JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Inhibition of Buckwheat Starch Digestion by the Formation of Starch/Bile Salt Complexes: Possibility of Its Occurrence in the Intestine

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ABSTRACT: During the digestion of starch in foods, starch is mixed with bile in the duodenum. Because fatty acids and some kinds of polyphenols could bind to starch, it was postulated that bile salts might also bind to starch. The purpose of this paper is to study the effects of bile and bile salts on starch/iodine complex formation and pancreatin-induced starch digestion. Bile suppressed starch/iodine complex formation and inhibited pancreatin-induced starch digestion slightly in control buckwheat starch, but did so significantly in buckwheat starch from which fatty acids and polyphenols had been extracted. Such significant suppression and inhibition by bile were also observed in a reagent soluble starch. The effects of cholate and taurocholate on the starch/iodine complex formation and the pancreatin-induced starch digestion were essentially the same as those of bile. Bile, cholate, and taurocholate suppressed amylose/iodine complex formation more significantly than amylopectin/iodine complex formation and inhibited pancreatin-induced amylose digestion more effectively than the digestion of amylopectin. It is concluded from the results that bile salts could bind to starch, especially amylose, the helical structures of which were not occupied by other molecules such as fatty acids and polyphenols, and that the binding resulted in the inhibition of starch digestion by pancreatin. The conclusion suggests that the function of bile salts can be discussed from the point of not only lipid digestion but also starch digestion.

KEYWORDS: amylase-catalyzed digestion, buckwheat starch, starch/bile salt complexes, starch/iodine complexes

INTRODUCTION

It has been reported that various organic and inorganic compounds can bind to starch, occupying the interior of the amylose helix.[1,2] In the compounds, fatty acids such as palmitic, stearic, oleic, linoleic, and linolenic acids are included; the occupation of the helical structures of amylose by these acids results in the suppression of amylose/iodine complex formation and the inhibition of amylase-catalyzed digestion of amylose.[3−11] Quercetin, rutin, and epicatechin-dimethylgallate (Figure 1), which have hydrophobic moieties in the molecules, can also bind to starch, and the binding results in not only the suppression of starch/iodine complex formation but also the inhibition of amylase-catalyzed starch digestion.[12] Furthermore, an isoflavone genistein forms complexes with amylose,[13] and condensed procyanidins bind to polysaccharides including starch.[14] These findings suggest that components with flavonoid structure have the ability to associate with polysaccharides including starch. It has been reported that isoflavones bind to receptors of a steroid hormone estrogen.[15,16] The results prompted us to postulate that bile salts (Figure 1) might also bind to starch to inhibit its digestion by amylase. Many studies have been conducted on the binding of bile salts to polysaccharides,[17−22] but only one study concerns the effect of bile salts on amylase-catalyzed digestion of starch.[23]

In the present study, the effects of bile and bile salts on starch/iodine complex formation and pancreatin-induced starch digestion were studied using control (nonextracted) buckwheat starch and organic solvent-extracted buckwheat starch. Organic solvents such as methanol can extract fatty acids and polyphenols from buckwheat starch.[12] Furthermore, the effects of bile and bile salts on starch/iodine complex formation and pancreatin-induced starch digestion were studied using amylopectin and amylose. Taking the results obtained in this study into consideration, we discuss (i) that bile salts can make complexes with starch, the helical structures of which are not occupied by other molecules such as lipids and polyphenols, in the duodenum, and (ii) that the formation of starch/bile salt complexes makes starch amylase-resistant in the small intestine.

MATERIALS AND METHODS

Reagents. Sodium cholate, sodium taurocholate, soluble starch, pancreatin from hog pancreas, and bile powder from ox were obtained from Wako Pure Chemical Industries (Osaka, Japan). The bile powder contained cholic acid conjugates approximately 40%. Amylose (molecular weight, ca. 160,000) and amylopectin were obtained from Tokyo Kasei Ind. (Tokyo, Japan). 4-Hydroxybenzhydrazide or *p*-hydroxybenzoic acid hydrazine was from Sigma-Aldrich Japan (Tokyo, Japan). Buckwheat flour (cv. Mancan) was from Itsuki Shokuhin (Kumamoto, Japan). Amylose contents and degree of polymerization of amylose of buckwheat starch have been reported to be 16−18% and 1020−1380, respectively.[24]

Preparation of Solutions. Potassium iodide (1.5 g) was dissolved in 12.5 mL of water. Iodine (0.635 g) was added to the solution, and the volume of the mixture of potassium iodide and iodine was brought to 50 mL by adding water. The solution (equivalent to 100 mM iodine) was kept in the dark to be used as iodine solution. Pancreatin (10 mg/mL) was suspended in water. Sodium cholate (100 mM), sodium taurocholate (100 mM), and bile powder (100 mg/mL) were dissolved in water. The concentration of total cholic acid conjugates in the bile solution was estimated to be 85 mM, under the postulation that the molar ratio of glycocholate to taurocholate was 3:1.

