CFW RESEARCH

A Rapid Method To Determine Quantitatively the In Vitro Adsorption of Taurocholate to Soluble Fiber

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ABSTRACT

A new method was developed to study the adsorption of taurocholate to cereal β-glucan and other commercially available soluble fibers and gums. The method involved mixing soluble fiber with ^{14}C-labeled taurocholate, incubation at 37°C for 1 hr, centrifugal microfiltration, and scintillation measurement. Scintillation measurement showed that the filtrate, collected after repeated washing of the mixture through the membrane filter, was free of unbound taurocholate. The level of radioactivity measured in the filtrate of the blank sample was very close to 100% of that added, indicating there was no adsorption of bile salts to the membrane material. The amount of bile salt adsorbed was calculated as the difference between the amount of bile salt added and the amount detected in the filtrate. A coefficient of variation of <5% was achieved, indicating the method is reproducible and sensitive enough to distinguish the taurocholate adsorption capability of cereal β-glucan and commonly used soluble fibers. Among the soluble fibers and gums evaluated, barley β-glucan, oat β-glucan, and guar gum showed higher taurocholate adsorption compared with locust bean gum, carboxymethyl cellulose, high- and low-methoxy pectin, xanthan, gum arabic, microcrystalline cellulose, λ-carrageenan, and κ-carrageenan.

There are two categories of dietary fiber: soluble and insoluble. Although both categories confer hypocholesterolemic activity, the former is more effective than the latter (2,6,7). The hypocholesterolemic activity of these fibers has been attributed to their viscosity, fermentability, and bile acid-binding properties (1,5,6,8). The subject of interest in the current study was the bile salt adsorption capability of soluble fibers.

Bowles and coworkers (3) reported that the hypocholesterolemic activity of β-glucan, a soluble fiber, stems from its ability to entrap bile acids in viscous digesta rather than specific adsorption to bile acid micelles. Their method of analysis involved the use of solid-state ^{13}C-nuclear magnetic resonance (NMR) of a complex between β-glucan and Congo red dye. This method is time-consuming (takes longer than 5 hr) and complex and requires expertise in spectroscopy. In addition, background noise is a limiting factor in solid-state NMR. Cuesta-Alonso and Gilliland (4) used dialysis and membrane filtration to determine bile salt adsorption to soluble fiber. Their method is simple compared with that of Bowles and coworkers (3), but nevertheless requires more than 3 days to complete the analysis.

A simple method of determining bile acid or salt adsorption to insoluble fiber has been developed and utilized extensively in nutrition research. The method involves mixing insoluble fiber with ^{14}C-labeled bile acids or salts, incubation at 37°C for 2 hr, centrifugation, and liquid scintillation measurement of the supernatant. The amount of bile acid or salt bound is calculated as the difference between the amount added and the amount recovered in the supernatant (6). For obvious reasons, this method is not suitable for soluble fiber, but it can be modified for use with soluble fiber.

The main objective of the current study was to develop a method to quantitatively determine the extent of bile salt adsorption to soluble fiber. Story and Lord (12) employed a membrane filtration (ultrafiltration) technique to determine bile salt adsorption to soluble fiber. The intent was to combine the centrifugation step of the method of Goel and coworkers (6) with the membrane filtration step of the method of Story and Lord (12) into a single step to develop a simple method for measuring bile acid adsorption to soluble fiber.

