Comparison of the binding sites of GSH S-transferases of the Y_a- and Y_b-subunit classes: effect of glutathione on the binding of bile acids

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Abstract We have previously observed that the Y_a subunit-containing glutathione (GSH) S-transferases from rat liver exhibit a common high affinity binding site for lithocholic acid, bilirubin, and sulfobromophthalein (BSP) (1984. *J. Lipid Res.* **25:** 1177–1183). Subsequently we found that cholic acid and its amidates bound to a site on the Y_a subunit separate for the lithocholic acid/bilirubin site (1986. *J. Lipid Res.* **27:** 955–966). We now have extended this work by showing that amidates of lithocholic acid as well as chenodeoxycholic acid and its amidates competitively displace [^{14}C]lithocholic acid from the Y_a subunit. GSH did not inhibit binding of any of the ligands to the high affinity Y_a site, but did inhibit binding to the cholic acid site on the Y_a subunit. We have also defined the binding sites and effects of GSH on the Y_b class of subunits. Lithocholic, chenodeoxycholic, and cholic acids (and amidates) shared a common site on the Y_b or Y_b' subunit, whereas BSP and bilirubin were bound at a different site. Both the bile acid and organic anion sites on the Y_b subunit were inhibited by GSH. The inhibition by GSH in all cases (Y_a cholic acid site or Y_b bile acid or bilirubin sites) was saturable, of the competitive type, and incomplete at maximal GSH concentrations, suggesting that when GSH binds to its distinct substrate site, it induces a conformational change in the proteins affecting the other binding sites. — **Takikawa, H., and N. Kaplowitz.** Comparison of the binding sites of GSH S-transferases of the Y_a- and Y_b-subunit classes: effect of glutathione on the binding of bile acids. *J. Lipid Res.* 1988. **29:** 279–286.

Supplementary key words flow dialysis • equilibrium dialysis • bilirubin • sulfobromophthalein • lithocholic acid • chenodeoxycholic acid • cholic acid

The glutathione S-transferases are a unique family of cytosolic proteins for which a dual role has been suggested (1, 2). These enzymes catalyze important detoxification reactions, but also bind nonsubstrate ligands, such as bilirubin and bile acids. The large content of these proteins in liver, the organ that translocates bilirubin and bile acids from blood to bile, suggests that the interaction of these ligands with the glutathione S-transferases is an important aspect of the process (1, 2). The only direct data pertaining to this possibility indicate the probable role of the binding interaction of ligands with these cytosolic proteins in minimizing the free intracellular concentration available for back-diffusion into plasma (3).

We and others have extensively characterized the binding properties of GSH S-transferases from rat liver (4–12). The majority of these enzymes can be divided into two groups: one group consists of homo- and heterodimers of Y_a or Y_c subunits (Y_aY_a, Y_aY_c, Y_cY_c) and the other group consists of Y_b or Y_b' subunits (Y_bY_b, Y_bY_b', $Y_b'Y_b'$). Our work has especially focused on the binding properties for bile acids (8, 9). We previously have found marked differences in the binding of lithocholic acid versus cholic acid (8, 9). Lithocholic acid binds to a unique high affinity site on the Y_a subunit which is shared by bilirubin and BSP (8). Cholic acid binds to a different site on the Y_a subunit (9). The site on the Y_a subunit responsible for chenodeoxycholic acid binding has not been determined. The number of different sites on the Y_b subunits for bile acids and organic anions also has not been defined. However, it is known that the Y_b family does not exhibit high affinity binding of lithocholic acid (9) or bilirubin (6).

In examining the binding properties of GSH S-transferases, an important issue, only touched upon in our previous work, is the effect of GSH on binding of bile acids and organic anions (9). In some cases, GSH inhibited binding but the type of inhibition was not characterized. Since it is expected that GSH will be present physiologically at saturating concentrations (millimolar), the effect of GSH on binding may be very important and sites unaffected by GSH may be expected to preferentially

Abbreviations: BSP, sulfobromophthalein; ANS, 1-anilino-8-naphthalene sulfonate; GSH, reduced glutathione; GSSG, oxidized glutathione; DTT, dithiothreitol.

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bind ligands. Therefore, we undertook to assess the effects of GSH on ligand binding while further studying the differences in binding properties of the so-called Y_a and Y_b classes of enzymes.

METHODS

Chemicals

All bile acid samples, bilirubin, sulfobromophthalein (BSP), 1-anilino-8-naphthalene sulfonate (ANS), reduced (GSH) and oxidized (GSSG) glutathione, and dithiothreitol (DTT) were purchased from Sigma Chemical Co. (St. Louis, MO). The following radioactive materials were used: [^{14}C]lithocholic acid (55 mCi/mmol) and [^{14}C]chenodeoxycholic acid (59.8 mCi/mmol) from Amersham, Arlington Heights, IL; [^{14}C]cholic acid (52 mCi/mmol), [^{14}C]glycocholic acid (52.5 mCi/mmol), [^{3}H]taurocholic acid (3.8 Ci/mmol), and [^{3}H]GSH (1.0 Ci/mmol) from New England Nuclear, Boston, MA.

