Kinetics of Exchange of a Resin-Bound Bile Acid by Chloride Ion under Mild Flow Conditions[†,‡]

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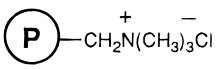
Received May 15, 1998

Revised Manuscript Received June 16, 1998

Introduction

Strongly basic anion-exchange resins are currently used as therapeutic agents for the treatment of hypercholesterolemia.[1] Thus, when polymers such as **1** are ingested and excreted, bile acids that become ionically bound to them are removed from the enterohepatic circulation. This loss is then sensed, the biosynthesis of bile acids from cholesterol in the liver is upregulated, and the increased consumption of cholesterol manifests itself as a therapeutically useful decrease in serum cholesterol. Although the physiology and biochemistry that connect biliary concentrations of bile acids and serum concentrations of cholesterol in vivo are reasonably well understood, the details of the ion-exchange processes leading to excretion are not presently clear.



1, cross-linked polystyrene (2% divinylbenzene) 90% ring substitution

A common characteristic of anion-exchange resins used to sequester bile acids is their extraordinarily low efficacy (i.e., only ca. 2% of the exchangeable sites on an excreted resin remain populated with bile acids). The source of this low efficacy is a point of contention but is probably due to a competition between the rates of three processes that occur in the ileum, the portion of the lower small intestine where bile acids are removed from the lumen by an active transport mechanism and recycled back to the liver. The first process is the active transport of bile acids from the lumen through the wall of the ileum; the second is the desorption of bile acids off of the anion-exchange resin into the lumen; and the third is the passage of the resin through the ileum, the region of the active transport. Such a model, in fact, readily accounts for the modest progress that has been made in the design of ionic sequesterants; e.g., resin **1**, which was introduced almost 40 years ago for the lowering of cholesterol in serum, remains as a polymer of choice.[2] Although the adsorption of bile acids onto ion-exchange resins under equilibrium conditions has received considerable attention, to the best of our knowledge, no published reports have yet appeared concerning the kinetics of desorption. However, it is exactly the rate of desorption that appears to have a major influence of the efficacy of an anion-exchange resin as a therapeutic agent.

In this paper, we report the results of a study that was aimed at defining the kinetics of exchange of resin-bound taurocholate by chloride ion. A primary goal of this work was to identify the rate-determining step for this process under physiologically relevant flow conditions, i.e., *mild* agitation. Thus, we sought to clarify whether the rate of bile acid release is governed by diffusion of ions through a thin quiet liquid layer surrounding the polymer beads (film diffusion) or by the diffusion of ions within the polymer (particle diffusion).[3] In principle, such fundamental insight could help to guide the rational design of improved resins for the sequestration of bile acids. Although mild agitation is known to favor film diffusion, the possibility exists that bile acids may exhibit unusually slow particle diffusion due to their facial amphiphilicity and/or their size. Specifically, strong nearest-neighbor association via hydrogen bonding and van der Waals forces could significantly retard their diffusion throughout the gel by imparting a "stickiness" between them (Scheme 1).[4−6] In addition, large bile acid anions might be expected to diffuse slowly within the resin, especially at high loadings where there is considerable steric congestion within the polymer.

Experimental Section

General Methods. Taurocholic acid sodium salt hydrate was purchased from Aldrich Chemical Co. and used directly. A commercial anion-exchange resin [**1**, Amberlite IRA 400 (Cl), 20−50 mesh] was purchased from Aldrich Chemical Co. House-deionized water was purified using a Millipore Milli-Q-filtering system containing one carbon and two ion-exchange stages. All UV measurements were made using a Milton Roy Spectronic 1201 spectrometer.

Resin-Bound Taurocholate. Prior to loading taurocholate onto the Amberlite IRA 400 resin, a regeneration procedure was employed in order to remove impurities. Specifically,

[†] L.Z., experimental design, preliminary studies, resin preparation, and Figure 4; V.J. experimental design; E.R.S., concepts; S.L.R., concepts, experimental design, and wrote paper.

