The Glutathione S-Transferases of the Small Intestine in the Rat

G. Clifton and N. Kaplowitz

Clinical Investigation Center, Naval Regional Medical Center, Oakland, California 94627 [G. C.], and the Gastroenterology Division, Medical and Research Services, Veterans Administration, Wadsworth Hospital Center, Los Angeles, California 90073 [N. K.]

SUMMARY

Glutathione S-transferase activities have been identified in the small intestine of the rat. Three activities obtained with p-nitrobenzyl chloride (aralkyl), 1,2-epoxy-3-(p-nitrophenoxy)propane (epoxide), and ethacrynic acid (alkene) as substrates were present in significant amounts. Gel filtration indicated an elution volume for the intestinal transferase activities that was similar to those activities in the liver and kidney. The induction of the intestinal transferases by polycyclic aromatic hydrocarbons and phenobarbital is similar to those effects observed previously for the hepatic and renal enzymes. The highest concentration of transferase activities occurs in the proximal small intestine; these activities are reduced upon fasting. Parallel observations have been reported for aryl hydrocarbon hydroxylases. Because only low or negligible levels of epoxide hydrides have been reported in the small intestine, the glutathione S-transferases may be the primary epoxide-detoxifying system in that organ.

INTRODUCTION

The glutathione S-transferases are detoxifying enzymes that exist in the cytosol of liver cells. These enzymes catalyze the conjugation of glutathione with a variety of exogenous substrates (2—4, 8, 15) including the products of microsomal mixed-function oxygenases (13, 22, 23). These 2 enzyme systems may be considered complementary; thus, in the biotransformation of foreign compounds, 1 means of detoxification of microsomal activated metabolites is interaction with glutathione catalyzed by the transferases. Aside from this biochemical association of toxification-detoxification, both enzyme systems are responsive to the 2 major kinds of enzyme inducers, phenobarbital and polycyclic aromatic hydrocarbons (6, 9, 18). Furthermore, in addition to the liver, the occurrence and inducibility of both enzyme systems have been reported for the kidney (5, 10).

The microsomal mixed-function oxygenases also have been identified in the small intestine and may have an important role in the metabolism of absorbed drugs and carcinogens (12). Aryl hydrocarbon hydroxylase activity has been induced in the small intestine of animals (7, 9), and levels of cytochrome P-450 and hydroxylase activity appear to be under dietary control (14, 27, 29). Thus, it seemed of interest and importance to consider the possibility of the parallel occurrence and distribution of the glutathione S-transferases in the small intestine as well as the effect of fasting and the possibility of drug induction in this organ.

MATERIALS AND METHODS

Animals, Treatment, and Preparation of Intestinal Cytosol. Three groups, each containing 8 male 270- to 330-g Sprague-Dawley rats, were given phenobarbital (Sigma Chemical Co., St. Louis, Mo.), 8 mg/100 g i.p. in 0.9% NaCl solution daily for 10 days; 3,4-benzo(a)pyrene (Sigma), 1 mg i.p. in corn oil twice daily for 10 days; or 3-methylcholangthrene (Calbiochem, San Diego, Calif.), 1 mg i.p. in corn oil twice daily for 10 days. Two 6-animal groups were deprived of food but not water for 24 and 48 hr, respectively. A control group of 8 rats was given i.p. 0.9% NaCl solution. Animals were killed with ether anesthesia 24 hr after the last treatment. Small intestines were removed; washed with 0.01 M sodium phosphate buffer, pH 7.4; and trisected into proximal, middle, and distal segments. For each weighed segment, 20% (w/v) homogenates (0.01 M sodium phosphate-0.25 M sucrose buffer, pH 7.4) were prepared and centrifuged at 106,000 x g for 60 min in a Beckman Model L2-65B ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). Lipid layer was removed by suction. Cytosol fractions were decanted and stored at −15°.

Gel Filtration. Gel filtration at 4° was done with Sephadex G-100 (Pharmacia, Uppsala, Sweden) columns, 35 x 2.5 cm, with 0.01 M sodium phosphate buffer, pH 7.4, as the mobile phase. Flow rate was 30 ml/hr; 10 fractions/hr were collected. For gel filtration experiments, concentrated intestinal cytosol, 20% original volume, was prepared by ultratfiltration at 4° with Diaflo Model PM10 ultrafilters (Amicon Corp., Lexington, Mass.). In certain experiments sulfobromophthalein (3.0 mg) was added to the concentrated cytosol immediately before placement on the column. Sulfobromophthalein binding to protein in column fraction was determined by alkalization and measurement of absorbance at 580 nm.

