Equilibrium and Kinetic Factors Influencing Bile Sequestrant Efficacy

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In vitro bile salt binding equilibria and kinetic studies were performed with cholestyramine to determine how these factors influence bile sequestrant efficacy *in vivo*. Chloride ion at physiologic concentrations caused more than a twofold reduction in glycocholate (GCH) binding, compared to binding in the absence of salt, over a range of GCH concentrations and was also observed to displace bound GCH. In addition, chloride ion displaced from cholestyramine as a result of bile salt binding was measured using a chloride selective electrode, and the results show that bile salt binding is due to ion exchange. Comparison of the results of the equilibrium binding experiments to human data shows that the effect of anion binding competition alone cannot account for the lack of efficacy of cholestyramine. Consideration of other effects, such as additional binding competition or poor availability for binding, based on data from the literature, shows that adequate bile salt binding potential exists and that these interferences are not major factors influencing resin efficacy. In kinetic studies, both binding uptake of GCH and displacement of GCH from cholestyramine by chloride ion were relatively rapid, indicating that cholestyramine should equilibrate rapidly with bile salts in the GI tract. Based on these findings, it is suggested that the low efficacy of cholestyramine is a result mainly of its relatively poor ability to prevent bile salt reabsorption in the ileum.

KEY WORDS: bile sequestrants; cholestyramine; bile salts; bile salt binding.

INTRODUCTION

Bile sequestrant resins are an important class of drug used in the management of elevated serum cholesterol levels. However, their effectiveness is often compromised because the large doses needed for therapy reduce patient compliance and give rise to side effects. The actual amount of bile salts sequestered *in vivo* (1) is much less than the theoretical capacity of the resins. The reasons for this discrepancy have not been fully established. Although introduced almost 30 years ago, there has been little progress toward developing more efficacious bile sequestrants.

Over the past several years, however, there has been renewed interest in bile sequestrants. This is due in part to recent findings in large-scale clinical studies that bile sequestrants (specifically cholestyramine) effectively lower cholesterol levels and reduce the risk of developing cardiovascular disease. For this reason and because of their proven safety, bile sequestrants have become the drug of first choice in the management of type II hyperlipoproteinemia (2). In addition to issues of noncompliance due to the large amount of drug that must be administered and side effects due to the large doses, there are economic factors related to the need for improving bile sequestrants. A recent study of cholestyramine in the prevention of coronary heart disease has shown that bile sequestrant therapy is not cost-effective for several segments of the hyperlipidemic population and that drug costs greatly influence estimates of the cost per year of life saved (3). Thus, any improvement in the efficacy or potency of sequestrants that lowers the dosage and consequently lowers the cost of therapy would increase the usefulness of sequestrants and have a significant impact on the hypercholesterolemic population.

Novel approaches used in improving sequestrants, including the synthesis of new resins or modification of existing ones (4,5), have not yet been commercially successful. Since *in vitro* studies are not necessarily predictive of *in vivo* activity (5,6), it is not clear which aspects of bile salt binding behavior *in vitro* are most relevant to *in vivo* activity. Past reports on bile sequestrants have served to characterize various details of bile salt binding behavior, but a clear understanding of why bile sequestrants are so inefficient *in vivo* is still lacking. The fundamental process that governs the pharmacologic action of bile sequestrants is the removal of bile salts from the enterohepatic cycle while within the gastrointestinal (GI) tract. In order to devise strategies for improving these resins, it is necessary to identify how sequestrants function and what factors influence their activity in the GI tract. The approach in this report is to develop a mechanistic understanding of bile sequestrant action by examining the physical–chemical aspects of *in vitro* bile salt binding to cholestyramine in relation to the physiology of the GI tract.

MATERIALS AND METHODS

Cholestyramine of pharmaceutical grade was obtained from Sigma Co. (St. Louis, MO) in powder form (100–200 mesh). Sodium glycocholate (GCH; 99% pure) was purchased from Sigma Co., sodium taurodeoxycholate (TDC; 95% pure) was obtained from Aldrich Chemical Co. (Milwaukee, WI), and both were dried under vacuum and stored in a desiccator and used without further purification. Sodium chloride and phosphoric acid were reagent grade. HPLC-grade methanol was used in the HPLC assay and the water employed in these experiments was distilled and deionized. The moisture content of the commercial resin was determined to be 10% and this was used as a correction factor for the mass of cholestyramine used in each sample.

Equilibrium Studies

Known amounts of resin (approximately 0.02 g) were weighed out into vials and 20 ml of a bile salt solution at a given concentration was added. The suspensions were agitated on a test tube rocker at room temperature for at least 24 hr. Aliquots were then removed and filtered through 0.22-μm filters (GSWP, Millipore, Milford, MA) and the filtrate was assayed for bile salts by HPLC as described below.