[‡] The following members of the Chemistry 58 Class of 1998, who are coauthors of this paper, obtained all of the data in Figures 1−3: Deborah A. Althoff, Robert W. Amareld, Christopher T. Bakker, Tara S. Baney, Scott J. Bollinger, Amy E. Bridgeman, Daniel S. Brockman, Andrew P. Butler, Tarik M. Clarke, Paul A. Cooper, Martha B. Corrozi, Patricia Crowley, James K. T. Cullen, Diane A. Daniels, Keith E. Dombrowski, Jenniefer, M. Dunmire, Robert A. Engleman, Danielle M. Frikker, Ian M. Ftaiha, Francesca L. Garcia, Gorka Garcia-Malene, Rachel A. Garrison, David R. Gevry, Kathryn Grigg, Jennine M. Hannaway, Matthew E. Haynes, Troy F. Hendry, Ryan D. Horst, Meredith S. Hughes, Rebekah Hunter, Chad E. Jarrah, Anita V. Ketty, Kris Kotsay, Andrew D. Kraft, Kathryn M. Kryzanek, Kristy D. Kulick, R. Quinn Kurtz, Matthew P. Lawson, Paul S. Lockner, Alison B. Loupos, Maciej Ludwinski, Kristin M. Margiotto, Fred M. Mason, Lynn, M. McCourt, Kristen J. McKenna, Angela M. Mendel, Kinshasa C. Morton, Alison M. Newman, John R. Nicholson, Goddony Normil, Eugenie M. O'Connor, Sara Pickens, Kevin A. Pinkos, Michael K. Price, Michal Lee Radovici, Jaclyn J. Ramirez, Richie Rana, Mandira Ray, Jodi L. Renner, Daniel S. Rhoads, Sharon A. Romano, Tara A. Santoroski, Jennifer L. Scott, Dana M. Sepe, John W. Shabaker, Ednan S. Sheikh, Alan W. Slowick, Eunbyul Sou, Matthew Stannard, Kristen J. Stead, Eunbyul Sou, Danielle M. Teagno, Janine R. Trindade, Scott A. Troutman, Vicki A. Uremovich, Erica Vanzandt, Tanya Vega, Gregory A. Vincent, Christina S. Vitt, Veronica M. Vollmer, Amy S. Wall, Melissa A. Weintraub, Hiroki Yanagisawa, and Lusia S-S. Yi.

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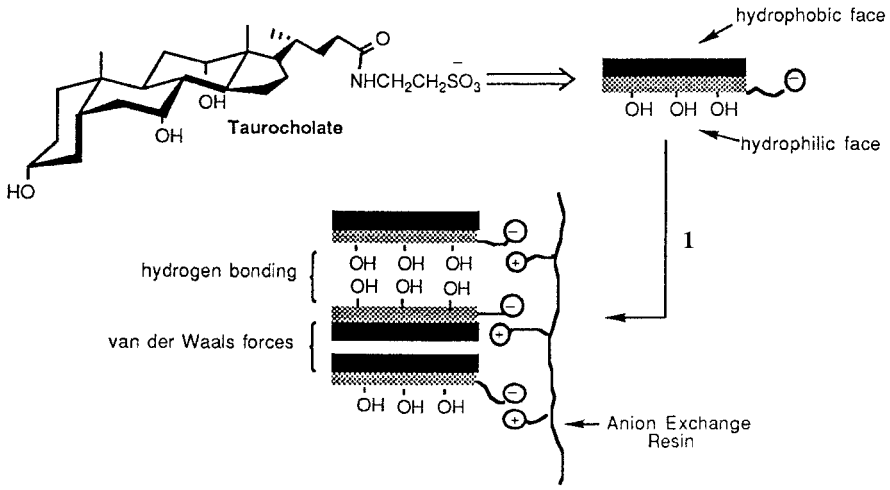
S0024-9297(98)00781-5 CCC: $15.00
Published on Web 07/22/1998

