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DO THE SOLUBLE GLUTATHIONE *S*-TRANSFERASES HAVE DIRECT ACCESS TO MEMBRANE-BOUND SUBSTRATES?

THOMAS D. BOYER,*† DAVID ZAKIM‡ and DONALD A. VESSEY‡

†‡Liver Studies Unit, Department of Medicine, Veterans Administration Medical Center, San Francisco, CA 94121, and the Departments of †‡Medicine and ‡Pharmacology, and the †‡Liver Center, University of California, San Francisco, CA 94143, U.S.A.

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Abstract—The ability of the soluble glutathione *S*-transferases to bind the membrane (liposome) bound substrates 1-chloro-2,4-dinitrobenzene and sulfobromophthalein was determined. The transferases were found to have access only to substrates in the aqueous phase. They could not bind membrane-bound substrates and, thus, enzymatic activities were reduced by the membrane partitioning of the substrates. The reduction in enzymatic activity was directly proportional to the lipid solubility of the substrate. The liposomes had no direct effect on the enzyme *per se*. [^{35}S]Sulfobromophthalein and [^{14}C]chlorodinitrobenzene bound to liposomes were found to have rapid rates of release into the aqueous phase. Rates of hydration of chlorodinitrobenzene from liposomes were rapid enough such that rates of catalysis (measured in a stopped-flow spectrophotometer) were affected only by the partition coefficient of substrate between lipid phase and water, and not by the rate of transfer of substrate from lipid to water phase.

Many drugs and toxins are non-polar compounds and, as such, are soluble in lipids but poorly soluble in water. When added to an aqueous suspension of biological membranes, they partition preferentially into the lipid phase of the membrane [1, 2]. Similar partitioning into the internal membranes of the cell *in vivo* would reduce greatly the aqueous concentration of these non-polar molecules such that only small amounts of these molecules per unit time would be expected to move through the aqueous phase to their sites of metabolism. The soluble forms of the enzyme glutathione *S*-transferase (EC 2.5.1.18) bind a variety of non-polar molecules and, thus, have been suggested to increase the rate of movement of these non-polar molecules from the plasma membrane to intracellular sites of metabolism [3]. However, direct experimental proof of this idea is lacking, and this could only occur if rates of release from the membrane are rapid. Rates of release for several molecules are slow (minutes to hours) [4–8], and this could limit the ability of the glutathione *S*-transferases to facilitate intracellular diffusion. However, if the soluble glutathione *S*-transferases could interact directly with membrane-bound molecules in a manner similar to phospholipid exchange protein [7], and perhaps α-tocopherol transfer protein [9], then their function as both transport proteins and enzymes would not be limited by the solubility properties of ligands/substrates.

In this paper, we report that the soluble glutathione *S*-transferases are unable to remove non-polar electrophiles from artificial membranes. In addition, we show the importance of rates of release from the membrane in determining rates of catalysis.

MATERIALS AND METHODS

Materials. 1-Chloro-2,4-dinitrobenzene (CDNB)§ sulfobromophthalein (BSP), reduced glutathione (GSH) and egg lecithin were purchased from the Sigma Chemical Co., St. Louis, MO. 1-Chloro-2,4-dinitro-[U-^{14}C]benzene (5.87 mCi/mmole) and [^{35}S]sulfobromophthalein (125 mCi/mmole) were purchased from Amersham, Arlington Heights, IL.

Spectrophotometric assay methods. Enzyme activity was determined spectrophotometrically as described previously [10]. Assays were performed at 25° in 0.1 M potassium phosphate, pH 6.5, with CDNB as substrate, or at pH 7.5 with BSP as substrate. The appearance of *S*-2,4-(dinitrophenyl)-glutathione was monitored at 340 nm ($\varepsilon = 9.6 \times 10^3$ $M^{-1}\,cm^{-1}$), and the glutathione conjugate BSP at 330 nm ($\varepsilon = 4.5 \times 10^3\,M^{-1}cm^{-1}$) [10]. The extinction coefficients were unaffected by the presence of liposomes. All enzyme activities were corrected for non-enzyme rates when necessary. Activities for a given set of conditions varied by less than 10%. CDNB was added from an ethanol solution, and the concentration of ethanol was kept at 1% (v/v) or less. Substrates (CDNB and BSP) were mixed with the liposomes and buffer in the assay cuvette and allowed to stand for 20–30 min at room temperature. The reaction was initiated by the addition of GSH and then enzyme (final volume = 1 ml). Initial rates of enzymatic activity were determined using a

* Author to whom all correspondence should be addressed at: 151K, Veterans Administration Medical Center, 4150 Clement St., San Francisco, CA 94121, U.S.A.

§ Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; BSP, sulfobromophthalein; and GSH, reduced glutathione. reduced.

recording spectrophotometer. Activities are expressed as μmoles of product formed per min per mg protein. Lines were drawn by least-squares analysis of kinetic data. Protein was determined by either the biuret method [11] or the method of Schaffner and Weissmann [12].

Preparation of enzyme. Fed, male Sprague–Dawley rats (250–300 g) (Simonsen Laboratories, Gilroy, CA) were killed by a blow to the head. The livers were removed rapidly and placed in chilled 0.25 M sucrose. All subsequent procedures were performed at 0–4°. Purified glutathione *S*-transferases A, B and C were prepared as described previously [13].

Preparation of liposomes and determination of partition coefficients. Liposomes were prepared from egg lecithin by sonication [5]. The partition coefficients of [^{14}C]CDNB and [^{35}S]BSP in a lipid–water system were determined by filtration [5] and equilibrium dialysis at 25° in 0.1 M phosphate, pH 6.5 (CDNB) of pH 7.5 (BSP). Corrections for binding of BSP and CDNB by the Amicon membrane were made. When using equilibrium dialysis, the concentration of the ligand was measured on both sides of the membrane after equilibrium was achieved (24 hr). Both methods gave the same partition coefficients. Concentration of phospholipid in the liposomal suspension was determined gravimetrically following evaporation of the water.

Meausrements of rates of release of substrates from liposomes to water and effect of this release step on catalysis. Rates of release of [^{14}C]CDNB and [^{35}S]BSP from liposomes into buffer [phosphate, pH 6.5 (CDNB) or 7.5 (BSP)] were determined at room temperature as described previously [5]. Both [^{14}C]CDNB and [^{35}S]BSP bound to the membrane. The data have been corrected for this binding. The effect of these rates of release on catalysis was determined in a stopped-flow spectrophotometer (Dionex, Sunnyvale, CA, modified by O.L.I.S., Jefferson, GA). Liposomes (20 mg/ml) in 0.1 M phosphate, pH 6.5, were mixed with CDNB (final concn 1 mM) and allowed to stand for 20 min at room temperature. This solution was then diluted 1:10 in the recording cell of the stopped-flow spectrophotometer with a solution containing 0.01 mg/ml purified glutathione *S*-transferase A, 1 mM GSH, and 0.1 M phosphate, pH 6.5. The appearance of product was monitored at 340 nm over the first 2 sec of the reaction. All solutions and the recording cell were maintained at 25°. The amount of CDNB present at equilibrium in the aqueous phase in the presence of liposomes was calculated before (0.047 μmole) and after (0.332 μmole) dilution. Enzymatic rates were determined for the latter concentrations of CDNB in the stopped-flow spectrophotometer following a 1:10 dilution with the same enzyme/GSH solution (liposomes absent). All assays were performed in triplicate. Slopes were determined by least-squares analysis, and statistical analysis was performed using Student's *t*-test. All values are expressed as means ± S.E.M.

RESULTS

Interaction of glutathione S-transferases with membrane-bound and aqueous substrates. These experiments were performed to determine whether the glutathione *S*-transferases functioning in a liposome/water system interact with substrate in the aqueous phase, the membrane phase, or both. This is a significant question because most of the substrates of the soluble glutathione *S*-transferases partition preferentially into the lipid phase of a lipid/water system. For example, the partition coefficients of [^{14}C]CDNB and [^{35}S]BSP were, respectively, 4.5 ± 0.42 (S.E.M.) $\times 10^4$ and $9.2 \pm 0.53 \times 10^5$ in favor of the lipid phase. With regard to function *in vivo*, these substrates were also found to partition preferentially into liver microsomal membranes when the latter were suspended in an aqueous medium.