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Keeping the mass of resin small with respect to the volume of solution ensured that sorption of bile salts due to the embibment of the solution from the swelling of the resin was negligible. The experiments were done at room temperature since this variable does not have a large effect on ion-exchange equilibrium (7,8). Since cholestyramine is a quaternary amine and the pK_a of the bile acids was below 3.5, it was not necessary to control the pH of the solutions with buffers. In preliminary experiments the pH of the bile salt–cholestyramine suspensions was above 6.5, indicating that the bile salts were more than 99% ionized.

Bile salt binding in the presence of NaCl was determined in a similar manner, except that the bile salt solutions were made up in 0.1 and 0.025 *M* NaCl. Studies on the effect of concentration of NaCl on bile salt binding were conducted by first adding bile salt solution and equilibrating as above, then adding a 1-ml aliquot of a concentrated NaCl solution so that the resultant NaCl concentration ranged from 0 to 200 m*M*. These samples were then equilibrated for 24 hr, filtered, and assayed for bile salt.

Kinetic Studies

Kinetic studies of GCH adsorption to cholestyramine were conducted in the presence of 0.1 *M* NaCl at GCH concentrations of 2 and 10 m*M* GCH. Twenty milliliters of GCH solution was added directly to known amounts of resin and aliquots of the suspension were removed at appropriate time intervals, filtered, and assayed for GCH. Three individual samples were used for each time point and the vials were agitated for the duration of the experiment. Displacement kinetics were studied using a batch technique. Fifty milligrams of resin was hydrated in 10 ml of water for several hours; and then 35 ml of a GCH solution was added so that the total initial concentration of GCH (12 m*M*) was in the region where binding saturation occurred based on the equilibrium binding results. After 24 hr the suspension was centrifuged and the supernatant was sampled. The solution was decanted and the infranatant was mixed with 40 ml of water and centrifuged again. This procedure was repeated four more times in order to wash the excess GCH from the resin. Loss of a small fraction of the resin occurred during the decantation process due to ultrafine particles that remained suspended, but this amount was judged to be insignificant. Finally, the resin was transferred into a 400-ml polyethylene vessel with 250 ml of water, and 100 ml of a NaCl solution was added so that the final concentration of Cl^- was 0.1 *M*. A sample was taken prior to the addition of sodium chloride and was used as the zero-time point (no bile salt was detected). One-milliliter aliquots were removed at each time point, filtered, and analyzed for bile salt as described below. The suspension was constantly stirred at 50 rpm with a small paddle stirrer.

Chloride Exchange Experiments

The chloride released from the resin due to exchange with organic anions was measured using a chloride-selective electrode in an additional set of binding experiments. The resin used in these experiments was washed several times in water and methanol to remove impurities and dried under vacuum before use. Twenty milliliters of distilled deionized water was added to 20 mg of resin and the suspension was allowed to hydrate overnight. Aliquots of stock solutions of the binding anion (GCH, TDC) were then added and the suspensions were allowed to equilibrate for 24 hr with occasional agitation. Preparation for bile salt analysis was the same as in the equilibrium binding studies described previously. The apparatus for the chloride analysis consisted of a chloride selective electrode (Orion 94-17B), a double-junction reference electrode (Orion 90-02), and an ion/pH meter with a millivolt display (Orion EA 920), all available from Orion Research Inc., Cambridge, MA. A portion of the filtrate from each sample was analyzed in the presence of a fixed amount of ionic strength adjustor ($NaNO_3$). The ionic strength adjustor provides a sufficiently high and constant background ionic strength so that the chloride activity remains directly proportional to concentration. The amount of chloride in the unknown samples was calculated from calibration curves prepared from chloride standards. All chloride analyses were done at 30°C under gentle stirring. Chloride concentrations fell within the region of linear electrode response. In preliminary tests for interference in the chloride measurement due to the presence of bile salts, it was found that within certain bile salt concentration ranges at low Cl^- concentrations, falsely low potential readings resulted. Analysis of the results from the bile salt binding data indicated that the chloride measurements were in the range of bile salt and chloride concentrations where readings were true. Calculations for these experiments were based on the molecular weight of the anionic species and a chloride exchange capacity of 3.5 mEq/g for cholestyramine (9).

HPLC

Glycocholate and taurodeoxycholate were quantified by HPLC assay with a C_{18} column (HiBar, RP-18, 10 μm, 25 cm, EM Science, Cherry Hill, NJ) and a methanol/water mobile phase, 70:30 for GCH and 57.5:42.5 for TDC, adjusted to pH 2.0 with phosphoric acid. The absorbance was measured using a Spectroflow 783 variable-wavelength detector (Kratos Analytical Instruments, Ramsey, NJ) at a wavelength of 210 nm and the flow rate was 1 ml/min. The amount of organic anion bound to the resin was calculated by the difference between the initial amount added and the final amount in solution at equilibrium or at the sampling time. The amount of bile salt bound per gram of resin is based on the dry weight of the resin.