Received: February 15, 2011
Revised: May 1, 2011
Accepted: May 2, 2011
Published: May 02, 2011

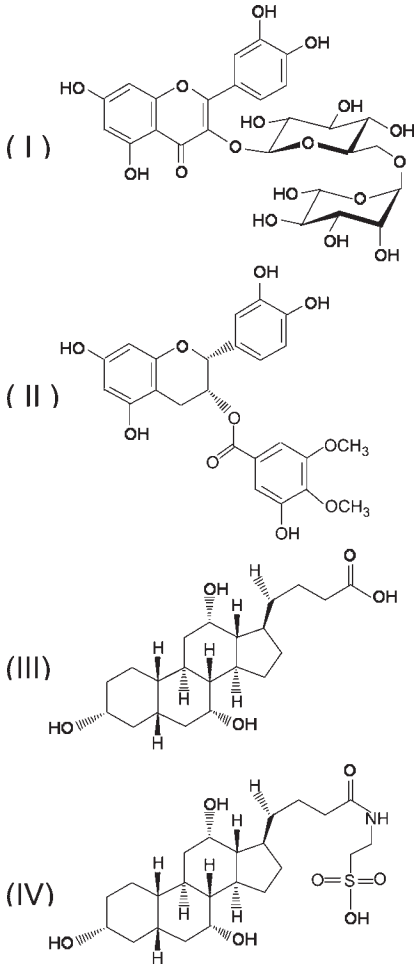

Figure 1. Chemical structures of components included in this study: (**I**) rutin; (**II**) epicatechin-3,4-dimethylgallate; (**III**) cholic acid; (**IV**) taurocholic acid.

Preparation of Extracted Buckwheat Starch. Extracted buckwheat starch was prepared as described previously.[12] Buckwheat flour (0.3 g) was extracted with 5 mL of methanol three times and then extracted with 5 mL of petroleum ether twice. The extracted buckwheat flour and the nonextracted flour were suspended each in 10 mL of water, and the suspensions were heated with a microwave oven (800 W) until they boiled. It took about 15 s for the boiling. The boiled buckwheat flour suspensions were cooled to room temperature and centrifuged at 3500*g* for 1 min. The gelatinous sediments were dried in vacuo over NaOH. In the following, dried starch preparations, which were prepared from nonextracted buckwheat flour and extracted buckwheat flour, are referred to as control buckwheat starch and extracted buckwheat starch, respectively.

Preparation of Starch Suspensions. Control buckwheat starch and extracted buckwheat starch (5 mg) were suspended each in 1 mL of water. The suspensions were kept for 0.5 h at room temperature to make the starch swell, and then 7.5 mL of 50 mM KCl−HCl (pH 2.0) and 7.5 mL of 100 mM sodium phosphate (pH 7.1) were added successively to the suspensions, simulating pH changes in the stomach and the duodenum, where starch passed through and reached, respectively. The pH of the starch suspensions after the addition of the two buffer solutions was 6.8. The suspensions were homogenized using a glass homogenizer and then sonicated for 10 s with a sonicator (UR-200P, Tomy Seiko Co., Ltd., Tokyo, Japan) to be used for experiments. The concentration of starch in the suspensions was 0.31 mg/mL.

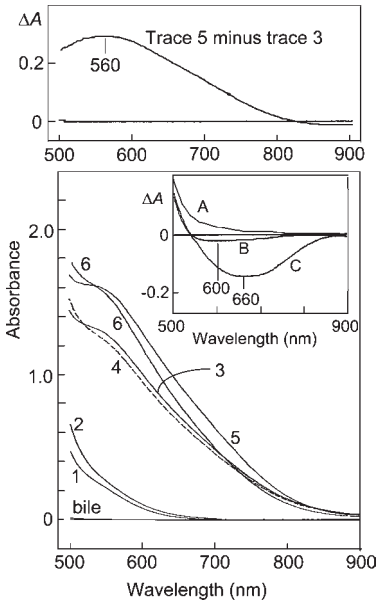

Figure 2. Effects of bile on the formation of starch/iodine complexes. (Bottom) *A* spectra of starch/iodine complexes: (1) no addition; (2) 1 + bile; (3) control buckwheat starch; (4) 3 + bile; (5) extracted buckwheat starch; (6) 5 + bile. The concentration of bile was equivalent to 1.7 mM cholic acid conjugates. (Inset) Δ*A* spectra: (A) 2 minus 1; (B) 4 minus 3; (C) 6 minus 5. (Top) Δ*A* spectrum of 5 minus 3 in the bottom panel.

