Chemical modification of rat liver microsomal glutathione transferase defines residues of importance for catalytic function

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Amino acid residues that are essential for the activity of rat liver microsomal glutathione transferase have been identified using chemical modification with various group-selective reagents. The enzyme reconstituted into phosphatidylcholine liposomes does not require stabilization with glutathione for activity (in contrast with the purified enzyme in detergent) and can thus be used for modification of active-site residues. Protection by the product analogue and inhibitor *S*-hexylglutathione was used as a criterion for specificity. It was shown that the histidine-selective reagent diethylpyrocarbonate inactivated the enzyme and that *S*-hexylglutathione partially protected against this inactivation. All three histidine residues in microsomal glutathione transferase could be modified, albeit at different rates. Inactivation of 90% of enzyme activity was achieved within the time period required for modification of the most reactive histidine, indicating the functional importance of this residue in catalysis. The arginine-selective reagents phenylglyoxal and 2,3-butanedione inhibited the enzyme, but the latter with very low efficiency; therefore no definitive assignment of arginine as essential for the activity of microsomal glutathione transferase can be made. The amino-group-selective reagents 2,4,6-trinitrobenzenesulphonate and pyridoxal 5'-phosphate inactivated the enzyme. Thus histidine residues and amino groups are suggested to be present in the active site of the microsomal glutathione transferase.

INTRODUCTION

Glutathione transferases (EC 2.5.1.18) are a family of enzymes which aid in the detoxification of numerous carcinogenic, toxic and pharmacologically active substances (Jakoby, 1978; Chasseaud, 1979). Substrates for glutathione transferases are hydrophobic compounds bearing electrophilic centres, and these are converted to less reactive water-soluble conjugates that are excreted in urine and bile (Mannervik, 1985). The glutathione transferases occur in multiple cytosolic forms and one microsomal form (Morgenstern & DePierre, 1985, 1988). The microsomal glutathione transferase resembles the cytosolic glutathione transferases in having a broad substrate specificity (Morgenstern & DePierre, 1983), but differs significantly in other important characteristics such as amino acid sequence (Morgenstern *et al.*, 1985), subunit M_r (Morgenstern *et al.*, 1982), immunological properties (Morgenstern *et al.*, 1982) and ability to be activated by thiol reagents (Morgenstern & DePierre, 1983) and trypsin (Morgenstern *et al.*, 1989).

The microsomal enzyme is localized predominantly in the endoplasmic reticulum, but is also found in the mitochondrial outer membrane (Morgenstern & DePierre, 1988). Rat liver contains a high concentration of the microsomal glutathione transferase (3% of the microsomal protein) and has been used as the dominant source for purifying this protein.

A few studies on catalytically important residues in cytosolic glutathione transferases have appeared (Mannervik *et al.*, 1978; Schasteen *et al.*, 1983; Awasthi *et al.*, 1987), but no such studies have been performed with the microsomal glutathione transferase. Therefore we have carried out a thorough evaluation of the catalytic requirements of the membrane-bound enzyme. Also, in view of the similar functions yet unrelated amino acid sequences of glutathione transferases in the different subcellular

compartments (Mannervik & Danielsson, 1988), it was of interest to compare the results with those obtained with cytosolic glutathione transferases. It appears that histidine residue(s) and amino group(s) are essential for the catalytic activity of the microsomal glutathione transferase from rat liver.

MATERIALS AND METHODS

Chemicals

2,4,6-Trinitrobenzene (TNB) was kindly donated by Nobel Chemicals, Karlskoga, Sweden. All other chemicals were of high purity and were obtained from common commercial sources.

Purification of microsomal glutathione transferase

Microsomal glutathione transferase was purified from male Sprague–Dawley rat livers as previously described (Morgenstern & DePierre, 1983). Specific activity ranged from 2 to 4 $\mu\text{mol}/\text{min}$ per mg for the unactivated enzyme and from 30 to 60 $\mu\text{mol}/\text{min}$ per mg for the *N*-ethylmaleimide (NEM)-activated enzyme.