RESULTS AND DISCUSSION

The sodium salts of glycocholic acid and taurodeoxycholic acid were chosen for use in these experiments because they span the range of affinity for cholestyramine for conjugated bile salts (7) and GCH accounts for about 75% of the bile salt pool in cholestyramine-treated patients (10). TDC has binding properties and physicochemical properties similar to those of taurochenodeoxycholate (7,11) and differs from it only in the position of a hydroxyl group. While secondary bile acids are not found in the bile salt pool of cholestyramine-treated patients (10), TDC was used as a model dihydroxy conjugate with a high affinity for cholestyramine in lieu of the primary chenic species because of greater availability.

Bile Salt Binding, Chloride Competition, and Equilibria Considerations

The binding behavior of GCH to cholestyramine is shown in Fig. 1a. The initial concentrations of bile salts used in these experiments are within the range known to exist in the GI tract (12). Other bile salts show similar binding trends (7). At saturation, based on the data in Fig. 1a, cholestyramine can bind about 1.7 g of GCH per g of resin. An average daily excretion of 1.5 g of bile salts, which correlates well with the cholesterol lowering effect, is generally observed in patients taking cholestyramine (1). So ideally there is more than adequate binding potential in just 1 g of cholestyramine to result in the therapeutic action.

The shape of the binding curve in Fig. 1a resembles a typical Langmuir adsorption isotherm. The data in Fig. 1b were obtained in an experiment analogous to that in Fig. 1a, but in this case the amounts of both adsorbent and adsorbate were held constant and the system was diluted with water to adjust the concentration (in previous experiments a constant volume of bile salt solutions over a range of concentrations was added to a constant amount of resin). Based on a one-component Langmuir interpretation, one would expect the data in Fig. 1b to follow the same trend as in Fig. 1a. However, the binding is independent of concentration for the experiment shown in Fig. 1b because the amount of GCH and chloride initially present on the resin relative to the number of charged sites was held constant by using the same mass of bile salt and resin in each sample. This result demonstrates that bile salt binding is a mass action process and that, under certain conditions, a simple Langmuir adsorption model fails to describe the system adequately because it does not account for the effects of a competing species (13). For this reason and because analysis of the data in Fig. 1a showed that the binding potential of cholestyramine for GCH is very high, a Langmuir analysis has not been used here to interpret the binding data.

While in the GI tract resin will be exposed to various endogenous anions. Chloride ion is one of the predominant anions present in the GI tract and is ubiquitous throughout the length of the intestine (14,15). Figure 2a shows the results of binding studies similar to those in Fig. 1a but performed in the presence of 0.025 and 0.10 *M* sodium chloride. Chloride has a pronounced effect on both the magnitude of binding and the shape of the binding profiles. GCH binding in 0.10 *M* NaCl is only about one-third that in water at an equilibrium GCH concentration of 1 m*M* (see Fig. 1a). The effect of an additional anion on bile salt binding to sequestrants has usually not been studied over an extended range of bile salt concentrations. Figure 2b illustrates the effect of increasing amounts of NaCl on bile salt binding to cholestyramine from GCH solutions initially at a concentration of 3 m*M*, which represents a nominal bile salt concentration found in the intestine. The binding of GCH was reduced as

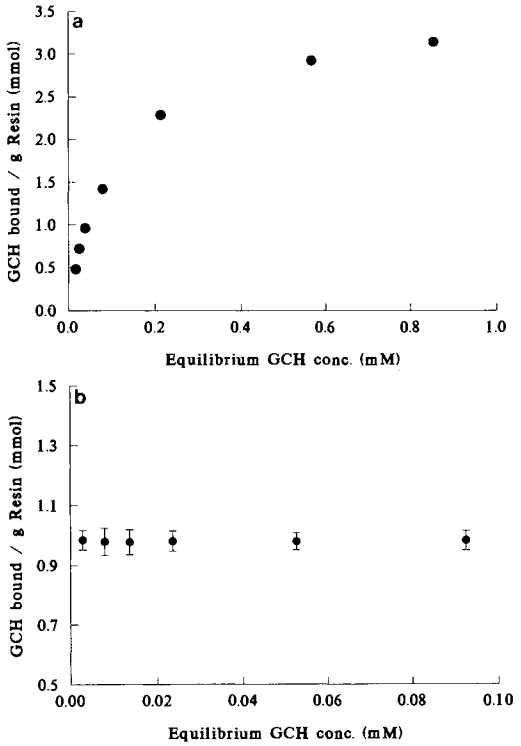

Fig. 1. (a) GCH binding to cholestyramine as a function of GCH concentration. Initial concentration range was 0.50–4.0 m*M*. SE is within the symbols and $n = 3$. (b) Experiments similar to that in a but performed by keeping the mass of GCH constant. Concentration was changed by dilution with water (see text). The initial concentration range was 0.2–4.0 m*M* GCH. Error bars correspond to SE and $n = 5$.