Soluble starch (10 mg/mL), amylose (10 mg/mL), and amylopectin (10 mg/mL) were suspended each in gently boiling water.

Starch/Iodine Complexes. It is known that the starch/iodine complexes can be used to estimate the concentration of starch and that the color of the amylose/iodine complexes (blue; λ_{max} = 560−660 nm) is different from that of the amylopectin/iodine complexes (purple; λ_{max} = 500−520 nm).[28−26] Then, the effects of bile salts on the formation of starch/iodine complexes were studied by recording absorption (*A*) spectra from 900 to 500 nm using a 557 double-beam spectrophotometer (Hitachi, Tokyo, Japan). The light path of the measuring beam was 4 mm. When control buckwheat starch and extracted buckwheat starch were used, bile or bile salts were added to 0.9 mL of the buckwheat starch suspensions. Immediately after the addition of bile or bile salts to the starch suspensions, the two solutions were mixed by top and bottom inversion of the cuvette, and then 0.1 mL of iodine solution was added to the suspensions. Recording of *A* spectra was started within 1 min after the preparation of mixtures of starch and bile or bile salts. When soluble starch, amylose, or amylopectin was used, 20 μL of soluble starch suspension (equivalent to 0.2 mg of starch), 30 μL of amylose suspension (equivalent to 0.3 mg of amylose), or 20 μL of amylopectin suspension (equivalent to 0.2 mg of amylopectin) was added to 0.9 mL of the mixture of 50 mM KCl−HCl (pH 2.0) and 100 mM sodium phosphate (pH 7.1) (1:1, v/v), and then bile or bile salts and iodine solution were added successively as described above to measure *A* spectra.

Pancreatin-Induced Starch Digestion. Pancreatin-induced starch digestion was also studied using a 557 spectrophotometer. To digest starch, 1 μL of pancreatin suspension (10 mg/mL) was added to 0.9 mL of a starch suspension that was prepared as above. Immediately after the addition of pancreatin, the two solutions were mixed by top and bottom inversion of the cuvette. The suspension was incubated for defined periods at about 25 °C, and then 0.1 mL of iodine solution was added. By the addition of iodine solution, pancreatin-induced starch digestion was terminated. The degree of the digestion and digested starch species were estimated from difference (Δ*A*) spectra of starch/iodine complexes after and before the digestion.

Pancreatin-induced starch digestion was also studied by quantifying reducing sugars.[12,27] 4-Hydroxybenzhydrazide (0.33 M) was dissolved in 0.6 M HCl and used as solution I. Solution II contained 0.042 M sodium citrate, 0.007 M calcium chloride, and 0.5 M sodium hydroxide. The mixture of solutions I and II (1:9, v/v) was prepared every day and stored on ice between uses. A starch suspension (0.5 mL) was digested by pancreatin (10 μg/mL) for defined periods. The reaction was terminated by adding 2.5 mL of the mixture of solutions I and II and then incubated in boiling water for 6 min. After removal of precipitates by centrifugation, absorbance (A) at 410 nm was determined.

Data Presentation. Each experiment was repeated two or three times and, essentially, the same results were obtained. Typical data or averages of two or three experiments are presented in the figures.

RESULTS AND DISCUSSION

Spectrophotometric Characteristics of Starch/Iodine Complexes. At the beginning of this study, starch/iodine complexes of control and extracted buckwheat starch were characterized. Figure 2 (bottom) shows A spectra of iodine solution (spectrum 1), starch/iodine complexes of control buckwheat starch (spectrum 3), and starch/iodine complexes of extracted buckwheat starch (spectrum 5). The more intense A in spectrum 5 than in spectrum 3 indicates that more iodine bound to the helical structures of extracted buckwheat starch than to those of control buckwheat starch. The ΔA spectrum of spectrum 5 minus spectrum 3 had a peak around 560 nm (Figure 2, top), indicating that the extraction of buckwheat flour by organic solvents resulted in the increase in the concentration of amylose that could bind to iodine. According to a previous paper, the extraction of fatty acids and polyphenols contributed to the increase in the amylose concentration.[12] The presence of free fatty acids and polyphenols in buckwheat flour has been reported elsewhere.[24,29−31]