Determination of Enzyme Activity. Five glutathione S-
Comparison of enzyme activities in 3 segments of intestine, the liver, and kidney of the rat

Activity for glutathione S-transferase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Small Intestine</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Middle</td>
<td>Distal</td>
</tr>
<tr>
<td>Ethacrynic acid</td>
<td>29.1 ± 1.9</td>
<td>21.1</td>
<td>13.1</td>
</tr>
<tr>
<td>1,2-Epoxy-3-(p-nitrophenoxy)propane</td>
<td>3.65 ± 0.33</td>
<td>2.52</td>
<td>2.46</td>
</tr>
<tr>
<td>p-Nitrobenzyl chloride</td>
<td>5.63 ± 0.45</td>
<td>2.95</td>
<td>2.95</td>
</tr>
<tr>
<td>3,4-Dichloronitrobenzene</td>
<td>Trace</td>
<td>Trace</td>
<td>113</td>
</tr>
<tr>
<td>Methyl iodide</td>
<td>ND</td>
<td>ND</td>
<td>16.6</td>
</tr>
</tbody>
</table>

Intestinal Glutathione S-Transferases

Enzyme activities were determined by previously described methods (11, 18, 26). The following substrates were used in 2.0-mL reaction volumes: 1.0 mM 3,4-dichloronitrobenzene (aryl substrate) at 37°C (Aldrich Chemical Co., Milwaukee, Wis.); 0.5 mM p-nitrobenzyl chloride (aryl substrate) at 37°C (Aldrich); 0.5 mM 1,2-epoxy-3-(p-nitrophenoxy)propane (epoxide substrate) at 37°C (Eastman Kodak Co., Rochester, N. Y.); 0.2 mM ethacrynic acid (alkene substrate) at 37°C (a gift from Merck Sharp & Dohme Research Laboratories, Rahway, N. J.); 1.5 mM [14C]methyl iodide (alkyl substrate) at 20°C (New England Nuclear, Boston, Mass.). Excess glutathione (10 mM; Sigma) was used for each reaction except for the ethacrynic acid activity for which 0.25 mM glutathione was used. Reactions were initiated by the addition of 200 µl of intestinal cytosol except for the alkene activity for which 50 µl were used. Volumes were selected to minimize the error involved in determination of raw activities and yet meet the requirement that the enzymatic reactions be linear with respect to time and protein concentration. Nonenzymatic reaction rates of substrates were subtracted from the enzymatic rates.

Protein concentrations were determined by the method of Lowry et al. (21).

Comparison of activities between groups of rats was done by unpaired Student's t test (1).

RESULTS

Distribution of Enzyme Activities in the Small Intestine and Comparison with Values of Hepatic and Renal Transferase Activities. Three glutathione S-transferase activities were present in significant amounts in the small intestine of the rat: epoxide, p-nitrobenzyl chloride, and ethacrynic acid (Table 1). Activity with 3,4-dichloronitrobenzene was present in trace amounts. Methyl iodide activity was not detectable. For the 3 measurable transferases, the level of enzymatic activity in the proximal intestine was significantly greater than the levels in either the middle or distal intestine. The levels of specific activity in the proximal intestine were significantly lower than values previously determined for the hepatic and renal transferases.

Gel Filtration of Intestinal Transferases. Concentrated intestinal cytosol (3.0 ml) was filtered through a Sephadex G-100 column (Chart 1). Glutathione S-transferase activities with ethacrynic acid, epoxide, and p-nitrobenzyl chloride had superimposable elution peaks when the individual column fractions were assayed. This elution volume (90 ml) corresponds to that found for the hepatic and renal transferases determined with the same column conditions (17, 19). In contrast to hepatic cytosol, there was no detectable binding of sulfobromophthalein to the column fractions containing the intestinal transferases. This observation is consistent with our previous demonstration of the relationship between sulfobromophthalein binding to column fractions of liver and kidney cytosol and the presence of 3,4-dichloronitrobenzene activity (aryltransferase) (16, 17).

Effect of Fasting on Enzymatic Activities. The transferase activities for the 3 readily detectable substrates were diminished in the intestines from animals that had been fasted for 48 hr (Chart 2). Transferase activities determined with epoxide and p-nitrobenzyl chloride substrates were reduced significantly in the proximal and distal intestine after 48-hr starvation. Activity with epoxide substrate

Table 1

Comparison of enzyme activities in 3 segments of intestine, the liver, and kidney of the rat