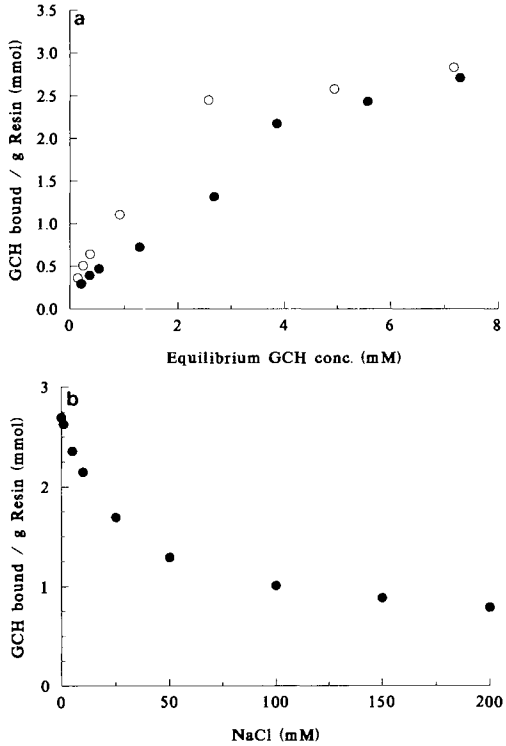

Fig. 2. (a) GCH binding to cholestyramine as a function of GCH concentration in the presence of a constant concentration of salt: (○) 0.025 *M* NaCl; (●) 0.10 *M* NaCl. Initial GCH concentration range was 0.5–10.0 m*M*. SE is within the symbols and $n = 5$. (b) GCH binding to cholestyramine from a 3 m*M* solution of GCH (initial concentration) as a function of added NaCl. SE is within the symbols and $n = 5$.

the concentration of chloride ion was increased. Binding values changed very little beyond 80 m*M* NaCl and there were no abrupt changes in binding as a function of NaCl in the physiologic range of 80–150 m*M*. The results in Fig. 2b also indicate that bile salt binding is reversible to a large extent since the chloride was added subsequent to equilibration of the resin with only bile salt. Thus, the difference in total binding between the no-added salt point and subsequent samples with added salt represents the amount of bile salt displaced by the added chloride. The finding that bile salt binding is reversible is contrary to the results of Johns and Bates (7). However, their test of reversibility involved dilution with water and did not evaluate the ion-exchange process. Reversibility is a fundamental property of ion exchange (8) and has been noted previously for bile salt–cholestyramine systems with the addition of fatty acids (16) and citrate ion (17).

It is important to determine whether the reduction in bile salt binding due to the presence of chloride is responsible for the observed binding inefficiency *in vivo*. In Table I the theoretical doses of resin needed to sequester a therapeutic amount of bile salt have been calculated based on the binding data from the previous experiments and nominal values for bile salt concentration. These figures are based on an average fecal excretion of bile salts of 1.5 g/day on a dose of 32 g/day of cholestyramine being necessary for cholesterol lowering therapy (1). While the binding conditions are somewhat idealized, these calculations show, assuming that all of the bound bile salts are excreted, that the bile salt binding capacity of the resin is much greater than the net *in vivo* binding based on fecal excretion data. When the effect of a competing anion is included, the calculated dose is one-tenth that normally required. Under fasting conditions when bile salt concentrations are very low, there is still adequate binding potential for the dose of cholestyramine to be reduced by two-thirds and still obtain a therapeutic effect. The calculations show that the actual dose of resin is approached only when 10% of the binding capacity, under conditions of nominal bile salt concentration and 0.1 *M* NaCl, is considered. Since other bile salts in the bile salt pool have a greater affinity for the resin than GCH, these values represent conservative estimates. Thus, based on an equilibrium binding assumption, the effect of Cl^- alone cannot account for the lack of *in vivo* activity of cholestyramine.

Table I. Dose of Cholestyramine Necessary for the Cholesterol Lowering Effect Calculated from Binding Data[a]

Conditions	g bile salt bound/ g resin	Dose required (g)
Clinical use	0.047	32.0
Binding at capacity in nominal bile salt concentration		
2 m*M* [eq][b]	1.75	0.86
2 m*M* [eq] in 0.1 *M* NaCl	0.50	3.0
Binding at bile salt concentrations found in fasting ileum		
0.2 m*M* [eq]	1.10	1.4
0.2 m*M* [eq] in 0.1 *M* NaCl	0.15	10.0
Binding at 10% maximum capacity at 2 m*M* [eq] in 0.1 *M* NaCl	0.05	30.0

[a] A dose of 32 g of cholestyramine will generally result in cholesterol lowering and fecal bile salt excretion of 1.5 g/day of bile salts (1). Data from the binding curves in Figs. 1a and 2a were used to calculate the amount of resin needed to bind 1 g of bile salt.