The aqueous concentration of substrate in a mixed lipid/water system was calculated from the partition coefficient of the substrate and the known amounts of lipid and aqueous phases. Initial rates of enzyme activity for that aqueous phase concentration of substrate were determined separately and compared with the rate observed in a two-phase system. If the enzyme interacts only with the substrate in the aqueous phase, rates measured at equal aqueous phase concentrations of substrate will not be altered by the presence of liposomes. On the other hand,

Table 1. Comparison of observed and expected enzymatic activities with CDNB and transferase B in the presence of liposomes*

$[CDNB]_t$ (mM)	Lipid (mg)	$[CDNB]_{H_2O}$ (mM)	Observed rate ($\mu moles \cdot min^{-1} \cdot mg^{-1}$)	Expected rate ($\mu moles \cdot min^{-1} \cdot mg^{-1}$)
0.05	1.04	0.024	1.4	1.5
0.10	1.04	0.049	2.5	2.8
0.20	1.04	0.097	4.3	4.8
0.50	1.04	0.243	8.3	8.3
0.05	2.1	0.016	1.0	1.0
0.10	2.1	0.032	1.9	2.0
0.50	2.1	0.162	6.8	6.7

* Each assay contained 0.1 M potassium phosphate, pH 6.5, 5 mM GSH, and 1.3 μg transferase B. The aqueous-phase concentration of 1-chloro-2,4-dinitrobenzene (CDNB) in the presence of liposomes was calculated based on a partition coefficient of 4.5×10^4, and the expected rate for that aqueous concentration of CDNB was obtained from a double-reciprocal plot of rate versus CDNB performed in the absence of liposomes. $K_{m(app)} = 0.27$ mM in the absence of liposomes. $[CDNB]_t$ = total concentration of CDNB in assay, and $[CDNB]_{H_2O}$ = aqueous concentration of CDNB in assay.

Table 2. Comparison of observed and expected enzymatic activities with BSP and transferase C in the presence of liposomes*

$[BSP]_t$ (μM)	$[BSP]_{H_2O}$ (μM)	Observed rate ($\mu moles \cdot min^{-1} \cdot mg^{-1}$)	Expected rate ($\mu moles \cdot min^{-1} \cdot mg^{-1}$)
10	4.7	0.11	0.12
15	6.9	0.14	0.14
20	9.2	0.18	0.16
30	13.9	0.19	0.18
50	23.1	0.22	0.21
100	46.2	0.25	0.23

* Each assay contained 0.1 M potassium phosphate, pH 7.5, 5 mM GSH, 4 μg transferase C, and 0.16 mg lipid as liposomes. The expected rate and the aqueous concentration of sulfobromophthalein (BSP) were obtained as described in Table 1. The partition coefficient for BSP was 9.2×10^5. $K_{m(app)} = 5\ \mu M$ in the absence of liposomes. $[BSP]_t$ = total concentration of BSP in assay; and $[BSP]_{H_2O}$ = aqueous concentration of BSP in assay.

if the enzymes has access to both aqueous and membrane-bound substrates, the rate measured in the presence of liposomes is likely to be consistently greater than that measured in their absence for equal concentrations of substrate in the water phase of the assay system. Rates measured at equal aqueous phase concentrations of substrate were the same in the presence and absence of liposomes (Tables 1 and 2). Soluble glutathione *S*-transferases appear, therefore, to have access to substrates in the bulk aqueous phase but do not appear to interact with substrates within artificial membranes.

Effects of liposomes on enzymatic activities of glutathione S-transferase. The failure of the glutathione *S*-transferases to remove substrates from the membrane means that catalytic activity will be related to the aqueous concentration of the substrate which can be determined directly from the partition coefficient of the substrate in a lipid–water system. Because BSP partitions more into liposomes than does CDNB, it is predicted that for a given concentration of lipid the rate of conjugation of BSP would be reduced to a greater extent than that of CDNB. As shown in Table 3, BSP conjugation was reduced to a much greater extent than was CDNB conjugation. The total concentrations of BSP and CDNB in these experiments were 5-fold greater than their respective K_m values.