Purification of GSH S-transferases from rat liver

GSH S-transferases were purified from male Sprague-Dawley rat livers as we previously reported (9). Y fraction was obtained by gel filtration of cytosol on Sephadex G75 superfine and further purified with octyl-GSH affinity chromatography and chromatofocusing on polybuffer exchanger 118 (Pharmacia). The four major forms of GSH S-transferase (Y_aY_a, Y_aY_c, Y_bY_b and Y_bY_b') were homogeneous as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 12.5% gel according to the method of Laemmli (13). According to the nomenclature of Jakoby, Ketterer, and Mannervik (14), these proteins, respectively, are GSH S-transferase 1-1, 1-2, 3-3, and 3-4. All proteins were stored at −70°C for no longer than 2 weeks before use in binding studies. Protein concentrations were determined by amino acid analysis.

Binding studies

Binding of lithocholic acid by equilibrium dialysis. Binding of lithocholic acid by GSH S-transferases was studied by equilibrium dialysis as previously reported (5, 8, 9) at 4°C. A tracer amount of [^{14}C]lithocholic acid (1 nCi) with various concentrations of the unlabeled lithocholic acid (0.2–2 μM) was added to the protein compartment (0.5 ml of 0.79 μM Y_aY_a, 1.0 μM Y_aY_c, 9.4 μM Y_bY_b, or 10 μM Y_bY_b') in the absence and presence of GSH (0.5 or 1 mM). The other compartment contained 0.5 ml of 0.01 M sodium phosphate buffer, pH 7.4. After equilibrium was reached (16 hr), radioactivity on both sides of the Spectrapor 2 membrane (Spectrum Medical Industries, Los Angeles, CA) was determined. In the experiments to examine the effects of various substrates on lithocholic acid binding, various concentrations of GSH, oxidized glutathione (GSSG), dithiothreitol (DTT), sulfobromophthalein (BSP), or various bile acids were added to the mixture of fixed concentrations of [^{14}C]lithocholic acid and protein and equilibrium dialysis was performed. The dialysis cells were gassed with N_2; autoxidation of GSH under these conditions (4°C for 16 hr) was negligible.

Binding of bile acids by flow dialysis. Binding studies of cholic, glycocholic, taurocholic, and chenodeoxycholic acid by GSH S-transferases were performed by flow dialysis as previously reported (9) at 4°C. A Spectrapor membrane was placed between the upper and lower chambers. For control studies, 0.5 ml of 0.01 M sodium phosphate buffer, pH 7.4 containing a tracer amount of respective radiolabeled bile acids (0.1 μCi) was placed in the upper chamber. The lower chamber was eluted with the same buffer (30 ml/hr) and the outflow was collected every 2 min and radioactivity of each fraction was counted. Presence of GSH did not change the transfer rate of radioactivity from the upper to the lower chamber in the absence of protein. In the experiment with cholic acid, the same volume of buffer containing GSH S-transferase and labeled cholic acid was placed in the upper chamber. Protein concentrations were 52 μM for Y_aY_a, 83 μM for Y_bY_b, and 108 μM for Y_bY_b'. The lower chamber was perfused with the same buffer and the outflow was collected every 2 min. Various amounts of unlabeled cholic acid were added stepwise to the upper chamber every 8 min and the radioactivity of each fraction was counted. The experiment with GSH was performed in the same way with 3 mM GSH in the buffer of the upper chamber and the eluate. Free fraction (f) at each bile acid concentration was calculated from the ratio of the fraction of radioactivity transferred with protein and that with buffer (9). The free (C_f) and bound (C_b) concentrations of bile acids were calculated from f and the total concentrations (C_f) of bile acids as previously described (9). In the experiments that assessed the effect of GSH on the binding of bile acids by GSH S-transferases, various amounts of GSH (0.1–7 mM) were added stepwise every 8 min to the upper chamber with fixed concentrations of labeled and unlabeled bile acids and the same protein concentrations as above. The lower chamber was eluted with the buffer, radioactivity was counted, and C_b and C_f values were calculated.

Analysis of the binding studies

Data of bile acid binding with various concentrations of bile acids in the absence and presence of GSH were expressed as Scatchard plots (15). Number of binding sites (n) and K_d values were calculated from the untransformed data by nonlinear least squares using the following equation:

$$C_b = \frac{n(p) \cdot C_f}{K_d + C_f}$$

where P is protein concentration. K_d values for flow dialysis experiments with single concentrations of bile acids were directly calculated using the above equation, where C_b, C_f, and P are known, and n values from experiments with various concentrations of cholic acid were used.