[b] Denotes equilibrium bile salt concentration.

Other Anions and Additional Binding Effects

Other anions present in the GI tract or in food have been shown to reduce bile salt binding to cholestyramine; however, the effect of these ions is generally not as great as the effect of Cl^-. Experiments done by Toda *et al.* with cholestyramine and HCO_3^- (5), similar to those presented in Fig. 2b with chloride ion, display the same trend with respect to bile salt binding as the bicarbonate concentration is increased. Since HCO_3^- affects bile salt binding in a manner similar to Cl^-, but not to as great an extent as Cl^- (7), the net effect of both ions can be approximated solely by chloride ion. Kos *et al.* recently investigated the reduction in binding of cholate to cholestyramine as a result of sequential exposure to simulated GI fluids, competition with citrate ion, and exposure to fruit juices as suspending fluids (17). Although cholate binding was reduced because of the various treatments, the binding capacity of the resin was not lowered to the extent seen with physiologic Cl^- (Fig. 2b).

Dietary fatty acids have also been shown to bind to cholestyramine in a manner similar to bile salts (18). Bile salt binding in the presence of sodium salts of fatty acids is reduced as a function of fatty acid concentration and increasing chain length, with maximum reduction of bile salt binding seen with fatty acids of chain length greater than C_{11} (16). However, the actual availability of fatty acid molecules for binding may be less than that encountered in experiments performed with salts of fatty acids *in vitro* due to solubility limitations, partitioning, and rapid absorption (19,20). Also, feeding of long-chain triglyceride vs medium-chain triglycerides along with cholestyramine resulted in no significant difference in overall bile salt excretion in rats or humans (21,22). Consequently, it is not likely that the effect of fatty acids *in vivo* is a major factor influencing resin efficacy.

Kuron *et al.* (23) have postulated that poor dispersion of the resin and interference with bile salt binding by intestinal contents are responsible for the poor efficacy of sequestrants. However, binding studies performed with aspirated intestinal contents and measurement of postprandial intestinal bile acid concentrations subsequent to resin administration show that substantial binding occurs and the bile salt concentration in the upper jejunum is reduced by about half (24). Also, the effect of a mucin coating on the resin (resulting from contact with the intestinal mucosa) has been investigated as a potential factor reducing bile salt binding to cholestyramine and determined to be insignificant based on the uptake profiles of model compounds to a cholestyramine analog of larger particle size (25). These various findings related to the conditions of the upper GI tract support the assumption that, on an equilibrium basis, the extent of bile salt binding in the upper small intestine is sufficient for a therapeutic effect despite the presence of binding competition and other nonspecific effects.

Adsorption and Exchange

Since GCH binding to cholestyramine was found to be reversible, it was necessary to examine further the nature of bile salt binding to cholestyramine and establish the mechanism of binding. Studies performed at low pH where bile salts exist as nonionized species have shown that significant binding occurs (26), and this provides evidence that hydrophobic interactions with the resin matrix may contribute substantially to overall binding. Adsorption of short-chain fatty acids to Dowex 1-X2 (chemical equivalent of cholestyramine) has been shown to involve superposition of molecular sorption on ion exchange (27) and irreversible binding of benzoate ion to Dowex 1-X4 (analogue to cholestyramine with slightly higher cross-linking) has been observed (28).

These findings indicate that nonelectrostatic interactions are potentially significant and suggest that there may be a component of the overall binding that is independent of ion exchange which could contribute to the *in vivo* activity of the resin. By comparing the amount of chloride exchanged to the total amount of bile salts bound, it is possible to discriminate between the stoichiometric ion-exchange binding and additional nonspecific adsorption. Figure 3 shows the total bile salt anion binding and chloride exchange for GCH and TDC, respectively. The amount of organic anion bound was equivalent to or slightly less than the amount of chloride exchanged for each bile salt. This establishes that ion exchange is the primary adsorptive component involved for these compounds binding to cholestyramine and supports previous results (29).

The small differences in chloride exchange and total bile salt anion binding are probably attributable to several factors. Because the pH of the TDC solutions is about 9, the small amount of hydroxide ion present competes for binding sites and reduces the organic anion binding relative to the total amount of chloride evolved from the resin. However, the amount of hydroxide ion in these solutions based on the pH is not sufficient to account for the total difference between the two measurements. Residual chloride on the resin not removed by washing and slight impurities in the organic anions would also have a similar influence on the results. A charge effect as a result of bile salt micelles formed in the resin particle from unbound bile salts may also be partly responsible for the additional displacement of chloride ion since the exchange difference tends to increase with increasing bile salt concentration and is more pronounced with the more polar TDC bile salt. More detailed experiments and sensitive techniques are needed to identify fully the cause of this discrepancy.