MATERIALS AND METHODS

Oat and barley fibers (Viscofiber), concentrated forms of β-glucan (45–65% [w/w] β-glucan), were provided by Cevena Bioproducts Inc. (Edmonton, AB, Canada). Barley and oat β-glucans (BBG and OBG, respectively) were further purified on a laboratory scale (Fig. 1) using traditional aqueous extraction followed by alcohol precipitation of β-glucan to obtain a purified product containing >80% (w/w, db) β-glucan. High-methoxy pectin (HMP), low-methoxy pectin (LMP), locust bean gum (LBG), xanthan gum (XAN), guar gum (GUA), gum arabic (GAR), and carboxymethyl cellulose (CMC) were obtained from TIC Gums Inc. (Belcamp, MD). λ-Carrageenan (λ-CAR), κ-carrageenan (κ-CAR), and microcrystalline cellulose (MCC) were procured from FMC BioPolymer (Philadelphia, PA). Cholestyramine resin (CHO), an antihypercholesterolemic drug, was procured from Sigma Aldrich Inc. (St. Louis, MO). Cellulose fiber (CEL) was procured from Quadra Chemicals (Burlington, ON, Canada). All gum powders were >80% (w/w) pure. Nonradioactive sodium taurocholate was obtained from MP Biomedicals, Inc. (Aurora, OH). Radioactive sodium taurocholate (specific activity of 57.0 mCi/mmol) was purchased from Amersham Biosciences (Piscataway, NJ). Mono- and di-basic potassium phosphate (K_2HPO_4 and KH_2PO_4, respectively) were procured from BDH Inc. (Toronto, ON, Canada). Centrifugal filter devices (30,000 molecular weight cut off range; 0.5-mL capacity) were obtained from Millipore Corp. (Bedford, MA).

Preparation of Radioactive Bile Salt (Taurocholate) Stock Solutions

Stock solutions of unlabeled taurocholate salts were prepared in phosphate buffer (20 μ*M*; pH 6.5) at various concentrations ranging from 5 to 40 m*M*. Appropriate amounts of radioactive taurocholate were mixed to ensure scintillation counts of 100,000 dpm/mL of labeled solution.

Bile Salt Adsorption Assay

Soluble fiber (12.5 mg) was added to 2.5 mL of phosphate buffer (20 μ*M*; pH 6.5) and heated in a boiling water bath for 45 min or until completely dispersed. Labeled bile salt (2.5 mL), prepared in different concentrations (5–40 m*M*), was added to the 2.5 mL of fiber dispersion. This ensured the final mixture contained 0.25% (w/w) fiber and a

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2.5–20 m*M* taurocholate concentration for the assay. Next, the mixture (5 mL) was incubated in a shaking water bath at 37°C for 1 hr. An aliquot of 50 µL was transferred into a centrifugal filter device (the filter of the device was preconditioned by centrifuging 25 µL of phosphate buffer at 30,000 × *g* for 10 min) and centrifuged for 10 min at 30,000 × *g*. The membrane filter device was selected to have a molecular weight cut-off of 30,000 because all of the soluble fibers included in the study had molecular weights of >30,000. The retentate (residue) left on the membrane after the first centrifugation was subjected to four additional washings with 25 µL of phosphate buffer each time and centrifuged at 30,000 × *g* for 10 min. Filtrates of all washings from the fiber samples were pooled in the same vial.

Bile salt adsorption to fiber was monitored by counting the radioactivity of the pooled filtrate in a liquid scintillation counter (Beckmann Coulter Canada Inc., Mississauga, ON, Canada). A blank devoid of soluble fiber was also run to account for the amount of taurocholate bound to the membrane. The level of radioactivity measured in the filtrate of the blank sample was very close to 100% of that added, indicating there was no adsorption of bile salts to the membrane material. The amount of bile salt adsorbed by the fiber was calculated as the difference between the amount of bile salt added and the amount detected in the filtrate.

Testing of Soluble Fibers and Gums

For method development purposes, OBG, BBG, and reference compounds (such as CHO and CEL) were tested for their bile adsorption capacities over a range of bile salt concentrations (2.5–20 m*M*). Bile salt concentration was kept constant at 12.5 and 15 m*M* when testing the bile salt adsorption capacities of food gums used in the study: LBG, GUA, HMP, GAR, XAN, LMP, CMC, κ-CAR, λ-CAR, and MCC.