Inhibition constants (K_i) for various bile acids which inhibited the binding of lithocholic acid by Y_aY_a or Y_bY_b' were calculated by nonlinear least squares method as described in our previous report (8).

Binding of bilirubin by GSH S-transferases

Binding of bilirubin by Y_aY_a and Y_bY_b' were studied at room temperature by absorbance difference as previously reported (8). To cuvettes containing 2 ml of 0.01 M sodium phosphate buffer, pH 7.4, with 1 μM of bilirubin and either Y_aY_a (1 μM) or Y_bY_b' (2 μM), GSH or lithocholic acid was added stepwise and the absorbance difference (A_{472}–A_{405}) was monitored.

RESULTS

Effect of GSH on lithocholic acid binding by GSH S-transferases

GSH enhanced the binding of 0.1 μM lithocholic acid by Y_aY_a and Y_aY_c, whereas GSH showed little effect at 1.2 μM lithocholic acid (**Fig. 1**). This enhancement of binding at a lower concentration of lithocholic acid was reproducible in repeated experiments and was not affected by the order of addition of GSH, i.e., adding GSH first or last (data not shown). In contrast, GSH inhibited the binding of lithocholic acid by Y_bY_b and Y_bY_b' in a saturable, concentration-dependent manner (**Fig. 2**).

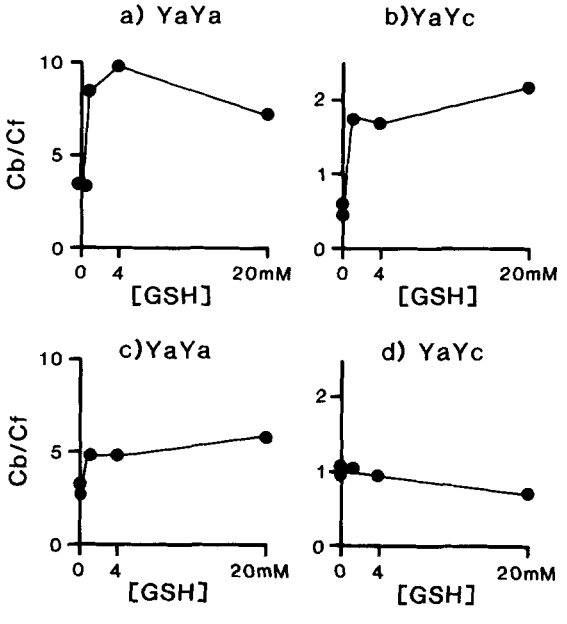

Fig. 1. Effect of GSH on the binding of lithocholic acid by Y_aY_a and Y_aY_c. Binding studies were performed by equilibrium dialysis with 0.1 μM (a,b) or 1.2 μM (c,d) lithocholic acid and 0.79 μM Y_aY_a (a,c) or 1.0 μM Y_aY_c (b,d). X-axis represents the initial concentration of GSH in the protein compartment.

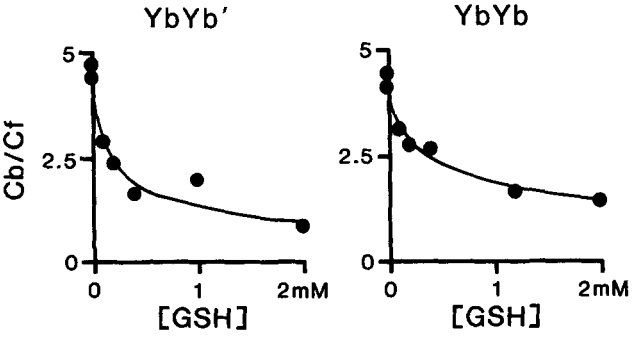

Fig. 2. Effect of GSH on the binding of lithocholic acid by Y_bY_b' and Y_bY_b. Binding studies were performed by equilibrium dialysis with 4 μM lithocholic acid and 10 μM Y_bY_b' or 9.4 μM Y_bY_b. X-axis represents the initial concentration of GSH in the protein compartment.

Fig. 3 shows Scatchard plots of binding of lithocholic acid by GSH S-transferases in the absence and presence of 0.5 or 1 mM GSH. Lithocholic acid binding by Y_aY_a or Y_aY_c was not affected by 1 mM GSH with the exception that the binding was enhanced at lower concentrations of lithocholic acid, consistent with the findings in Fig. 1. GSH competitively inhibited the binding of lithocholic acid by Y_bY_b or Y_bY_b'. Thus, GSH lowered the affinity but not the capacity to bind lithocholic acid.