Reconstitution of microsomal glutathione transferase into liposomes

Phosphatidylcholine (from egg lecithin; 20 mg) dissolved in chloroform/methanol (2:1, v/v) was dried under a stream of N_2 . Cholate (0.2 ml; 20%, w/v) was added and the mixture was sonicated in a water bath (Branson 2200) at room temperature under N_2 for 4×60 s. A 1.8 ml portion of a buffer containing 10 mM-potassium phosphate, pH 7.0, 20% (v/v) glycerol, 0.1 mM-EDTA, 50 mM-KCl (referred to as buffer E), and 2 mg of purified microsomal glutathione transferase in 10 mM-potassium phosphate (pH 7.0)/0.1 mM-EDTA/1% (v/v) Triton X-100/1 mM-GSH/20% (v/v) glycerol/0.1 M-KCl (volume 2–4 ml)

Abbreviations used: NEM, *N*-ethylmaleimide; TNBS, 2,4,6-trinitrobenzenesulphonate; PG, phenylglyoxal; DEPC, diethylpyrocarbonate; PMSF, phenylmethanesulphonyl fluoride; TNM, tetranitromethane; CDNB, 1-chloro-2,4-dinitrobenzene; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; TNB, 2,4,6-trinitrobenzene.

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were added (Morgenstern & DePierre, 1983). The enzyme/phosphatidylcholine solution was then dialysed for 72 h against buffer E containing 1 mM-glutathione and 0.05% cholate (two changes/24 h) and then for an additional 96 h against buffer E (two changes/24 h) until no detergent was present in the liposome solution [confirmed by experiments with radioactive Triton X-100 included (results not shown)]. The proteoliposome solution was kept under N_2 and stored at 4 °C. The resulting protein concentration was 0.2–0.5 mg/ml. Specific activity ranged from 0.8 to 2 μ mol/min per mg for the unactivated proteoliposomes and from 4 to 20 μ mol/min per mg for the NEM-activated proteoliposomes.

Protein determination

The protein content of microsomal glutathione transferase-containing liposomes was determined by the method of Peterson (1977) and by amino acid analysis on a Beckman 121 M analyser and a LKB 4151 Alpha-Plus analyser.

Enzyme assays

Enzyme activities with 1-chloro-2,4-dinitrobenze (CDNB) (0.5 mM) were measured as described (Keen *et al.*, 1976; Morgenstern *et al.*, 1988). In no case was carry-over of reagent from incubation to assay large enough to interfere significantly with the GSH concentration or the rate measurement.

Activation of microsomal glutathione transferase in liposomes

Enzyme in liposomes was activated by incubation with 150 μ M-NEM (final concentration) for 10 min at 20 °C. Activation was terminated by adding 100 μ M-dithiothreitol. Activity normally increased 3–12-fold upon NEM treatment (depending on the batch of liposomal enzyme used).

Modification of microsomal glutathione transferase in liposomes with different reagents

Diethylpyrocarbonate (DEPC). For measurement of enzyme activity, with all reagents, portions of the enzyme/reagent mixture (0.1–0.25 mg of enzyme/ml) were removed at the times indicated in the Figure legends and the activity towards CDNB was determined (minimal dilution factor was 20). Proteoliposomes were mixed with an equal amount of 0.1 M-potassium phosphate, pH 7.0. The reaction was started by addition of the desired concentration of DEPC dissolved in 99.5% ethanol and was carried out at 0 °C. The incubation contained 5% (v/v) ethanol (final concentration), which did not influence control activity.

For spectral analysis, the rate of histidine modification was monitored at 240 nm ($\epsilon_{240} = 3.2 \text{ mM}^{-1} \cdot \text{cm}^{-1}$) by adding 400 μ M-DEPC to proteoliposomes (protein concentration 0.45 mg/ml; final ethanol concentration of 5%) which had been mixed with 2 vol. of 0.1 M-potassium phosphate, pH 7.0, and 0.3% Lubrol PX. Experiments were carried out at 30 °C.

The concentration of DEPC was determined as described previously (Miles, 1977).