Scheme 1



the resin was rinsed with an aqueous NaCl solution (64 g NaCl/L) in a column that was maintained at 49 °C, until the eluent showed no significant change in absorbance at 205 nm. The resin was then rinsed thoroughly with deionized water. The complete removal of excess chloride ions was judged by UV measurement and by use of a $AgNO_3/HNO_3$ test; i.e., no significant absorbance could be observed at 205 nm, and no chloride ion could be detected in the eluent. The resin was then partially dried under reduced pressure in order to simplify weighing. The total exchange capacity of the resin (ca. 2.8 mmol of chloride/g of swelled resin) was then determined by the modified Volhard method.[7] In a typical preparation of a taurocholate-loaded resin, 1 g of purified Amberlite IRA 400 was added to 400 mL of a sodium taurocholate solution; the concentrations of taurocholate that were used ranged between 7×10^{-4} and 21×10^{-4} M, which determined the extent of loading. Each of these ion-exchange steps was carried out over 72 h at room temperature. The concentration of sodium taurocholate that remained in solution was determined by UV (205 nm), based on a calibration curve. The amount of resin-bound taurocholate was then calculated by subtracting the amount of residual sodium taurocholate in solution from the initial quantity. The taurocholate-loaded resin was then separated from the free taurocholate in solution by filtration, and the resin was then rinsed with deionized water until no significant absorbance could be detected at 205 nm. The resin was collected, quantitatively, by filtration, partially dried (to 27% moisture), weighed, and stored in a sealed bottle for subsequent ion-exchange kinetic experiments.

Kinetics of Ion Exchange. Rates of ion exchange were determined by use of a batch technique. Typically, 0.100 g of water-swollen resin was introduced into a 125-mL polyethylene bottle. After adding 100 mL of an aqueous solution of NaCl, the suspension was immediately subjected to gentle agitation by means of "hand-rocking". Five 1-mL aliquots were then withdrawn over a period of 90 min and analyzed directly by UV (205 nm) using appropriate calibration curves. All experiments were carried out at 22 ± 1 °C. The fraction of ammonium groups having taurocholate as a counterion (Θ) was determined as function of time by use of the following equation: $\Theta = \Theta_o - CV/WF$, where Θ_o is the fraction of ammonium groups having taurocholate as the counterion at the start of the exchange, C is the concentration of taurocholate that has been released into the solution, V is the volume of aqueous solution, W is the initial weight of the polymer, and F is the initial moles of ammonium groups per gram of polymer. Initial rates were estimated by fitting the rate data (first 30−40 min) according to the following equation: $\Theta = ae^{-bt} + c$; here ab is taken as the initial rate of ion exchange. Each of the kinetic curves included in Figures 1 and 2 was based on data that were obtained from at least five independent researchers. Thus, variations in rate data due to variations in "hand rocking" were negligible.

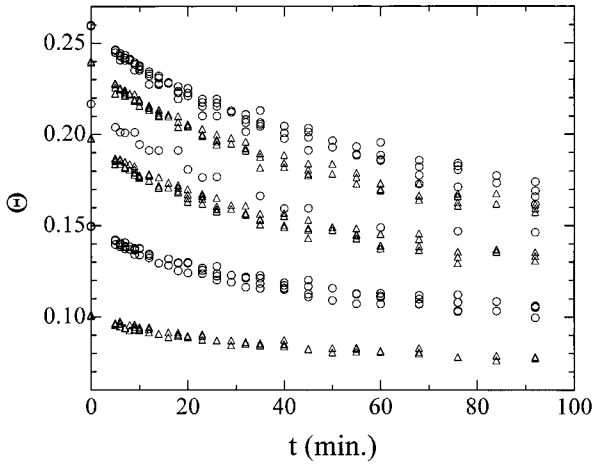


Figure 1. Plot of fraction of pendant ammonium groups that contain taurocholate (Θ) as a function of time in the presence of 50 mM NaCl. Initial loadings of taurocholate (Θ_o) were 0.10, 0.15, 0.20, 0.22, 0.24, and 0.26, respectively.

"Interruption" Test. The same procedure was followed as in Kinetics of Ion Exchange (see above), except that the experiment was interrupted after 10 min by separating the resin from solution using filtration. After a 40-min delay, the resin was returned to the NaCl solution (containing partially released taurocholate), and the kinetics of the exchange was further monitored as a function of time. In this experiment, the initial loading and NaCl concentration used were $\Theta_o = 0.22$ and 50 mM, respectively.