Although GSH enhanced the binding of lithocholic acid (0.2 μM) by Y_aY_a, GSSG and DTT had no effect on binding (**Fig. 4**). Both GSH and GSSG inhibited the binding of lithocholic acid by Y_bY_b' whereas DTT had no effect on the binding.

Effect of GSH on binding of various other bile acids by GSH S-transferases

GSH inhibited the binding of cholic acid by Y_aY_a, Y_bY_b, and Y_bY_b' in a saturable, concentration-dependent fashion (not shown). Similar to Fig. 2 with lithocholic acid, maximum inhibition with GSH (up to 10 mM) was ~50%. Nearly identical inhibition by GSH of glycocholic and taurocholic acid binding to Y_aY_a and Y_bY_b' was observed (not shown). **Fig. 5** shows the binding of cholic acid by GSH S-transferases in the absence and presence of 3 mM GSH. GSH competitively inhibited the binding of cholic acid by all isozymes. GSH lowered the affinity but not the capacity to bind cholic acid.

The effect of GSH on the binding of chenodeoxycholic acid (not shown) by GSH S-transferases was nearly identical to that of lithocholic acid, as was seen in Figs. 1 and

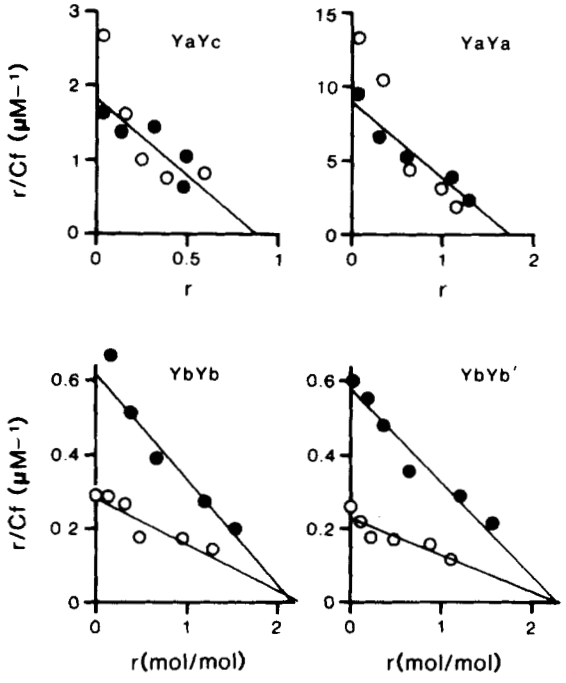

Fig. 3. Scatchard plots showing the effect of GSH on the binding of lithocholic acid by rat GSH S-transferases. Binding studies were performed by equilibrium dialysis with isozymes (1 μM Y_aY_c, 0.79 μM Y_aY_a, 9.4 μM $Y_bY'_b$ and 10 μM Y_bY_b) in the absence (●) or presence (○) of GSH (1 mM for experiments with Y_aY_c or Y_aY_a, and 0.5 mM for experiments with $Y_bY'_b$ or Y_bY_b); r, mol bound lithocholic acid/mol protein and C_f, unbound lithocholic acid concentration. Calculated parameters were K_d = 0.49 μM, n = 0.88 (n refers to binding stoichiometry, mole/mole) for Y_aY_c without GSH; K_d = 0.19 μM, n = 1.73 for Y_aY_a without GSH; K_d = 3.5 μM, n = 2.16 for Y_bY_b without GSH; K_d = 8.1 μM, n = 2.24 for Y_bY_b with GSH; K_d = 3.9 μM, n = 2.27 for $Y_bY'_b$ without GSH; and K_d = 10 μM, n = 2.29 for $Y_bY'_b$ with GSH. The standard deviations of the parameters computed from the fits were within 20% of the mean values. The open circles on the upper panels were not fit due to enhanced binding at low concentrations of lithocholic acid in the presence of GSH.

2. GSH exhibited no effect on the binding of chenodeoxycholic acid by Y_aY_a whereas GSH inhibited the binding of chenodeoxycholic acid by Y_bY_b' in a saturable, concentration-dependent fashion. As was the case with lithocholic acid binding by Y_bY_b', the inhibition by GSH was competitive and maximal inhibition was ~50%.

Table 1 summarizes the dissociation constants for binding of bile acids by GSH S-transferases. All data were in good agreement with our previous report, as listed in parentheses (9).

Binding of bilirubin and BSP by transferase Y_bY_b'

The effect of GSH on the binding of bilirubin by Y_aY_a and Y_bY_b' was examined by its effect on the difference spectra of bound bilirubin (**Fig. 6**). GSH did not inhibit the binding of bilirubin by Y_aY_a whereas GSH inhibited the binding of bilirubin by Y_bY_b'. Lithocholic acid had no effect on the binding of bilirubin by Y_bY_b' (not shown). This contrasts with our previous report showing that lithocholic acid and bilirubin compete for binding by Y_a subunits (8). In addition, BSP did not inhibit the binding of lithocholic acid by Y_bY_b' (not shown).