Statistical Analysis

All experiments were replicated twice. Three determinations of adsorbed bile salt concentration were performed for each replicate. Thus, all data reported are averages of six values. Data were analyzed using a general linear model (SAS Institute, Inc., Cary, NC), and the significance ($P \leq 0.05$) of the differences between mean values was established using Tukey's studentized range test.

RESULTS AND DISCUSSION

Method Development

Cereal β-glucans (BBG and OBG) and reference compounds (CHO, an antihypercholesterolemic drug, and purified CEL) were selected for method development purposes. After incubating fiber samples with labeled taurocholate, they were subjected to centrifugal filtration, the radioactivity of the filtrate was measured, and the retentate (residue) left on the membrane was washed repeatedly (up to six times) to ensure complete removal of free bile salt. The four washings performed on the retentate after centrifugal filtration (Fig. 2) were sufficient to wash away any free or unbound bile salt left in the retentate, as reflected by the small changes in scintillation counts (disintegrations per minute) observed in the filtrate after the fourth washing. The radioactivity of the filtrate, obtained after each washing up to the third, decreased substantially and did not change with subsequent washings. Repeated washing of the residue is a critical step in the assay because incomplete removal of free bile salt can contribute to overestimation of adsorption levels for the test fiber. Also, it is important to account for the bile salt interacting with the membrane of the filter

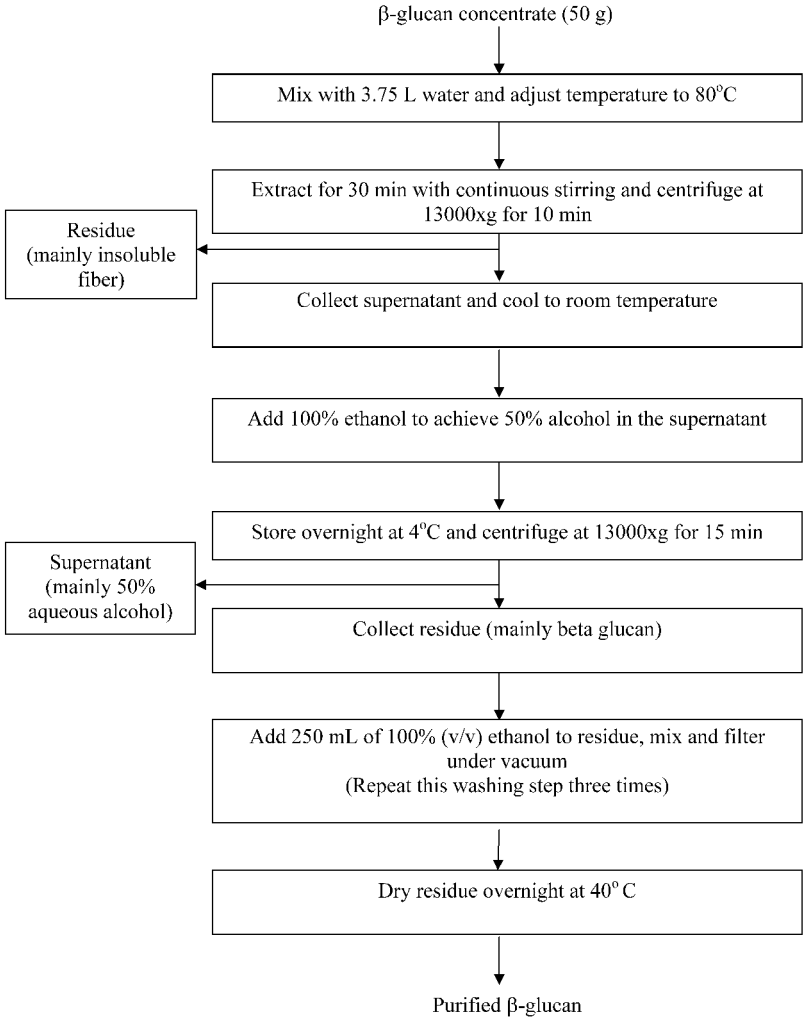

Fig. 1. Flow-chart depicting the steps involved in further purification of β-glucan from a concentrate at laboratory scale.