Results and Discussion

In this study, we have investigated the exchange of resin-bound taurocholate by chloride ion. Taurocholate was specifically chosen because of its strong acidity ($pK_a = 2$), which ensures nearly complete ionization in pure water in the absence of buffer. In addition, the strong UV absorption of sodium taurocholate at 205 nm allows for a convenient quantitative analysis in water.

Using procedures that are described in the Experimental Section, a series of resins were prepared from **1** having various loadings of taurocholate. Ion exchange was then carried out in the presence of 50 mM NaCl using gentle agitation. Analysis of the fraction of taurocholate that was liberated in solution as a funtion of time afforded release profiles that are shown in Figure 1. Here, Θ represents the fraction of ammonium groups having taurocholate as a counterion. A similar

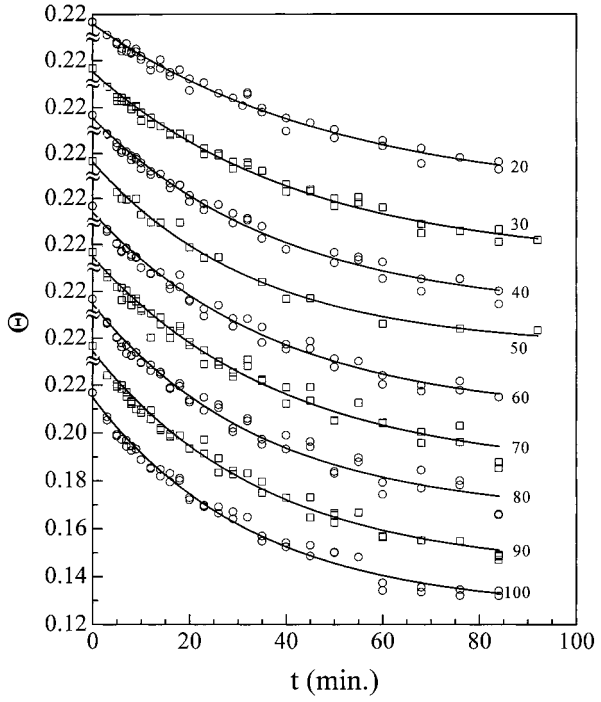


Figure 2. Plot of fraction of pendant ammonium groups that contain taurocholate (Θ) as a function of time. Numbers that are given represent the concentration of NaCl that was used (mM). The initital loading in all cases was $\Theta_o = 0.22$.

series of experiments was carried out in which the loading of resin-bound taurocholate (Θ_o) was held constant and the NaCl concentration varied (Figure 2). Plots of the initial rate of ion exchange, $(-d\Theta/dt)_o$, as a function of the loading of taurocholate and the NaCl concentration are shown in Figure 3A,B, respectively. On the basis of these data, the rate of release of taurocholate was found to obey eq 1.

$$(-d\Theta/dt)_o = k(\Theta_o)^{1.2}[NaCl]^{0.4} \quad (1)$$

To establish whether film diffusion or particle diffusion is rate-limiting for the ion-exchange process, we have carried out an "interruption test".[8] As noted by Helfferich, the interruption test is the best technique for distinguishing between particle and film diffusion control; other methods, which include the dependence of the observed rate on particle size, solution concentration, and degree of agitation, are known to be less reliable.[9] In the case of particle diffusion control, an interruption in the exchange by removal of the resin from solution provides time for concentration gradients within the beads to level out. Reimmersion then leads to an ion exchange rate that is greater than that observed prior to the interruption. With film diffusion control, no concentration gradients in the beads exist and the interruption has no effect on the rate. As can be seen in Figure 4, interruption has no effect on the rate of exchange of resin-bound taurocholate by chloride ion. Thus, these results provide compelling evidence for rate-limiting film diffusion.

To the extent that the desorption rates of sequestered bile acids from anion-exchange resins influence their efficacy in vivo, the results reported herein offer encouragement that improved resins should be possible by rational design. Specifically, the goal then becomes one of either slowing down film diffusion further (e.g., by changing the macroscopic shape and surface morphology of the resin particle in order to increase the thickness of the laminar boundary layer) or converting the rate-limiting step to particle diffusion (e.g., by increasing the cross-link density of the polymer).[10] In principle, either approach should help to retain more bile acids on the resin that are ultimately destined for excretion, and thus lead the way to improved therapeutic agents for the treatment of hypercholesterolemia.