Competitive displacement of lithocholic acid from GSH S-transferases by various bile acids

Fig. 7 shows the effects of various bile acids on the binding of lithocholic acid by Y_aY_a and Y_bY_b'. Amino acid conjugates of lithocholic acid and chenodeoxycholic acid and its glycine and taurine conjugates inhibited the binding of lithocholic acid by Y_aY_a. Cholic acid and its conjugates had some effects on binding but only at very high concentrations, an order of magnitude above their K_d values (see Table 1). Therefore, these effects are probably nonspecific. Chenodeoxycholic and cholic acid and their amino acid conjugates inhibited the binding of lithocholic acid by Y_bY_b'. Calculated inhibition constants (K_i) for bile acids from the data of Fig. 7 are listed in **Table 2.** These K_i values are in reasonable agreement with K_d values in Table 1 or our previously reported data (5, 9).

DISCUSSION

We previously characterized the binding of bile acids by the Y_a versus Y_b class of GSH S-transferases (8, 9). The Y_a subunit exhibited a high affinity site shared by lithocholic acid, bilirubin, and BSP, but not cholic acid (8, 9). Y_aY_a exhibited two, and Y_aY_c one high affinity site (9), indicating that each Y_a subunit contained a site whereas the Y_c subunit apparently did not. The Y_b class of enzymes bound lithocholic acid (9), bilirubin, and BSP (6) with lower affinity than the Y_a subunit, and each subunit of the dimeric proteins exhibited a binding site (9). Cholic acid was bound with comparable affinity by the Y_a and

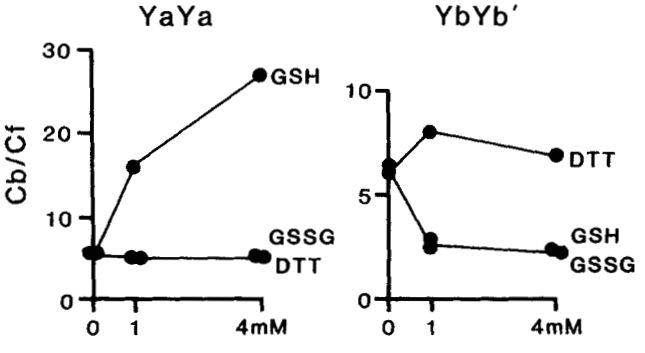

Fig. 4. Effect of GSH, GSSG, and dithiothreitol (DTT) on the binding of lithocholic acid by Y_aY_a or $Y_bY'_b$. Binding studies were performed by equilibrium dialysis with 0.2 μM lithocholic acid and 0.79 μM Y_aY_a; or 4 μM lithocholic acid and 10 μM $Y_bY'_b$. X-axis represents initial concentrations in the protein compartment.

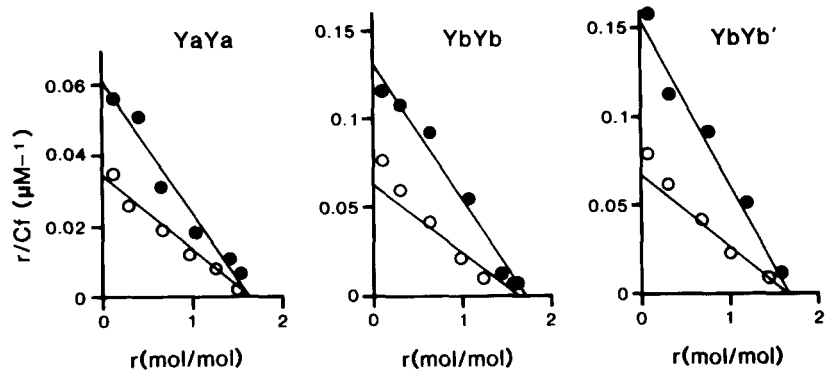

Fig. 5. Scatchard plots showing the effect of GSH on the binding of cholic acid by GSH S-transferases. Binding studies were performed by flow dialysis with 52 μM Y_aY_a, 83 μM Y_bY_b, or 108 μM $Y_bY'_b$ in the absence (●) and presence (○) of 3 mM GSH. Calculated parameters for cholic acid binding were K_d = 27 μM, n = 1.62 for Y_aY_a without GSH; K_d = 47 μM, n = 1.62 for Y_aY_a with GSH; K_d = 11 μM, n = 1.66 for $Y_bY'_b$ without GSH; K_d = 25 μM, n = 1.64 for $Y_bY'_b$ with GSH; K_d = 13 μM, n = 1.71 for Y_bY_b without GSH; and K_d = 26 μM, n = 1.64 for Y_bY_b with GSH. The standard deviations of the parameters computed from the fits were within 20% of the mean values.