Binding of Bile Components to Starch. The concentration of bile salts in the intestine has been reported to be 1−10 mM.[32−35] Then, effects of bile equivalent to 1.7 mM cholic acid conjugates on the formation of starch/iodine complexes were studied (Figure 2, bottom). Bile increased A of iodine solution in the wavelength range from 500 to 700 nm (compare spectra 1 and 2), indicating that components in bile made complexes with iodine. The effects of bile on starch/iodine complex formation in buckwheat starch suspensions show that bile decreased A of extracted buckwheat starch (compare spectra 5 and 6) more significantly than A of control buckwheat starch (compare spectra 3 and 4) in the wavelength range from 540 to 900 nm. The different degrees of A decrease indicate that more bile components bound to the extracted starch than the control starch to suppress starch/iodine complex formation. The peak of the ΔA spectrum with and without bile in the extracted starch (inset; spectrum C, peak wavelength = 660 nm) shows that bile mainly suppressed the formation of amylose/iodine complexes. ΔA spectra with and without bile in the control starch did not have any distinct peaks (inset; spectrum B), but the difference spectrum ($\Delta\Delta A$ spectrum) between spectra B and A (inset) had a peak around 540 nm (data not shown). The $\Delta\Delta A$ spectrum suggests that bile mainly suppressed the formation of amylopectin/iodine complexes in the control starch, but at present, we could not exclude the possibility of bile-dependent suppression of amylose/iodine complexes formation in the control starch. This is deduced from the reports that the A peak of amylose/iodine complexes shifts from 620 to 520 nm with the decrease in length of the helical structure of amylose.[25−27] The result in Figure 2 indicates that components in bile could bind to amylose if the helical structures of amylose were not occupied with other molecules.

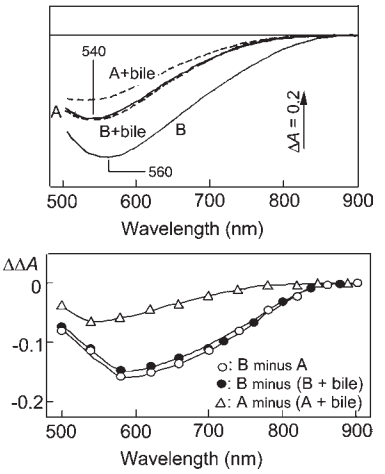

Figure 3. Changes in A spectra of starch/iodine complexes. (Top) ΔA spectra after and before pancreatin-induced digestion: (A) control starch; (A + bile) control starch plus bile; (B) extracted starch; (B + bile) extracted starch plus bile. Digestion time = 1 min. The concentration of bile was equivalent to 1.7 mM cholic acid conjugates. (Bottom) $\Delta\Delta A$ spectra between two spectra of the top panel.

Inhibition of Starch Digestion by Bile. Pancreatin-induced digestion of control buckwheat starch and extracted buckwheat starch was studied by recording ΔA spectra before and after the digestion (Figure 3, top). The result indicates (i) that the digestion was faster in the extracted starch (spectrum B) than in the control starch (spectrum A, solid line); (ii) that not only amylopectin but also amylose seemed to be digested in the control starch, because ΔA spectrum had a peak around 540 nm (spectrum A); and (iii) that amylose seemed to be digested mainly in the extracted starch, because the peak wavelength of the ΔA spectrum was 560 nm (spectrum B). The occurrence of amylose digestion in the extracted starch was supported by the $\Delta\Delta A$ spectrum between spectra B and A, which had a peak around 600 nm with a shoulder around 700 nm (Figure 3, bottom).

Bile inhibited pancreatin-induced digestion of control buckwheat starch slightly [compare spectrum A with spectrum (A + bile) in the top panel]. The $\Delta\Delta A$ spectrum of spectrum A minus spectrum (A + bile) (bottom; peak wavelength, approximately 540 nm) indicates that the inhibition was mainly due to inhibition of amylopectin digestion. Bile also inhibited pancreatin-induced digestion of extracted buckwheat starch [top panel, spectrum (B + bile) with a broken line]. The $\Delta\Delta A$ spectrum of spectrum B minus spectrum (B + bile) had a peak at approximately 600 nm with a shoulder around 700 nm (bottom), indicating that bile inhibited the digestion of amylose mainly in the extracted starch. We concluded from the results in Figures 2 and 3 that the binding of bile components to starch results in the inhibition of starch digestion by pancreatin.