To confirm further that the effect of the liposomes was due only to sequestration of the substrates and not to some other effect of the liposomes on the enzyme, the activities of glutathione *S*-transferases B and C were investigated at several concentrations of CDNB. If the effect of the liposomes were to sequester substrates, then they would appear to act as competitive inhibitors. This is what was observed (Figs. 1 and 2). At all but saturating concentrations

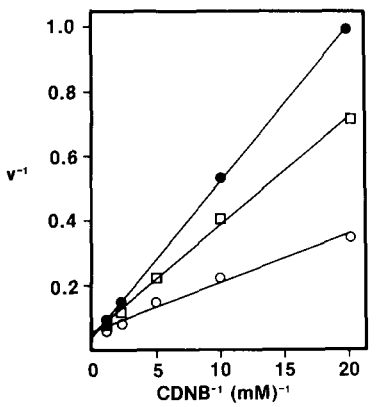

Fig. 1. Effect of liposomes on conjugation of CDNB by transferase B. Each assay contained 5 mM GSH; 0.1 M potassium phosphate, pH 6.5; 1.25 μg enzyme; and either none (○), 1 mg (□), or 2 mg (●) lipid as liposomes (egg lecithin). Rates are in μmoles per min per mg protein. Lines were drawn by least-squares regression analysis.

Table 3. Effect of liposomal lipids on activity of glutathione *S*-transferase C*

Amount of liposomal lipid (mg)	Rate ($\mu moles \cdot min^{-1} \cdot mg^{-1}$) BSP	CDNB
0	0.18	10.3
0.14	0.11 (39)†	9.3 (9)
0.27	0.09 (50)	9.3 (9)
0.55	0.03 (83)	8.2 (20)
1.37	0.0 (100)	7.7 (25)

* Each assay contained 0.1 M potassium phosphate (pH 6.5, CDNB; pH 7.5, BSP), 5 mM GSH, and 4 μg protein. The concentrations (total) of CDNB and BSP were 115 and 25 μM, respectively, which are 5-fold greater than the apparent K_m values when determined at 5 mM GSH.

† Percent inhibition.

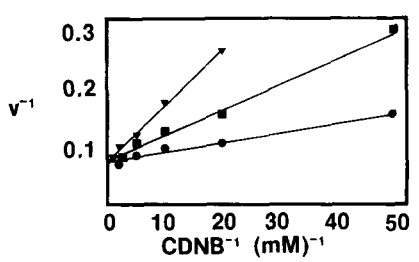

Fig. 2. Effect of liposomes on conjugation of CDNB by transferase C. The conditions of the assay were similar to those described in the legend of Fig. 1 except that each assay contained 2 μg of enzyme and either none (●), 4.4 mg (■), or 8.8 mg (▲) lipid as liposomes (egg lecithin). Rates are in μmoles per min per mg.

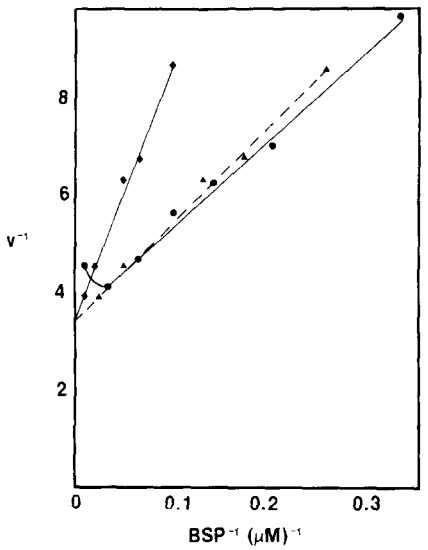

Fig. 3. Effect of liposomes on conjugation of BSP by transferase C. Each assay contained 5 mM GSH; 0.1 M potassium phosphate, pH 7.5; 4 μg enzyme; and either none (●) or 0.14 mg (◆) lipid as liposomes (egg lecithin). The aqueous phase concentration of BSP in the presence of liposomes was calculated from the partition coefficient, and the enzymatic rates in the presence of liposomes were plotted at the calculated aqueous concentration of BSP (▲). The dashed line was drawn from the latter data by least-squares regression analysis. The upward bending of the ● line represents substrate inhibition. Rates are in μmoles per min per mg.