<table>
<thead>
<tr>
<th>Substrate for glutathione S-transferase activity</th>
<th>Proximal</th>
<th>Middle</th>
<th>Distal</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethacrylic acid</td>
<td>29.1 ± 1.9</td>
<td>21.1 ± 1.6</td>
<td>13.1 ± 1.7</td>
<td>43.5 ± 1.9</td>
<td>47.0 ± 1.6</td>
</tr>
<tr>
<td>1,2-Epoxy-3-(p-nitrophenoxyl)propane</td>
<td>3.65 ± 0.33</td>
<td>2.52 ± 0.33</td>
<td>2.46 ± 0.13</td>
<td>11.6 ± 0.7</td>
<td>7.89 ± 0.40</td>
</tr>
<tr>
<td>p-Nitrobenzyl chloride</td>
<td>5.63 ± 0.45</td>
<td>2.95 ± 0.18</td>
<td>2.95 ± 0.18</td>
<td>169 ± 6</td>
<td>29.5 ± 2.7</td>
</tr>
<tr>
<td>3,4-Dichloronitrobenzene</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>113 ± 5</td>
<td>3.80 ± 0.29</td>
</tr>
<tr>
<td>Methyl iodide</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>16.6 ± 0.6</td>
<td>15.3 ± 0.8</td>
</tr>
</tbody>
</table>

* Mean ± S.D.  
+ Spontaneous conversion of substrate: 58% of proximal value for ethacrylic acid; 11% for 1,2-epoxy-3-(p-nitrophenoxyl)propane; and 19% for p-nitrobenzyl chloride.  
+ For statistical comparison with proximal intestine.  
d ND, not detectable.
dropped to 44\% (p < 0.005) of control in the proximal segment and to 38\% (p < 0.001) of control in the distal segment. Activity with \(p\)-nitrobenzyl chloride was 59\% (p < 0.0025) and 55\% (p < 0.025) of the respective control values. Activity determined with ethacrynic acid as substrate was affected only in the proximal segment where it was found to be 74\% (p < 0.025) of control. None of the detectable activities in the middle segment was significantly lower under these experimental conditions.

**Induction of Enzymatic Activities by Polycyclic Aromatic Hydrocarbons and Phenobarbital.** The level of glutathione S-transferase activities with ethacrynic acid and \(p\)-nitrobenzyl chloride increased significantly in the middle and distal intestinal segments after treatment with benzo(a)pyrene and 3-methylcholanthrene (Table 2). 3-Methylcholanthrene produced 33 and 37\% increases of ethacrynic acid activity and 23 and 21\% increases of \(p\)-nitrobenzyl chloride activity in the middle and distal intestine, respectively.

**DISCUSSION**

Several hepatic and renal glutathione S-transferases are induced by polycyclic aromatic hydrocarbons and pheno-
Intestinal Glutathione S-Transferases

In this study, we have reported the induction of glutathione S-transferase activities for ethacrynic acid and p-nitrobenzyl chloride by benzo(a)pyrene and 3-methylcholanthrene in the more distal portions of the intestine. Interestingly, the induction of microsomal benzo(a)pyrene hydroxylase in the small intestine by 1,2-benzanthracene is also greatest in the more distal intestine (30). From our observations about the distribution and inducibility of the transferase activities in small intestine, kidney, and liver as well as the demonstration of overlapping substrate specificities of the pure enzyme proteins from liver (11), it is apparent that the regulation and effects of inducing agents on this system are complex. In addition, p-nitrobenzyl chloride activity is induced by phenobarbital in the rat kidney (5). Recently, glutathione S-transferase activity in intestinal cells with 1-chloro-2,4-dinitrobenzene as substrate has been shown to increase after phenobarbital administration (20).

The distribution of glutathione S-transferases along the length of the small intestine is similar to that previously reported for the benzo(a)pyrene hydroxylase system (25). In both studies the bulk of activity is in the more proximal segments and drops off distally. Another similarity between the 2 enzyme systems occurs in animals that have been fasted. Fasting significantly decreases both benzo(a)pyrene hydroxylase and glutathione S-transferase activities in the small intestine of the rat (30). For the glutathione S-transferases, the effect appears to be more prominent in the proximal versus middle or distal segment.

The glutathione S-transferases parallel the mixed-function oxygenases and aryl hydrocarbon hydroxylases in various tissues including the small intestine. Aromatic hydroxides, the presumed carcinogenic intermediates produced by action of aryl hydrocarbon hydroxylases, are enzymatically detoxified either by conjugation with water (epoxide hydrase) or glutathione (glutathione S-transferases). In the small intestine low or undetectable levels of epoxide hydrase activity have been reported (24, 25, 28). Therefore, in the small intestine enzymatic detoxification of epoxide intermediates under the influence of the glutathione S-transferases may predominate. The influence of diet and inducing agents on the glutathione S-transferases may have a critical role in the metabolism and action of carcinogens in this organ.

REFERENCES

The Glutathione S-Transferases of the Small Intestine in the Rat

G. Clifton and N. Kaplowitz


Updated version  Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/37/3/788

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.