Figure 4 (top and middle panels) shows time courses of pancreatin-induced digestion of control buckwheat starch and extracted buckwheat starch. The digestion was slower and smaller in the control starch than in the extracted starch when estimated by the formation of starch/iodine complexes at 500 and 620 nm. Bile inhibited not only the initial rate but also the amount of digestion, and the degree of inhibition was larger in

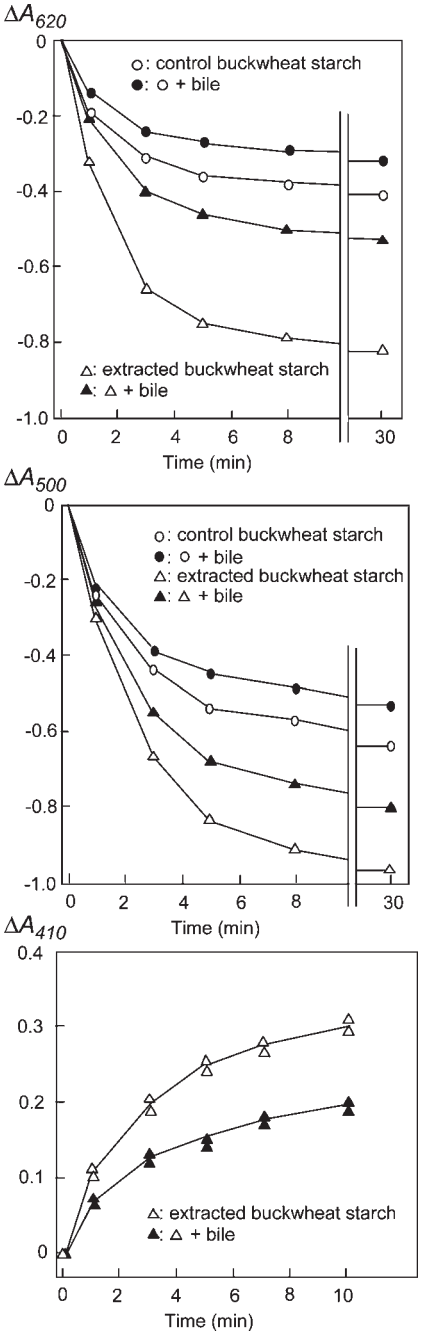

Figure 4. Bile-dependent inhibition of pancreatin-induced starch digestion. (Top and middle) Starch digestion estimated by starch/iodine complex formation (top, *A* decrease at 620 nm; middle, *A* decrease at 500 nm). (Bottom) Formation of reducing sugars. The concentration of bile was equivalent to 1.7 mM cholic acid conjugates.

the extracted than in the control starch, as expected from the conclusion deduced from the results in Figures 2 and 3. Figure 4 (bottom) shows that bile inhibited pancreatin-induced starch digestion by about 33% in extracted buckwheat starch when the digestion was estimated by the production of reducing sugars. This value was similar to the degree of bile-dependent inhibition of starch digestion (about 36%) in the top panel in extracted buckwheat starch. The similar degree of inhibition indicates that starch/iodine complex formation was useful to estimate the effects of bile on starch digestion.

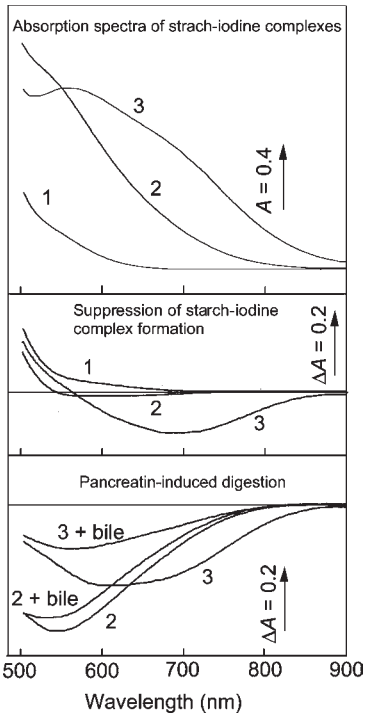

Figure 5. Effects of bile on the digestion of amylose and amylopectin. (Top) *A* spectra: (1) iodine; (2) amylopectin + iodine; (3) amylose + iodine. (Middle) Δ*A* spectra in the presence and absence of bile: (1) iodine solution; (2) iodine solution with amylopectin; (3) iodine solution with amylose. (Bottom) Δ*A* spectra of starch/iodine complexes after and before the digestion by pancreatin: (2) amylopectin; (2 + bile) amylopectin + bile; (3) amylose; (3 + bile) amylose + bile. The concentration of bile was equivalent to 1.7 mM cholic acid conjugates.