of CDNB, the enzymatic activities were less when liposomes were included in the assay solutions. Similar results were obtained with transferase C when BSP was used as a substrate (Fig. 3). When activities were corrected for membrane partitioning of substrates, however (dashed line, Fig. 3), no inhibition was observed. Thus, the liposomes are not competitive inhibitors in the classical sense, as this implies interactions with the enzyme *per se*. Substrate inhibition by BSP was present at concentrations equal to, or greater than, 50 μM (Fig. 3). In the presence of liposomes, however, substrate inhibition was absent at all concentrations of BSP (up to 100 μM).

We were interested in whether the phenomena observed with pure proteins were also observed with the transferases present in whole cytosol. The effect of liposomes on the activity of the glutathione *S*-transferases in the 100,000 *g* supernatant fraction was determined, and the results were similar to those observed with pure proteins (Table 4).

Release rates and the effects on catalysis. At equilibrium, the aqueous concentration of a non-polar molecule is determined by the partition coefficient of the molecule in a two-phase system. *In vivo*, however, the aqueous and membrane concentrations of a molecule will not be static but will vary, depending upon the rate of entrance of the molecule into the cell and its rate of metabolism. For example, as a non-polar molecule is metabolized by a cytosolic protein, the fall in concentration in the aqueous

Table 4. Effect of liposomes on the glutathione *S*-transferase activity of the 100,000 *g* supernatant fraction*

Protein/Lipid (mg/mg)	Rate (μmoles·min^{-1}·mg^{-1}) CDNB	BSP
0	0.85	0.0039
1:1	ND†	0.0008
1:2	0.79	0.00028
1:4	0.69	0

* Each assay contained 0.1 M phosphate, 3 mM GSH, and either 0.5 mM CDNB or 0.03 mM BSP and 0.6 mg protein. Substrates were incubated with the liposomes for 20–30 min preceding the addition of GSH and enzyme.
† Not done.

phase is buffered by movement of another molecule from the membrane into the aqueous phase. If rates of release are slow relative to catalytic rates, the former process may be rate-determining for metabolism. We were interested, therefore, in the rates of release of CDNB and BSP from liposomes into buffer.

Rates of release of [^{14}C]CDNB and [^{35}S]BSP were measured by dilution followed by rapid filtration. A new equilibrium level was achieved within 2–3 min. After dilution-induced perturbation of the equilibrium of the liposome–substrate–water system, more than 80% of the [^{14}C]CDNB and almost 50% of the

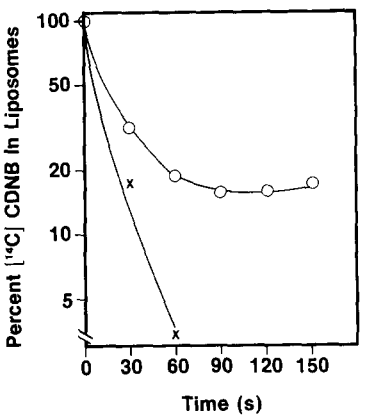

Fig. 4. Rate of release into buffer of [^{14}C]CDNB from liposomes. [^{14}C]CDNB (200 nmoles) was added to 6.1 mg lipid (egg lecithin liposomes) in 0.8 ml of 0.1 M phosphate, pH 6.5, and the liposomes were allowed to take up the [^{14}C]CDNB; 0.2 ml of the liposomes-[^{14}C]CDNB was then mixed with 9.8 ml of 0.1 M phosphate in a stirred cell containing an unbacked YM-10 membrane. Fractions were collected at 30-sec intervals, and the amount of [^{14}C]CDNB present in the filtrate was determined by counting an aliquot in a liquid scintillation counter. Binding of [^{14}C]CDNB by the membrane was corrected for by filtering [^{14}C]CDNB in the absence of liposomes. The decrease in total liposomal [^{14}C]CDNB (○) was plotted on a semilogarithmic scale versus time. The system had reached equilibrium when 84% of the [^{14}C]CDNB had entered the aqueous phase. The total amount of CDNB transferred (total [^{14}C]CDNB less liposomal [^{14}C]CDNB at equilibrium) was also determined, and the decrease in this liposomal [^{14}C]CDNB is also plotted (x). The latter line represents the rate of approach to equilibrium.