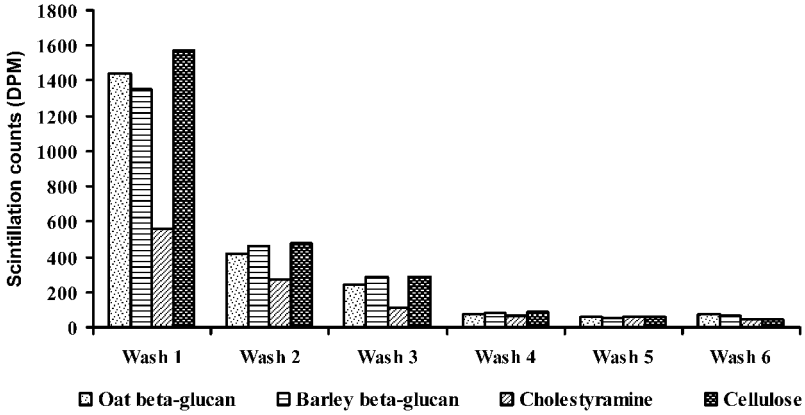

Fig. 2. Scintillation counts (measured as disintegrations per minute) of the filtrate collected separately during each successive washing of the retentate (residue) following incubation of ^{14}C-taurocholate with barley β-glucan, oat β-glucan, cholestyramine resin, and cellulose.

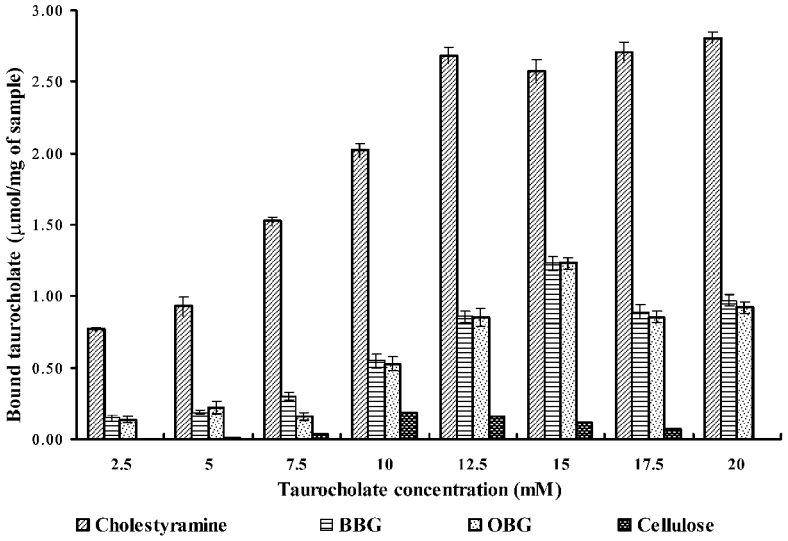

Fig. 3. Effect of sodium taurocholate concentration on taurocholate adsorption by 0.25% (w/w) aqueous dispersions of barley β-glucan (BBG), oat β-glucan (OBG), cholestyramine resin, and cellulose.