Effects of Bile on Digestion in Soluble Starch. The effects of bile on starch/iodine complex formation were studied using a reagent soluble starch that would not contain fatty acids, polyphenols, etc. Bile equivalent to 1.7 mM cholic acid conjugates suppressed the formation of starch/iodine complexes, and Δ*A* spectra with and without bile had a peak at approximately 660 nm (data not shown).

The above concentration of bile inhibited pancreatin-induced digestion of soluble starch. Δ*A* spectra of starch/iodine complexes before and after the digestion had a peak at approximately 600 nm when digested in the absence of bile and at approximately 550 nm when digested in the presence of bile (data not shown). The ΔΔ*A* spectrum of the Δ*A* spectra obtained in the presence and absence of bile had a peak at approximately 620 nm. The results obtained using soluble starch support the conclusion deduced from Figures 2 and 3 that bile components can bind to amylose, inhibiting its digestion if the helical structures are not occupied by other molecules.

Effects of Bile on the Digestion of Amylose and Amylopectin. The top panel of Figure 5 shows *A* spectra of amylopectin/iodine complexes (spectrum 2) and amylose/iodine complexes (spectrum 3) in addition to the *A* spectrum of iodine solution (spectrum 1). Addition of bile to iodine solution increased *A* in the wavelength range from 500 to 700 nm (middle panel, spectrum 1). Bile did not significantly affect the *A* spectrum of amylopectin/iodine complexes in the wavelength range from 500 to 700 nm (middle panel, spectrum 2), but the difference between spectra 1 and 2 (data not shown) showed that bile suppressed the formation

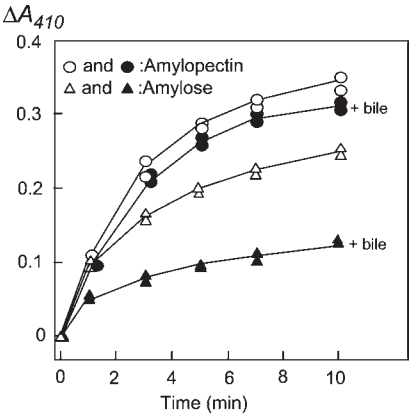

Figure 6. Inhibition of pancreatin-induced digestion of amylose and amylopectin by bile. Starch digestion was estimated by the production of reducing sugars. Each data point represents the average of two experiments. The concentration of bile was equivalent to 1.7 mM cholic acid conjugates.

of amylopectin/iodine complexes. Because iodine could bind to the helical structures of amylopectin, bile components might also bind to the helical structures to suppress the formation of amylopectin/iodine complexes. Spectrum 3 in the middle panel shows that suppression of amylose/iodine complex formation by bile accompanied *A* decrease around 700 nm.

Pancreatin-induced digestion of amylopectin and amylose was also studied by recoding Δ*A* spectra before and after the digestion (Figure 5, bottom). Bile inhibited pancreatin-induced digestion of amylopectin slightly [compare spectrum 2 with spectrum (2 + bile)], whereas the digestion of amylose was inhibited significantly by bile [compare spectrum 3 with spectrum (3 + bile)]. Pancreatin-induced digestion of amylopectin and amylose was studied by measuring the production of reducing sugars (Figure 6). Here again, bile inhibited the digestion of amylose more significantly than that of amylopectin. The results in Figures 5 and 6 indicate that bile-dependent inhibition of starch digestion was not due to the inhibition of amylase activity. If amylase activity itself was inhibited by bile, the degree of bile-dependent inhibition of amylose digestion should be the same as that of bile-dependent inhibition of amylopectin digestion. The results in Figures 5 and 6 also indicate that bile components preferred amylose to amylopectin for the binding and that the binding resulted in the inhibition of pancreatin-induced digestion, confirming the conclusion obtained using buckwheat starch and soluble starch.

Inhibition of Starch Digestion by Bile Salts. Because cholate conjugates were present in bile, we investigated the effects of cholate and taurocholate (Figure 1) on the formation of starch/iodine complex (Figure 7, top). At the beginning, the effects of these components on the *A* spectrum of an iodine solution were studied (A). No changes in *A* spectra were observed when 2 mM cholate (spectrum 1), 4 mM cholate (spectrum 2), or 2 mM taurocholate (spectrum 3) was added to the iodine solution, but the addition of 4 mM taurocholate resulted in an increase in *A* of the iodine solution between 500 and 700 nm (spectrum 4). The result indicates (i) that the taurine moiety of taurocholate contributed to the formation of taurocholate/iodine complex and (ii) that the *A* increase observed after addition of bile to an iodine solution (Figures 2 and 5) was due to the formation of bile acid conjugate/iodine complexes.