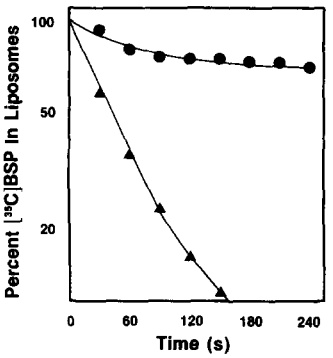

Fig. 5. Rate of release into buffer of [^{35}S]BSP from liposomes. [^{35}S]BSP (400 nmoles) was added to 1.84 mg lipid (egg lecithin liposomes) in 0.6 ml of 0.1 M phosphate, pH 7.5, and the liposomes were allowed to take up the BSP. The remainder of the experiment followed the procedures described in Fig. 4. Decrease in total liposomal [^{35}S]BSP (●) and the amount transferred (▲) were calculated as described in the legend of Fig. 4.

[^{35}S]BSP that entered the aqueous phase was released from the liposomes within the first 30 sec, which was the earliest time point that could be measured (Figs. 4 and 5). In light of the rapid release rates and the fact that corrections for binding of the ligands to the filters were necessary, it was not possible to determine kinetic constants accurately by this technique.

Experiments with a stopped-flow spectrophotometer were performed in order to determine whether the rate of release of CDNB bound to liposomes, although apparently rapid, was still slow enough to influence catalysis. If the rate of release is slow relative to catalysis, then either the rate of conjugation will be slow relative to that in the absence of liposomes or one will observe a time-dependent increase in enzymatic activity as substrate is released from the liposomes. The plan for the experiment was to put CDNB bound to liposomes in one syringe and the complete reaction mixture minus CDNB in the other syringe. On mixing of the two solutions, there was a 10-fold dilution of the liposome–CDNB

Table 5. Initial enzymatic rates determined in stopped-flow apparatus*

Concn of CDNB (μM)	Liposomes	Rate (ΔO.D.$_{340}$/sec ± S.E.M.)
4.7	Absent	$8.5 \pm 0.43 \times 10^{-3}$†
33.2	Absent	$36.8 \pm 3.04 \times 10^{-3}$†
100	Present	$34.7 \pm 2.39 \times 10^{-3}$†

* The final concentrations in the recording cell were: 0.9 mM GSH, 0.009 mg/ml protein (transferase A), 0.1 M phosphate, pH 6.5, and 2 mg/ml lipid (as liposomes). Each result is the mean of three experiments. The reference voltage was obtained against water. There was no measurable non-enzyme rate. The concentration of CDNB if no release occurred in the first 2 sec following dilution was 4.7 μM; the concentration of CDNB present if equilibrium was achieved instantaneously was 33.2 μM.

† $P < 0.01$.

