate and cholate are similar. Thus, hydrophobic interactions do not appear to be important under these conditions.

CONCLUSIONS

The in-vitro bile-salt-binding capacities of phosphonium-containing styrene-divinylbenzene analogs of cholestyramine are similar to those of cholestyramine. The interaction appears to involve ion-exchange. With increasing ionic strength of the medium there is a decrease in the bile-salt-binding capacities of the resins. The ionic content of the resins also plays an important role. Very low binding capacities

were obtained for Resin I with the low phosphonium ion content (0.63 mmol Cl^-/g resin). Increasing the ionic content of the resin to 3.0 mmol Cl^-/g resin resulted in a 3-fold increase in the binding capacity. A similar increase could be obtained by swelling Resin I prior to addition of the sorbate. However, a further doubling of the ionic content by attaching diphosphonium-containing pendants did not lead to any increase in binding capacity. Diffusion effects are, therefore, important.

Unlike cholestyramine, the phosphonium-containing resin, Resin II, did not show any difference in binding capacity when the bile salt was changed from sodium glycocholate to sodium cholate, a more hydrophobic bile salt. On the other hand, the bile-salt-binding capacity of Resin II was not as sensitive as cholestyramine to the presence of chloride ions. Further studies are required to determine if the phosphonium analogs of cholestyramine are more selective than cholestyramine in the small intestine.

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