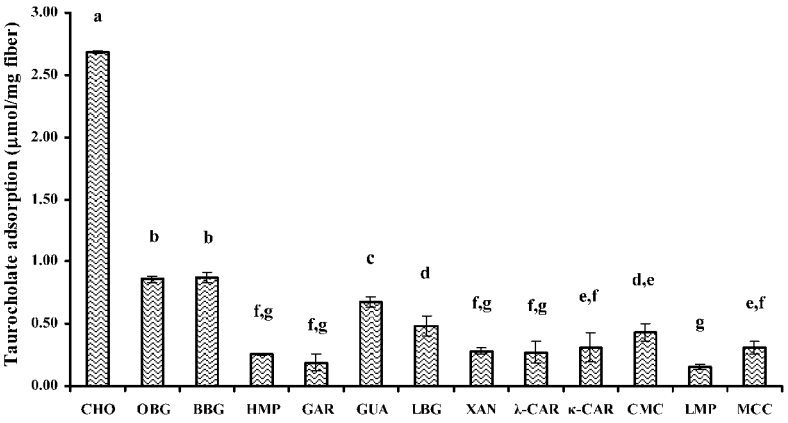

Fig. 4. Adsorption of taurocholate by various commercially available soluble fibers and gums evaluated at a sodium taurocholate concentration of 12.5 m*M*. Bars topped by the same letter are not significantly different ($P > 0.05$). CHO = cholestyramine resin; OBG = oat β-glucan; BBG = barley β-glucan; HMP = high-methoxy pectin; GAR = gum arabic; GUA = guar; LBG = locust bean gum; XAN = xanthan; λ-CAR = λ-carrageenan; κ-CAR = κ-carrageenan; CMC = carboxymethyl cellulose; LMP = low-methoxy pectin; and MCC = microcrystalline cellulose.

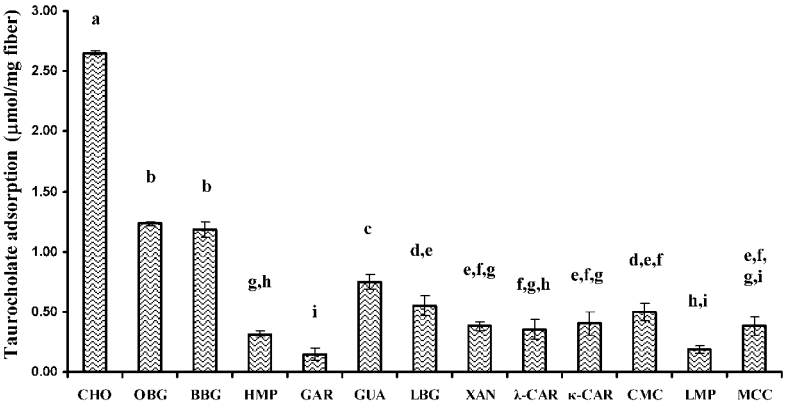

Fig. 5. Adsorption of taurocholate by various commercially available soluble fibers and gums evaluated at a sodium taurocholate concentration of 15 m*M*. Bars topped by the same letter are not significantly different ($P > 0.05$). CHO = cholestyramine resin; OBG = oat β-glucan; BBG = barley β-glucan; HMP = high-methoxy pectin; GAR = gum arabic; GUA = guar; LBG = locust bean gum; XAN = xanthan; λ-CAR = λ-carrageenan; κ-CAR = κ-carrageenan; CMC = carboxymethyl cellulose; LMP = low-methoxy pectin; and MCC = microcrystalline cellulose.

device. The membrane used in this study had a very low affinity to bile salts and large enough pores that they did not clog during filtration.

The bile salt adsorption capacities of all samples were tested at sodium taurocholate concentrations of 2.5–20 m*M*. Different taurocholate concentrations affect the bile salt adsorption capacities of soluble dietary fibers (4). Figure 3 shows bile salt adsorption as taurocholate concentration increased from 2.5 to 20 m*M*. The amount of bile salt adsorbed to the samples (Fig. 3) reached a plateau at a taurocholate concentration of 12.5 or 15 m*M*, after which no further change in bile salt adsorption was observed. At a taurocholate concentration of 12.5 m*M*, BBG demonstrated bile salt adsorption of ≈0.87 μmol/mg, whereas bile salt adsorbed to CHO was ≈2.68 μmol/mg. This agrees with the data reported for CHO by Lee and coworkers (9). CEL showed the least bile adsorption among the samples evaluated in this section. The bile salt adsorption capacities of both types of cereal β-glucans (OBG and BBG) was similar. Figure 3 suggests the soluble fibers reached their saturation level for bile salt adsorption at taurocholate concentrations higher than 15 m*M*. This agrees with previous data showing soluble fiber saturation at high taurocholate concentrations (4,12).