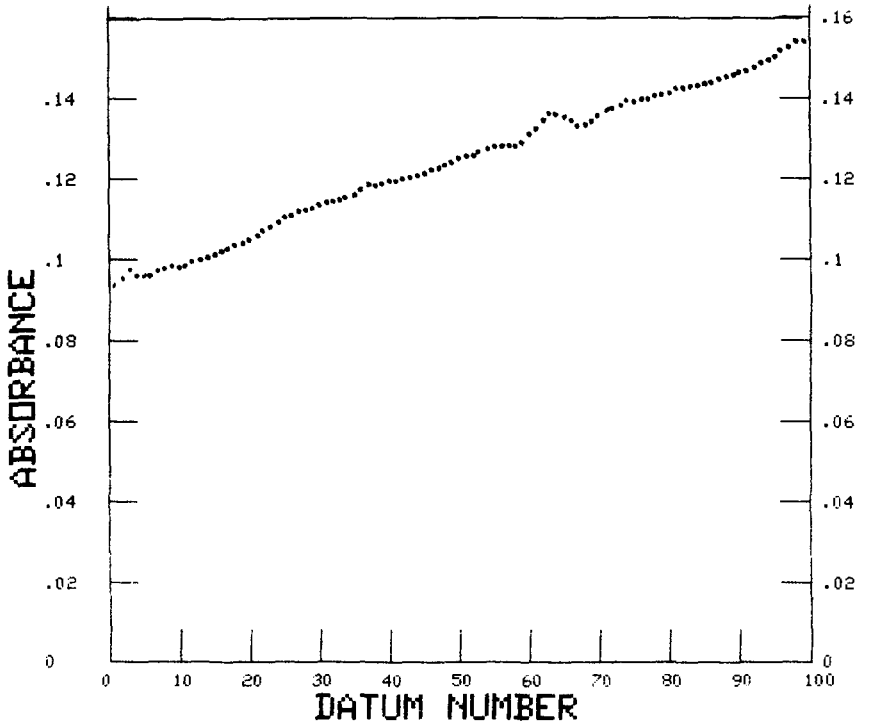

Fig. 6. Effect of rates of hydration on catalysis. Liposomes (20 mg/ml) in 0.1 M phosphate, pH 6.5, were mixed with 1 mM CDNB. This solution was diluted 1:10 in the recording cell of a stopped-flow spectrophotometer with a solution containing 0.01 mg/ml purified glutathione S-transferase A, 1 mM GSH, and 0.1 M phosphate, pH 6.5. The appearance of product was monitored at 340 nm over the first 2 sec of the reaction. Each datum number equals 0.02 sec.

complex. Control experiments were carried out in which enzymatic rates were determined in the stopped-flow spectrophotometer, in the absence of liposomes, at aqueous concentrations of CDNB that would be attained if on dilution there was (a) no hydration of CDNB bound to liposomes (4.7 μM CDNB), or (b) instantaneous attainment of a new equilibrium (33.2 μM CDNB). The difference in the rates of metabolism of CDNB at these aqueous phase concentrations was large (Table 5). In the presence (Fig. 6) and absence of the liposomes (not shown), enzymatic rates were linear over the time of observation, and there was no time-dependent increase in rates of conjugation. The actual enzymatic rate measured in the presence of liposomes was significantly greater than the rate obtained in the control experiment at the low concentration of CDNB (4.7 μM) and was similar to the rate obtained in the control experiment at the high concentration of CDNB (33.2 μM); that is, the concentration obtained when the rate of hydration was exceedingly rapid (Table 5). Thus, the rate of hydration of CDNB bound to a liposomal membrane was rapid and did not limit rates of catalysis.

DISCUSSION

The soluble glutathione *S*-transferases are important for detoxifying a wide range of xenobiotics that are ingested or formed within the hepatocyte [14]. Many of these xenobiotics are insoluble in water and would be expected to partition into cellular membranes, thus potentially limiting their access to the soluble glutathione *S*-transferases. How severely the partitioning of these substrates into membranes affects their subsequent metabolism or binding by soluble proteins depends on (a) whether the protein can interact with the membrane such that it can remove substrates directly; (b) the rate of hydration of the substrate from the membrane; and (c) the relationship of the aqueous concentration of the electrophile and the apparent K_m of the soluble enzyme.

Two proteins, phosphatidylcholine exchange protein and α-tocopherol transfer protein, have been shown to increase the rate of transfer of the respective non-polar molecules between membanes, both liposomal (including egg lecithin) and naturally occurring [7, 9, 15]. In the current investigation, the soluble glutathione *S*-transferases from rat liver were found not to interact directly with substrates present within liposomes made from egg lecithin. These enzymes had direct access only to substrates present in the aqueous phase. Although it is possible that some cell membranes could contain domains that would allow the glutathione *S*-transferases to interact directly with membrane-bound substrates, the nature of this interaction would have to be different than that described for the other exchange proteins. Thus, based on the results of this study, catalysis of membrane-bound substrates by the glutathione *S*-transferases will be determined by the extent of partitioning of the substrates into cellular membranes.