Y_b classes (9). We previously found that GSH inhibited the binding of cholic acid to both Y_a- and Y_b-containing proteins (9). In contrast, GSH had no effect on lithocholic acid binding to the Y_a subunit but inhibited its binding to the Y_b' subunit (9). However, we did not examine the concentration dependence of the effect of GSH or the type of inhibition. Our previous suggestion that GSH inhibited cholic acid binding to a greater extent with Y_a- than Y_b-containing proteins was not confirmed in the present studies. The previous studies of the effect of GSH were preliminary and used transferases stored for more than 1 year. We have now repeated and extended this work with freshly prepared proteins.

In the present studies of the effects of GSH, we have found different effects of GSH on the bile acid and organic anion binding sites on the Y_a and Y_b subunits. These differences in effects of GSH further identify the nature of the binding sites on the Y_a and Y_b classes of GSH. GSH had little effect on the binding of lithocholic acid by Y_a subunits at near-saturating concentrations of ligand (1 μM) but markedly enhanced the binding of lithocholic acid at concentrations near its K_d (0.1 μM). We missed this finding in our previous work because of the high concentration of lithocholic acid previously employed. This enhancement of binding was not exhibited in the presence of GSSG or DTT. The mechanism for this enhanced binding is not certain. We speculate that the enhanced binding of lithocholic acid at low concentration in the presence of GSH insures sequestration of this potentially toxic bile acid. Inhibition of cholic acid binding to the Y_a subunit was observed in the presence of increasing concentrations of GSH. Thus, opposite effects of GSH on the binding of lithocholic acid and cholic acid by the Y_a subunit were observed. Taken together with our previous finding that cholic acid does not compete with lithocholic acid binding (5), the present findings add further support for distinct binding sites on the Y_a subunit for these two bile acids.

Chenodeoxycholic acid and its conjugates, although binding to the Y_a subunit with lower affinity than lithocholic acid, appeared to share the lithocholic acid site rather than the cholic acid site. Thus, chenodeoxycholic acid displaced lithocholic acid and its binding by Y_a-containing transferases was not inhibited by GSH. We have previously shown that bilirubin and BSP share the same high affinity site which binds lithocholic acid on the Y_a subunit (8). In the present work, we have shown that

TABLE 1. Dissociation constants (μM) for bile acid binding by GSH S-transferases

	Y_aY_a	Y_aY_c	Y_bY_b	Y_bY_b'
Lithocholic acid	0.19[a] (0.15[c])	0.49[a] (0.17[c])	3.5[a] (4.6[c])	3.9[a] (3.9[c])
Chenodeoxycholic acid	7.7[b] (8.9[d])	–	–	8.3[b]
Cholic acid	27[a] (46[c])	– (79[c])	13[a] (27[c])	11[a] (24[c])
Glycocholic acid	16[b]	–	–	19[b]
Taurocholic acid	–	–	–	41[b]

Dashes indicate not determined. The standard deviations of the fitted parameters were all within 10% of the mean values listed.

[a] From Scatchard plots of data obtained by equilibrium or flow dialysis analyzed as described in Methods.

[b] From flow dialysis with single concentrations of ligand calculated as described in Methods.

[c] Values from our previous report (9) obtained by flow dialysis at room temperature.

[d] Values from our previous report (9) obtained by 1-anilino-8-naphthalenesulfonate displacement at room temperature.

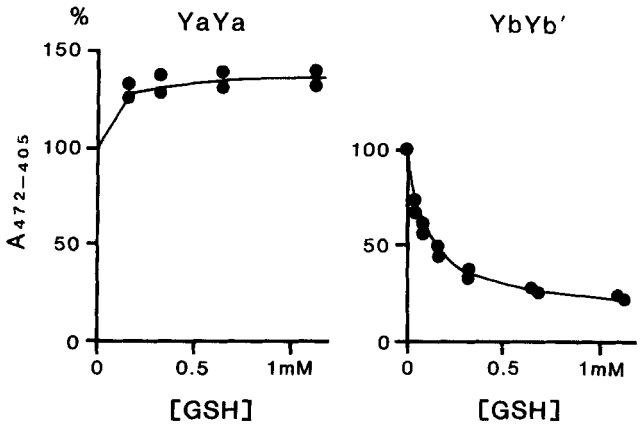

Fig. 6. Effect of GSH on the binding of bilirubin by Y_aY_a and Y_bY_b'. Binding of bilirubin was studied by absorbance difference as described in the text with fixed concentrations of bilirubin (1 μM) and protein (1 μM Y_aY_a or 2 μM Y_bY_b').

GSH does not affect bilirubin binding to this site as is the case with lithocholic acid.