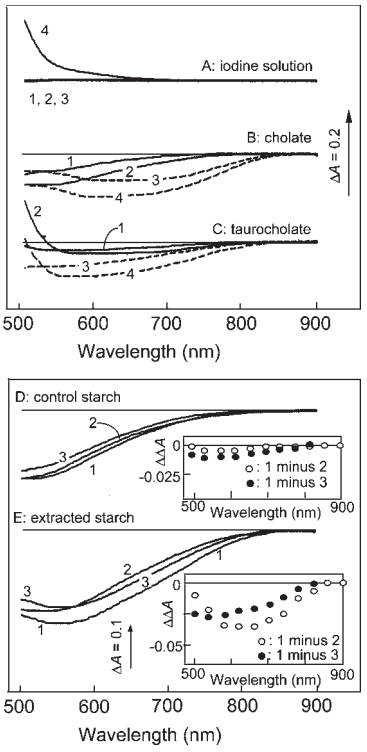

Figure 7. Effects of bile acids on starch/iodine complex formation and starch digestion. (Top, traces in A) Δ*A* spectra after and before the addition of cholate or taurocholate to 10 mM iodine in a buffer solution (pH 6.8): (1) 2 mM cholate; (2) 4 mM cholate; (3) 2 mM taurocholate; (4) 4 mM taurocholate. (Top, traces in B and C) Δ*A* spectra after and before the addition of cholate (B) or taurocholate (C) to starch suspensions: solid lines, control buckwheat starch; dashed lines, extracted buckwheat starch; (1 and 3) 2 mM cholate or taurocholate; (2 and 4) 4 mM cholate or taurocholate. (Bottom) Δ*A* spectra after and before pancreatin-induced digestion: (D) control buckwheat starch; (E) extracted buckwheat starch; (1) no addition; (2) 4 mM cholate; (3) 4 mM taurocholate. Digestion time = 1 min. (Insets) ΔΔ*A* spectra.

Starch/iodine complex formation was suppressed by cholate in control and extracted buckwheat starch (B). Δ*A* spectra in the presence and absence of cholate in the control and extracted starch had peaks around 520 nm (spectra 1 and 2) and between 600 and 700 nm (spectra 3 and 4), respectively. The results indicate that cholate mainly suppressed the formation of amylopectin/iodine and amylose/iodine complexes in the control and extracted starch, respectively.

The effects of taurocholate on the starch/iodine complex formation (C) show that taurocholate suppressed the formation of amylopectin/iodine complexes mainly in the control starch, although taurocholate-dependent suppression of amylose/iodine complexes could not be excluded (spectra 1 and 2). In the extracted starch, 2 mM taurocholate suppressed the formation of amylose/iodine complexes (spectrum 3), and the suppression became clear with 4 mM taurocholate (spectrum 4). Δ*A* spectra indicate that taurocholate suppressed not only amylose/iodine complex formation but also amylopectin/iodine complex formation, independent of buckwheat starch preparations.

Cholate and taurocholate inhibited pancreatin-induced starch digestion in control buckwheat starch and extracted buckwheat starch (Figure 7, bottom). Δ*A* spectra after and before the digestion of the control starch (D) indicate that cholate (spectrum 2) and

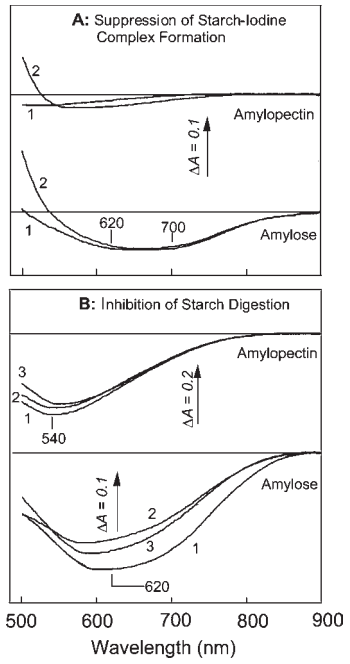

Figure 8. Interaction of bile salts with amylopectin and amylose. (A) ΔA spectra of amylopectin/iodine and amylose/iodine complexes in the presence and absence of bile acids: (1) 4 mM cholate; (2) 4 mM taurocholate. (B) ΔA spectra of amylopectin/iodine and amylose/iodine complexes after and before their digestion by pancreatin: (1) without bile acids; (2) 4 mM cholate; (3) 4 mM taurocholate. Digestion time = 1 min.