The effects of liposomes on the activities of glutathione *S*-transferases depend on the electrophilic substrate used. This is expected since the amount of substrate removed from the aqueous phase by the liposomes will vary with the physical and chemical properties of the substrate. In addition, one would predict that the effect of liposomes on rates of enzymatic activity will depend on the ratio of the concentration of the electrophile in the water phase to its K_m. The apparent K_m values of glutathione *S*-transferase C for CDNB and BSP at 5 mM GSH are 23 and 5 μM respectively. The data in Table 3 were obtained using an amount of each substrate per ml assay volume equal to five times the apparent K_m. Since the partition coefficient of CDNB is 20-fold smaller than that for BSP, the ratio $[S]_{H_2O}/K_{m(app)}$ was much larger after addition of liposomes for CDNB as compared with BSP, and, as expected, the liposomes had less effect on the rate of conjugation of CDNB versus BSP. The experiments can be designed, however, to show liposome-induced inhibition of the conjugation of either substrate to any extent desired. From the perspective of extrapolating rate data obtained *in vitro* to the function of the glutathione *S*-transferases *in vivo*, the data show that one must consider the concentration of intracellular membranes in relation to the volume of cell water, the total amount of electrophile, and the ratio of $[S]_{H_2O}/K_{m(app)}$ at the concentration of GSH within cells *in vivo*.

The role of the soluble glutathione *S*-transferases in the intracellular transport of non-polar molecules is unclear. For molecules such as BSP and CDNB that have rapid rates of release, the transferases may facilitate intracellular transport in the manner suggested by Tipping and Ketterer [3]. However, if a molecule has a slow rate of release, then, based on the results of this study, the glutathione *S*-transferases are unlikely to facilitate movement within the hepatocyte. Also, based on our study, it appears unlikely that the glutathione *S*-transferases would interact directly with the plasma membrane and thus they are unlikely to affect the rate of influx of ligands into the hepatocyte. Our observations are consistent with studies using perfused livers which found that the intracellular levels of glutathione *S*-transferase B did not influence the rate of influx of bilirubin into the hepatocyte [16]. Further study is needed to resolve the *in vivo* role of the glutathione *S*-transferases as transport proteins.

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REFERENCES

1. E. Tipping, B. Ketterer and L. Christodoulides, *Biochem. J.* **180**, 319 (1979).
2. E. Tipping, B. Ketterer and L. Christodoulides, *Biochem. J.* **180**, 327 (1979).
3. E. Tipping and B. Ketterer, *Transport by Proteins* (Eds. G. Blauer and H. Sund), pp. 369–82. Walter deGruyter, New York (1978).
4. D. Zakim and D. A. Vessey, *J. biol. Chem.* **252**, 7534 (1977).

5. T. D. Boyer, D. Zakim and D. A. Vessey, *J. biol. Chem.* **255**, 627 (1980).
6. M. Ahmed, J. S. Burton, J. Hadgraft and I. W. Kellaway, *Biochem. Pharmac.* **29**, 2361 (1980).
7. K. W. A. Wirtz, P. Devaux and A. Bienvenue, *Biochemistry* **19**, 3395 (1980).
8. L. R. McLean and M. C. Phillips, *Biochemistry* **20**, 2893 (1981).
9. H. Mowri, Y. Nakagawa, K. Inoue and S. Nojima, *Eur. J. Biochem.* **117**, 537 (1981).
10. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
11. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
12. W. Schaffner and C. Weissmann, *Analyt. Biochem.* **56**, 502 (1973).
13. W. C. Kenney and T. D. Boyer, *Analyt. Biochem.* **116**, 344 (1981).
14. W. Jakoby, *Adv. Enzymol.* **46**, 383 (1978).
15. A. M. Kasper and G. M. Helmkamp, Jr., *Biochemistry* **20**, 146 (1981).
16. A. Wolkoff, C. Goresky, J. Sellin, Z. Gatmaitan and I. Arias, *Am. J. Physiol.* **236**, E638 (1979).