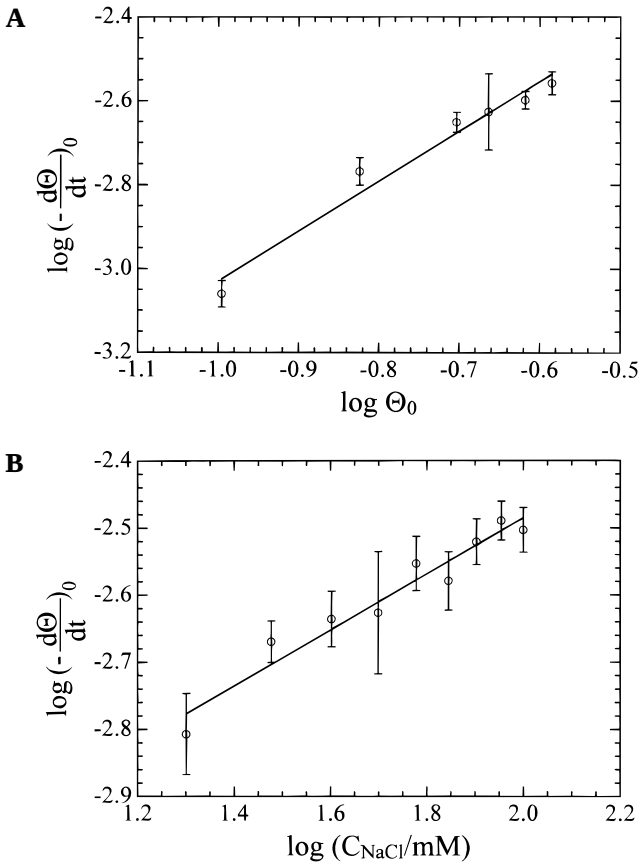


Figure 3. (A) Plot of initial rate of ion exchange $(-d\Theta/dt)_o$ as a function of Θ_o. (B) Plot of initial rate of ion exchange $(-d\Theta/dt)_o$ as a function of NaCl concentration.

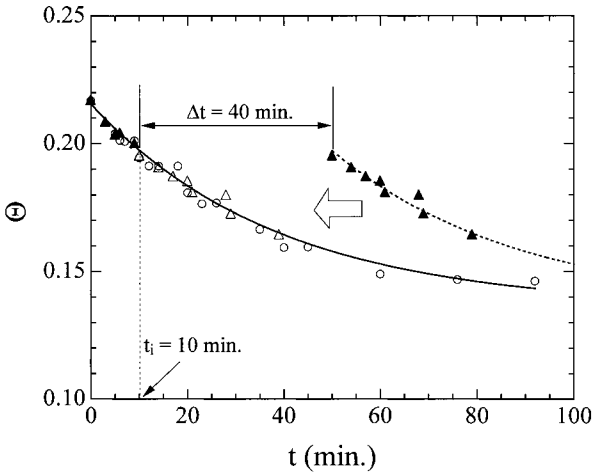


Figure 4. Plot of fraction of pendant ammonium groups that contain taurocholate (Θ) as a function of time in the presence of 50 mM NaCl: (▲) release kinetics observed for the first 10 min, followed by a 40-min interruption, and subsequent monitoring after reimmersion for an additional 30 min; (△) data obtained after the 40-min interruption, which have been "moved to the left" by 40 min; (○) release kinetics observed without an interruption under similar conditions. Initial loading of taurocholate (Θ_o) was 0.22.

Acknowledgment. This research was based on studies that were carried out as part of an undergraduate laboratory course (Chemistry 58) at Lehigh University during the Spring of 1998. We are grateful to the National Science Foundation (Grant CHE-9612702) and to Lehigh University for support of this research.

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(10) In preliminary studies, we have found that a ca. 10-fold decrease in the diameter of the anion-exchange resin results in ca. 10-fold increase in the initial rate of release of taurocholate by chloride ion ($\Theta_o = 0.30$; 50 mM NaCl). This result is fully consistent with film diffusion as the rate-limiting step.[3]

MA980781C