For reversal of carbethoxylation, DEPC-treated proteoliposomes (60 min, 20 °C) with no remaining activity were mixed with 50 mM-hydroxylamine hydrochloride, pH 7.0. This reaction mixture was incubated for 24 h at 4 °C and then dialysed for 15 h against two changes of 0.1 M-potassium phosphate (pH 7.0)/2 mM-glutathione.

Phenylglyoxal (PG). For measurement of enzyme activity, proteoliposomes were mixed with an equal volume of 0.1 M-potassium phosphate, pH 7.0. Freshly prepared PG was dissolved in 0.1 M-potassium phosphate, pH 7.0, to the desired concentration. Incubation was performed at 20 °C.

For amino acid analysis, proteoliposomes were mixed with an equal amount of 0.1 M-potassium phosphate, pH 7.0. PG (5 mM final concentration) was included to start the reaction and incubation was performed at 20 °C. After 5 min the tubes were put on ice and reactions were terminated by addition of 50 mM (final concentration) freshly prepared sodium borohydride dissolved in 1 mM-NaOH. The protein/reagent mixture was dialysed for 36 h against several changes of 0.1 M-sodium carbonate, pH 8.0, and finally against double-distilled water.

Butane-2,3-dione. For measurement of enzyme activity, the proteoliposomes were mixed with an equal amount of 0.1 M-sodium borate, pH 8.0, and the reaction was started by addition of 5 mM-butane-2,3-dione (final concentration). The incubation temperature was 20 °C.

2,4,6-Trinitrobenzenesulphonate (TNBS). For measurement of enzyme activity, proteoliposomes were mixed with an equal amount of 0.1 M-potassium phosphate, pH 7.5. TNBS dissolved in water was prepared fresh and added to give a concentration of 1 mM. The reaction was carried out at 20 °C.

Pyridoxal 5'-phosphate. For measurement of enzyme activity, pyridoxal 5'-phosphate was dissolved in 0.1 M-potassium phosphate, pH 7.5, just before use and protected from light. Proteoliposomes were mixed with an equal amount of 0.1 M-potassium phosphate, pH 7.5, and the reaction was started by addition of 1 mM-pyridoxal 5'-phosphate (final concentration). Reactions were carried out at 20 °C in tubes protected from light.

For amino acid analysis, proteoliposomes were mixed with an equal amount of 0.1 M-potassium phosphate, pH 7.5. Pyridoxal 5'-phosphate (1 mM) was included for 5 min in tubes protected from light at 20 °C. The tubes were put on ice and the pH was adjusted to 5.5 with 1 M-HCl. The reaction was terminated with 50 mM-sodium borohydride and the samples were dialysed as described for phenylglyoxal.

Tetranitromethane (TNM). For measurement of enzyme activity, TNM was dissolved in ethanol to the desired concentration and added to proteoliposomes that had been mixed with an equal amount of 0.1 M-potassium phosphate, pH 7.5 (5% ethanol, final concentration). The proteoliposome/TNM mixture was preincubated at 0 °C (NEM-activated enzyme).

For amino acid analysis, 2 mM-TNM was added to proteoliposomes at 20 °C. The reaction was terminated after 5 min by addition of 100 mM-cysteine. Dialysis was performed as described for PG.

Phenylmethanesulphonyl fluoride (PMSF). For measurement of enzyme activity, 1 mM-PMSF (final concentration) in ethanol was added to proteoliposomes which had been diluted with an equal amount of 0.1 M-potassium phosphate, pH 7.0 (5% ethanol, final concentration). Incubation was performed for up to 60 min at 20 °C.

1-Ethyl-3-(3-dimethylaminopropyl)carbodi-imide hydrochloride (EDC). For measurement of enzyme activity, 20 mM-EDC (final concentration) in ethanol (5% final concentration) was added to proteoliposomes which had been diluted with an equal amount of 0.1 M-potassium phosphate, pH 6.0. Incubation was performed for up to 90 min at 20 °C.