Bile Salt Adsorption Efficacy of Selected Food Gums

Several commercial food gums with varying molecular structures were evaluated using the newly developed bile adsorption assay. The bile salt adsorption capabilities of soluble fibers (BBG and OBG) were compared with that of CHO. For all experiments, a coefficient of variation (CV) of <5% was achieved, indicating the data are reproducible. Figures 4 and 5 depict the amount of bile salt bound to the food gums at 12.5 and 15 m*M* taurocholate concentrations, respectively. Purified BBG and OBG were similar ($P > 0.05$) to each other, but they showed significantly higher ($P \leq 0.05$) bile salt adsorption than any of the other fibers at both 12.5 and 15 m*M* salt concentrations. At both 12.5 and 15 m*M* taurocholate concentrations, GUA showed appreciable adsorption but was significantly lower than OBG and BBG ($P \leq 0.05$) (Figs. 4 and 5). This does not agree with the data reported by Cuesta-Alonso and Gilliland (4). In their study, GUA showed higher taurocholate adsorption compared with OBG. Other gums (LBG, HMP, GAR, XAN, LMP, κ-CAR, MCC, CMC, and λ-CAR) demonstrated lower sodium taurocholate adsorption capacities ($P \leq 0.05$) at both 12.5 and 15 m*M* taurocholate concentrations.

Although the mechanism of bile salt adsorption cannot be deduced from this assay, the assay's ability to distinguish differences in the bile salt adsorption capacities among the gums used in the study is clearly demonstrated. In recent literature (5,6), the mechanism of bile salt adsorption to BBG and

OBG, which lack positively charged groups, has been linked to their viscosity. Similarly, GUA and LBG have galactomannan structures that lack positively charged sites, and as a result, viscosity may play a role in their adsorption of bile salts as well. In the current study, galactomannans (LBG and GUA) bound almost twice as much of the bile salt as HMP and three times as much as LMP at both 12.5 and 15 m*M* taurocholate concentrations. Previous reports (9,10, 11,14) have indicated that viscosity, polysaccharide structure, and ionic characteristics are the principal factors contributing to bile acid adsorption. Although XAN dispersions have been reported to have five- to sixfold higher viscosity compared with OBG and BBG dispersions (13), bile salt adsorption capacities were almost one-third of those demonstrated by BBG and OBG dispersions.

These results demonstrate that the viscosity of dispersions may not be the only factor responsible for bile salt adsorption capability. HMP, LMP, and XAN carry negatively charged groups that can effectively hinder the entrapment of negatively charged taurocholate in the viscous polymer dispersion, resulting in low adsorption. The degree of methoxylation of pectin did not influence bile salt adsorption characteristics. CMC and LBG demonstrated similar levels of bile salt adsorption at both 12.5 and 15 m*M* taurocholate concentrations. κ-CAR and λ-CAR, differing only in their degree of sulfonate esters (≈24 and ≈27%, respectively), demonstrated similar bile salt adsorption capacities ($P > 0.05$) at both 12.5 and 15 m*M* taurocholate concentrations.

CONCLUSIONS

Membrane filtration coupled with centrifugation is a convenient means of quantitatively determining the extent of bile salt adsorption to β-glucan and other soluble fibers. A CV of <5% was achieved, indicating the results are reproducible. Among the food gums evaluated, BBG, OBG, and GUA showed higher taurocholate adsorption compared with LBG, CMC, HMP, LMP, XAN, GAR, MCC, λ-CAR, and κ-CAR.

Acknowledgments

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