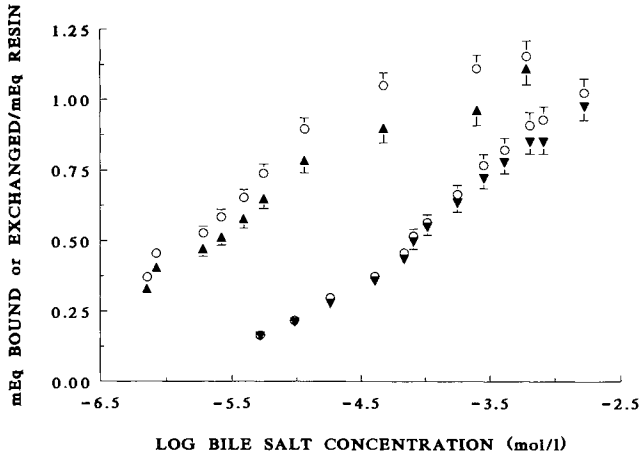

Fig. 3. GCH (▼) and TCH (▲) binding to and chloride exchange (○) from cholestyramine. Note that the X axis is on a log scale in order to separate out adjacent points and the amount bound or exchanged is on a per mEq resin basis. Error bars represent the standard deviation associated with each measurement method.

In Fig. 3 TDC binding is observed to slightly exceed the capacity of the resin and this phenomenon has also been noted previously (26,30) and attributed to hydrophobic interactions. However, since both TDC and Cl^- ions exceed the chloride exchange capacity of the resin, the additional TDC binding cannot be interpreted as nonexchange binding. This phenomenon may be related to increased capacity of the resin due to changes in swelling when binding different anions. While nonexchange binding is not present with respect to bile salts binding to cholestyramine (under the experimental conditions employed), nonelectrostatic interactions certainly play a significant role in influencing binding affinity and may be extremely important in retaining the bile salts on the resin while in the GI tract. The presence of a nonexchangeable binding component for benzoate on Dowex 1-X4 (28) is a strong indication that a similar phenomenon can occur with bile salts, considering their high degree of hydrophobicity.

Bile Salt Binding Kinetics

The enterohepatic system is extremely efficient and bile salts are almost completely reabsorbed in the ileum. Because of the finite residence time sequestrants have in the GI tract and the active reabsorption of bile salt, the time scale over which sequestrants can bind bile salts becomes an important factor to consider. Figure 4a shows the results for binding uptake studies with GCH, at initial concentrations of 2 and 10 mM, in the presence of 0.1 M NaCl. While the absolute amount of GCH bound in each treatment was different, binding rapidly approaches the equilibrium binding value, with over 90% of the binding occurring within 1 hr in both cases. Increasing the initial GCH concentration to 10 mM (in the presence of 0.1 M NaCl) did not greatly influence the apparent rate of uptake. While aspects of bile salt binding kinetics have been investigated previously (17,31), the results presented here give a much more definitive indication of the magnitude of binding relative to the solution conditions on the time scale of the GI transit time.

Since it was previously determined that bile salt binding was reversible, experiments were conducted on the kinetics of displacement of bound bile salts from cholestyramine by Cl^- to ascertain whether desorption might be a limiting kinetic factor influencing the efficacy of the cholestyramine. The results in Fig. 4b represent the consequence of a step change in bile salt concentration from an aqueous equilibrium concentration of 12 mM GCH to no free bile salt present in 0.1 M NaCl solution. Displacement of GCH by Cl^- occurs rapidly and 90% of the bound bile salts were released in 1 hr. While this is an extremely large perturbation

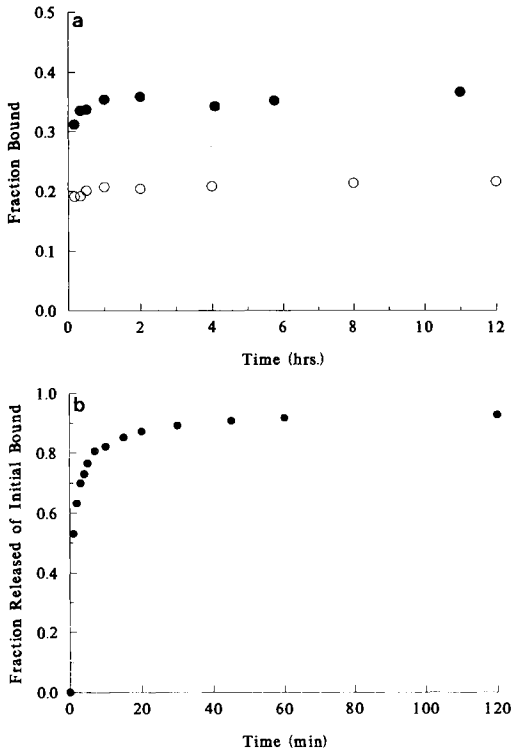

Fig. 4. (a) Binding kinetics of GCH to cholestyramine from 2 m*M* (○) and 10 m*M* GCH (●) solutions (initial concentration) in 0.1 *M* NaCl. The fraction bound is relative to the initial amount in solution. For 2 m*M* the initial amount of GCH was 4×10^{-5} mol, and 7.43×10^{-4} mol/g was bound at 48 hr. For 10 m*M* GCH the initial amount of GCH was 2×10^{-4} mol, and 1.98×10^{-3} mol/g was bound at 12 hr. SE is within the symbols and $n = 3$. (b) Displacement kinetics of GCH from cholestyramine by 0.1 *M* chloride ion. Initial amount of GCH bound was 1.51×10^{-4} mol, and 8.24×10^{-6} mol was bound at equilibrium.