The binding of lithocholic, chenodeoxycholic, and cholic acids by the Y_b subunit was inhibited by GSH. In addition, chenodeoxycholic acid, cholic acid, and their amidates displaced lithocholic acid from the Y_b subunit. Thus, all the bile acids appeared to share the same site on the Y_b subunit. Bilirubin binding by the Y_b subunit was inhibited in the presence of GSH. In the case of the Y_b subunit, BSP did not displace lithocholic acid and lithocholic acid did not inhibit bilirubin binding. Thus, organic anions (bilirubin and BSP) and bile acids appear to bind to two distinct sites on the Y_b subunit. We previously reported that the organic anion site on the Y_b subunit does not exhibit binding of the high affinity type seen with the Y_a subunit (6) and we now report that the organic anion site on the Y_b subunit does not bind lithocholic acid.

On the basis of this and our previous publications (8, 9), the subunits of the Y_a and Y_b class of transferases have markedly different binding properties which can be summarized as follows (see **Fig. 8**): *1*) Y_a subunit: each subunit exhibits a common binding site for lithocholic acid, chenodeoxycholic acid, their amidates, bilirubin, and BSP and GSH does not inhibit this site; each Y_a subunit exhibits a different site for cholic acid and its conjugates and this site is inhibited by GSH. *2*) Y_b subunit: each Y_b or Y_b' subunit exhibits a common bile acid site which is inhibited by GSH and a distinct common organic anion site which also is inhibited by GSH. The precise mechanism by which GSH (or GSSG) inhibits binding is uncertain. From the titration experiments with varying concentrations of GSH, inhibition by GSH is saturable and exhibits an IC_{50} for GSH in the 0.1–0.5 mM range. The K_m for GSH is 0.1–0.2 mM so that the inhibition by GSH parallels the binding affinity for GSH (2). The inhibition being dependent on GSH binding is further supported by the similar effect of GSSG which is known to competitively inhibit GSH binding (2). When GSH inhibited binding, the effect was apparently competitive with both Y_a and Y_b subunits, regardless of the bile acid. Three possibilities should be considered to explain the

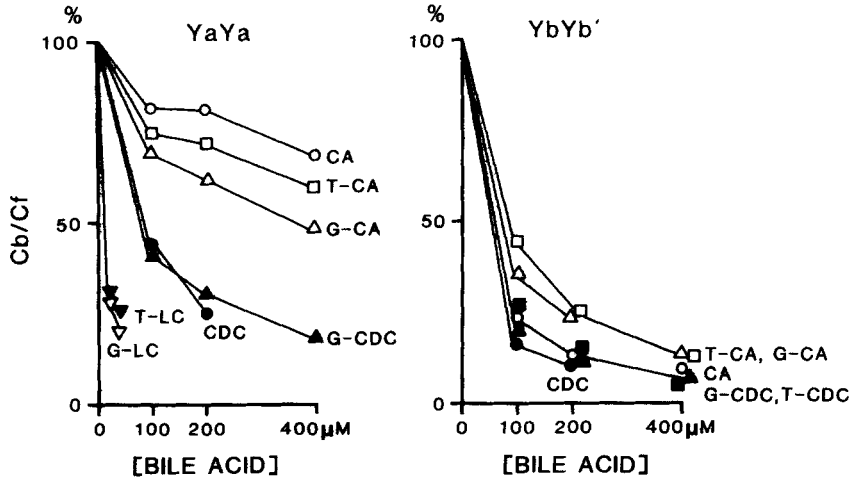

Fig. 7. Effect of various bile acids on the binding of lithocholic acid by Y_aY_a and Y_bY_b. Binding studies were performed by equilibrium dialysis with fixed concentrations of lithocholic acid and protein: 0.16–0.22 μM lithocholic acid and 0.79 μM Y_aY_a, or 4 μM lithocholic acid and 10 μM Y_bY_b. G-LC, glycolithocholic acid (▽); T-LC, taurolithocholic acid (▼); CDC, chenodeoxycholic acid (●); G-CDC, glycochenodeoxycholic acid (▲); T-CDC, taurochenodeoxycholic acid (■); CA, cholic acid (○); G-CA, glycocholic acid (△); T-CA, taurocholic acid (□).

TABLE 2. Inhibition constants (K_i) for bile acids determined from inhibition of the binding of radiolabeled lithocholic acid by Y_aY_a and Y_bY_b using equilibrium dialysis

Bile Acid	K_i	
	Y_aY_a	Y_bY_b
	μM	
Glycolithocholic acid	3.9	n.d.
Taurolithocholic acid	4.5	n.d.
Chenodeoxycholic acid	33	7.7
Glycochenodeoxycholic acid	37	11
Taurochenodeoxycholic acid	n.d.	11
Cholic acid	[a]	13
Glycocholic acid	[a]	25
Taurocholic acid	[a]	28

K_i values were calculated by the competitive inhibition model described in a previous report (8). The standard deviations of the fitted parameters were all within 10% of the mean values listed; n.d., not determined. See legend to Fig. 7 for further details.