taurocholate (spectrum 3) inhibited its digestion slightly, and $\Delta\Delta A$ spectra (inset) in the presence and absence of cholate and taurocholate had peaks at about 560 and 540 nm, respectively. This result suggests that both cholate and taurocholate mainly inhibited the digestion of amylopectin, although inhibition of amylose digestion by the bile salts could not be excluded. ΔA spectra after and before the digestion of the extracted starch (E) indicate that taurocholate (spectrum 3) as well as cholate (spectrum 2) inhibited A decrease in the wavelength range from 600 to 800 nm. $\Delta\Delta A$ spectra between spectra 1 and 2 and between spectra 1 and 3 (inset) had peaks around 620 and 540 nm, respectively. The results indicate that cholate inhibited the digestion of extracted buckwheat starch by binding to amylose and that taurocholate inhibited the digestion of amylopectin as well as amylose. The different effects of the two bile salts might be attributed to the presence and absence of a taurine moiety in the molecules.

Inhibition of Amylopectin and Amylose Digestion by Bile Salts. Figure 8A shows that cholate (spectrum 1) and taurocholate (spectrum 2) suppressed the formation of amylopectin/iodine complexes slightly, and the suppressive effect of taurocholate was somewhat larger than that of cholate. These bile salts suppressed the formation of amylose/iodine complexes significantly. Pancreatin-induced digestion of amylopectin was inhibited by cholate (spectrum 2) and taurocholate (spectrum 3), and the inhibitory effect of taurocholate was somewhat larger than that of cholate (Figure 8B). The more effective inhibition could be attributed to the larger affinity of taurocholate than that of cholate to amylopectin (panel A). This result coincides with the result that taurocholate inhibited the digestion of amylopectin in extracted buckwheat starch (Figure 7). Both cholate and taurocholate inhibited the digestion of amylose significantly as expected from the inhibition of amylose/iodine complex formation by the bile salts (panel A). In the present study, we did not investigate the effects of glycocholate on starch/iodine complex formation and pancreatin-induced starch digestion. However, we can deduce that glycocholate as well as cholate and taurocholate can suppress starch/iodine complex formation and can inhibit starch digestion because their molecular structures show that the physical characteristics of glycocholate are intermediate between those of cholate and taurocholate.

Many investigators have reported the binding of bile salts to starch and other polysaccharides, and the binding is discussed in relation to the fecal excretion of lipids and bile salts and the metabolism of lipids and cholesterol.[17−22,36−38] In the present study, it is shown that the binding of bile salts to starch, especially amylose, resulted in the suppression of formation of starch/iodine complexes and the inhibition of pancreatin-induced starch digestion. Although the digestibility of starch in the small intestine has been discussed from the point of physical characteristics such as particle size, surface area of starch granule, etc.[39] and from the point of complex formation between starch and fatty acids and/or polyphenols,[3−12] the results obtained in the present study suggest that the digestibility can also be discussed from the point of starch/bile salt complex formation. According to the present study, the increase in bile salt contents in the small intestine with the increase of amylose intake[41] can be explained by the formation of amylase-resistant starch/bile salt complexes in the duodenum but not by the binding of bile salts to starch that escaped digestion in the small intestine.[39] The binding of bile salts to starch in the duodenum is deduced from the results that bile salts bound starch rapidly. The rapid binding was supported by the results in Figures 2, 3, 5, and 7 that bile salt-dependent inhibition of starch/iodine complex formation was observed when iodine solution was added within 1 min after the preparation of the mixture of starch and bile salts. Bile salts have been reported to be able to enhance and inhibit the amylase-catalyzed digestion at low (<1 mM) and high concentrations, respectively.[23] The results of the present study suggest that the concentration of bile salts in bile (1−10 mM)[32−35] could inhibit starch digestion in the small intestine.

Starch/fatty acid complexes and starch/polyphenol complexes are resistant to amylase, suggesting that these complexes can contribute to slowing the increase in blood concentration of glucose.[3−12] Bile salts could also contribute to slowing the increase in blood concentration of glucose by transforming starch to amylase resistance by occupying the helical structures of starch, but the bile salt-dependent formation of amylase-resistant starch seemed to be suppressed by food components such as fatty acids and polyphenols (Figures 2−4 and 7). Therefore, if starch is ingested with fatty acid- and/or polyphenol-rich foods, these components can bind to starch in the mouth and the stomach, resulting in the decrease in binding of bile salts to starch in the duodenum. The decrease in binding of bile salts to starch affects starch-dependent metabolism of bile salts in the intestine. According to the above discussion, it is important to take the presence and absence of free helical structures in starch into consideration to discuss the interactions between starch and bile salts in relation to starch digestion, fecal excretion of bile salts, and cholesterol metabolism.

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