from equilibrium, smaller changes would be expected to take place within the same time scale.

A rigorous treatment of the data in these kinetic studies was not undertaken because no significant concentration effect was observed and comparison of the time scale of binding ($t_{90\%} < 1$ hr) to the resin GI residence time ($t_{1/2} = 3$ hr) showed that a large amount of binding occurred rapidly. The results from the uptake studies demonstrate that cholestyramine can equilibrate rapidly with bile salts and bind near its capacity (at a given bile salt concentration) within 1 hr and show that the binding process in the upper region of the GI tract is not a limiting factor for resin efficacy. However, since displacement occurs rapidly as well, bound bile salts are labile to desorption by other anions. These two results suggest that, to a large extent, cholestyramine will maintain binding equilibrium with the bile salts in the surrounding solution in the small intestine. Consequently, when the bile salt concentration is reduced through normal active reabsorption, in the presence of a constant supply of endogenous anions, the resin will reequilibrate with the ambient solution, other anions will replace the bile salts on the resin, and the displaced bile salts will be reabsorbed. This phenomenon, the reabsorption of bile salts initially instilled to the gut as cholestyramine-bound bile salts in the presence of NaCl, has been observed qualitatively *in situ* in animals (32).

CONCLUSIONS

In this report we have investigated aspects of the equilibrium and kinetic binding behavior of bile salts to cholestyramine. It has been demonstrated that bile salts bind to cholestyramine primarily by means of an ion-exchange mechanism governed by the principles of mass action, and bile salts can be displaced by additional competing anions. Competing anions, such as chloride, substantially reduce the *in vitro* binding capacity, however, even in the presence of a strongly competing ion, the binding capacity of cholestyramine is still sufficient potentially to sequester more bile salts than the net amount of fecal excretion observed *in vivo*. The result suggests that for an ion-exchange resin sequestrant, the affinity of the resin for bile salts relative to endogenous anions is of greater importance than the capacity of the resin for bile salts. Results from kinetic studies indicate that resin in the GI tract will rapidly equilibrate with bile salts. The finding that bile salt binding is reversible in the presence of a competing anion suggests that while substantial binding may occur in the intestine where bile salt concentrations are high, bile salts are displaced from the resin by competing anions in the lower ileum as the concentration of bile salts decreases as a result of active reabsorption. Thus, the reabsorption of bile salts may be the primary factor influencing the efficacy of bile sequestrants.

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REFERENCES

1. T. A. Miettinen. Effects of neomycin alone and in combination with cholestyramine on serum cholesterol and fecal steroids in hypercholesterolemic subjects. *J. Clin. Invest.* **64**:1485–1493 (1979).
2. Lipid Research Clinics Program. The lipid research clinics coronary primary prevention trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA* **251**:365–374 (1984).
3. G. Oster and A. M. Epstein. Cost-effectiveness of antihyperlipemic therapy in the prevention of coronary heart disease. *JAMA* **258**:2381–2387 (1987).
4. R. De Simone, F. Conti, M. R. Lovati, M. Sirtori, E. Cocuzza, and C. R. Sirtori. New microporous cholestyramine analog for treatment of hypercholesterolemia. *J. Pharm. Sci.* **67**:1695–1698 (1978).
5. H. Toda, K. Kihara, M. Hashimoto, and S. Mizogami. Bile acid binding and hypocholesterolemic activity of a new anion exchange resin from 2-methylimidizole and epichlorohydrin. *J. Pharm. Sci.* **77**:531–533 (1988).
6. W. T. Beher, G. J. Lin, K. K. Casazza, and J. Bertasius. Effects of anion-exchange polymers on bile acid metabolism in the rat. *Atherosclerosis* **16**:169–174 (1972).
7. W. H. Johns and T. R. Bates. Quantification of the binding tendencies of cholestyramine. I. Effect of structure and added elec-