[a] Nonspecific effects.

mechanism of inhibition. *a*) Bile acids might bind to the GSH substrate site. This is unlikely because bile acids are not pure competitive inhibitors of the enzyme activity of the GSH S-transferases with respect to GSH (5, 7, 9, 16). Moreover, in all cases maximum inhibition by GSH does not completely displace bile acids from the proteins. *b*) GSH might bind to a secondary site distinct from the GSH substrate site and this site might be shared with other ligands. This is unlikely because the inhibitory potency of GSH parallels its binding affinity for the GSH substrate site. Since GSH does not completely displace ligands, it is unlikely that GSH and ligands compete for the same site. *c*) The binding of GSH to its substrate site may result in a conformational change in the protein which leads to an alteration in the affinity of a structurally distinct ligand-binding site, i.e., an allosteric inhibitory effect. The apparent requirement for GSH binding to its substrate site to cause inhibition favors this possibility. The fact that GSH inhibits the binding of bilirubin and bile acids to two distinct sites on the Y_b subunit also favors this interpretation.

The hypothesis that the binding of GSH to the GSH S-transferases induces a conformational change which lowers the affinity of the low affinity ligand binding site is consistent with the previous findings of Vander Jagt et al. (17). They observed that the presence of GSH forces the transferases to adopt different conformations upon addition of bilirubin that retain their transferase activity.

Regardless of the mechanisms involved, the binding properties and physiologic role of the GSH S-transferases as ligandins must be viewed in relation to the effects of GSH on binding. GSH enhances or has no effect on the high affinity binding site on the Y_a subunit but inhibits the cholic acid binding site on the Y_a and both the bile acid and organic anion binding sites on the Y_b class of subunits. Under physiologic conditions, the GSH concentration is in the millimolar range and, therefore, the transferases are completely saturated with GSH. Consequently, the binding of bile acids and organic anions by the Y_b class of transferases will be inhibited, thereby leaving the high affinity site of the Y_a class of GSH S-transferases selectively available for binding of lithocholic acid, chenodeoxycholic acid, their amidates, and bilirubin. Thus, the differences in the effects of GSH on the binding properties of the two classes of transferases

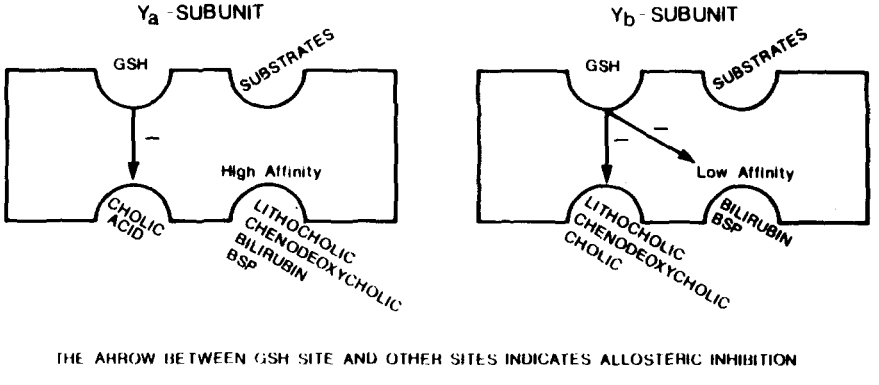

Fig. 8. Comparison of the binding properties of the subunits of the hepatic GSH S-transferases from the rat. The Y_a and Y_b subunits are schematically represented; the properties of the Y_b subunit would be the same as Y_b. Each subunit exhibits two non-substrate binding sites with different specificities. The Y_a subunit exhibits a high affinity site which is shared by lithocholic, chenodeoxycholic acids, their amino acid conjugates (not listed), bilirubin and BSP. This site is not influenced by GSH. The Y_a subunit exhibits a separate site for unconjugated and conjugated cholic acid which is inhibited (arrow) when GSH binds to its site. The Y_b subunit exhibits a common site for all bile acids and a separate site for bilirubin and BSP. The former binds lithocholic acid with low affinity and the latter binds organic anions with low affinity. Both non-substrate sites are inhibited when GSH binds to its site (arrows).

may largely determine the interaction of bile acids with these proteins. The precise physiological purpose of preferential binding of mono- and dihydroxy bile acids by a high affinity site on the Y_a subunits of the GSH S-transferases is unknown. However, the selectivity for mono- and dihydroxy bile acids as compared to trihydroxy bile acids suggests that binding of the former two may be important in detoxification by minimizing concentrations of their free forms in the cell.

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