trolyte on the binding of unconjugated and conjugated bile-salt anions. *J. Pharm. Sci.* **58**:179–183 (1969).
8. F. Helferrich. *Ion Exchange,* McGraw–Hill, New York, 1962.
9. S. Buadvari (ed.). *The Merck Index,* 11th ed., Merck & Co., Rahway, NJ, 1989, p. 342.
10. J. T. Garbutt and T. J. Kenney. Effect of cholestyramine on bile acid metabolism in normal man. *J. Clin. Invest.* **51**:2781–2789 (1972).
11. M. C. Carey, M. J. Armstrong, N. A. Mazer, H. Igimi, and G. Salvioli. Measurement of the hydrophillic-hydrophobic balance of bile salts: Correlation with physical-chemical interactions between membrane lipids and bile salt micelles. In G. Paumgartner, A. Stiehl, and W. Gerok (eds.), *Bile Acids and Cholesterol in Health and Disease,* MTP Press, Lancaster, PA, 1983, pp. 31–42.
12. T. C. Northfield and I. McColl. Postprandial concentrations of free and conjugated bile acids from the length of the normal human small intestine. *Gut* **14**:513–518 (1973).
13. G. E. Boyd, J. Schubert, and A. W. Adamson. The exchange adsorption of ions from aqueous solutions by organic zeolites. I. Ion-exchange equilibria. *J. Am. Chem. Soc.* **69**:2818–2829 (1947).
14. J. S. Fordtran and T. W. Locklear. Ionic constituents and osmolality of gastric and small-intestinal fluids after eating. *Am. J. Digest. Dis.* **11**:503–521 (1966).
15. C. Lentner (ed.). *Geigy Scientific Tables,* Ciba-Geigy Limited, Basle, 1981, p. 147.
16. L. M. Hagerman, D. A. Julow, and D. L. Schneider. In vitro binding of mixed micellar solutions of fatty acids and bile salts by cholestyramine. *Proc. Soc. Exp. Biol. Med.* **143**:89–92 (1973).
17. R. Kos, J. L. White, S. L. Hem, and M. T. Borin. Effect of competing anions on binding of bile salts by cholestyramine. *Pharm. Res.* **8**:238–241 (1991).
18. W. H. Johns and T. R. Bates. Quantification of the binding tendencies of cholestyramine. II. Mechanism of the interaction with bile salt and fatty acid salt anions. *J. Pharm. Sci.* **59**:329–333 (1970).
19. A. F. Hofmann and B. Borgstrom. Physico-chemical state of lipids in intestinal content during their digestion and absorption. *Fed. Proc.* **21**:43–50 (1962).
20. A. F. Hofmann and B. Borgstrom. The intraluminal phase of fat digestion in man: The lipid content of the micellar and oil phases of intestinal contents. *J. Clin. Invest.* **43**:247–257 (1964).
21. L. M. Hagerman and D. L. Schneider. Effect of cholestyramine on fecal bile salt excretion in rats fed diets containing medium-chain triglycerides or corn oil. *Proc. Soc. Exp. Biol. Med.* **143**:93–96 (1973).
22. R. B. Zurier, S. A. Hashim, and T. B. Van Itallie. Effect of medium chain triglycerides on cholestyramine-induced steatorrhea in man. *Gastroenterology* **49**:490–495 (1965).
23. G. W. Kuron, N. Grier, and J. W. Huff. The bile acid binding and hypercholesterolemic action of two water-soluble polymers. *Atherosclerosis* **37**:353–360 (1980).
24. T. A. Miettinen. Mechanism of nonabsorbable lipid-lowering drugs. In *Proc. Sixth Int. Congr. Pharmacol, Vol. 4,* Finn. Pharmacol. Soc., Helsinki, 1975, pp. 149–158.
25. P. E. Luner. Ph.D. dissertation, University of Michigan, Ann Arbor, 1990, pp. 142–146.
26. S. Lindenbaum and T. Higuchi. Binding of bile acids to cholestyramine at gastric pH conditions. *J. Pharm. Sci.* **64**:1887–1889 (1975).
27. G. L. Starobinets and I. F. Gleim. Ion exchange of weak organic electrolytes. *Russ. J. Phys. Chem.* **39**:1166–1169 (1965).
28. Y. Farag and J. G. Nairn. Rate of release of organic carboxylic acids from ion-exchange resins. *J. Pharm. Sci.* **77**:872–875 (1988).
29. J. Blanchard and J. G. Nairn. The binding of cholate and glycocholate anions by anion-exchanging resins. *J. Phys. Chem.* **72**:1204–1208 (1968).
30. S.-D. Clas. Cellulose ionomers as cholesterol-lowering drugs. *Polym. Preprints* **29**:438–439 (1988).
31. W. H. Johns and T. R. Bates. Quantification of the binding tendencies of cholestyramine. III. Rates of adsorption of conjugated bile salt anions onto cholestyramine as a function of added inorganic electrolyte concentration, temperature, and agitation intensity. *J. Pharm. Sci.* **59**:788–793 (1970).
32. A. Billiau and J. Van den Bosch. The influence of cholestyramine on the intestinal absorption of glycocholic acid. *Arch. Int. Pharmacodyn.* **150**:46–51 (1964).