Protection from inactivation by S-hexylglutathione

S-Hexylglutathione (400 μ M) was included in the different modification experiments as indicated in the Figure legends. All experiments were performed 2–5 times and representative experiments are shown.

Amino acid analysis

Amino acid analysis of native and modified microsomal glutathione transferase in proteoliposomes was carried out on samples hydrolysed for 24 h in 6 M-HCl in sealed and evacuated ampoules at 110 °C using a Beckman 121 M analyser and an LKB 4151 alpha plus amino acid analyser. These experiments were performed twice.

RESULTS

Modification by DEPC

Microsomal glutathione transferase in phosphatidylcholine liposomes was rapidly inactivated by addition of 50–400 μ M-DEPC (Fig. 1). When 400 μ M-S-hexylglutathione was included, the rate and extent of inactivation decreased (Fig. 1). To be able to exclude the possibility that DEPC reacts with the amino group of S-hexylglutathione, resulting in non-specific protection, the experiment was repeated with 800 μ M-DEPC. At this concentration of DEPC, S-hexylglutathione still protects the microsomal glutathione transferase (results not shown). The liposomal enzyme was sensitive to concentrations of DEPC

Fig. 1. Inactivation of microsomal glutathione transferase in liposomes by 400 μ M-DEPC

The proteoliposomes (0.45 mg/ml) were incubated as described in the Materials and methods section in the absence (□) or in the presence (■) of 400 μ M-S-hexylglutathione. Semilogarithmic curves of accessible activity (26% activity remaining after 5 min was subtracted from earlier time points) are shown in the insets: (a) ■, 50 μ M; □, 100 μ M; ●, 200 μ M; ○, 400 μ M-DEPC; (b) 400 μ M-DEPC + hexylglutathione (upper curve); – hexylglutathione (lower curve) (these values are from the large diagram). The units on the axes of the insets are the same as in the main Figure.

Fig. 2. Spectral analysis of histidine residue modification by 400 μ M-DEPC

A semilogarithmic plot is shown, in which 100% modification corresponds to reaction of all three histidines present (as shown in the right y-axis). The experiment was carried out as described in the Materials and methods section.

Fig. 3. Inactivation of NEM-activated (5-fold) microsomal glutathione transferase in liposomes by 400 μ M-DEPC

The proteoliposomes (0.45 mg/ml) were incubated as described in the Materials and methods section in the absence (□) or in the presence (■) of 400 μ M-S-hexylglutathione. A semilogarithmic curve of accessible activity (4% activity remaining after 5 min was subtracted from earlier time points) is shown in the inset. The units on the axes of the inset are the same as in the main Figure.

down to 50 μ M (2:1 molar ratio of reagent/histidine; 50% inactivation attained after 2 min). In order to be able to monitor the reaction of histidine with DEPC spectrophotometrically at 240 nm, 0.3% Lubrol PX was added to decrease the turbidity of the liposomes. Modification of all three histidine residues known to be present in the microsomal glutathione transferase (Morgenstern *et al.*, 1985) was completed within 10 min in this reaction system (Fig. 2). A total of 90% of the activity was lost within 1 min in this system (results not shown), which correlates with the modification of the most reactive histidine residue (Fig. 2).

The histidine modification could be divided into three phases, with decreasing rates indicating different reactivities for the three histidine residues (Fig. 2). During this time, no change in absorbance at 279 nm was detected, and therefore reaction of DEPC with tyrosine residues could be excluded (Miles, 1977).

When the activated enzyme was treated with DEPC, similar results to those with the untreated enzyme were obtained (Fig. 3),

Fig. 4. Spectral analysis of the Meisenheimer complex formed between GSH and TNB by rat liver microsomal glutathione transferase (0.9 mg/ml)

For spectrum a, 300 μ M-TNB was added to purified rat microsomal glutathione transferase containing 1 mM-GSH. For spectrum b, rat microsomal glutathione transferase was preincubated with 2 mM-DEPC for 5 min, after which 5 mM-GSH was added and incubated for a further 5 min, and then 300 μ M-TNB was included and the spectrum was recorded. Buffer blanks are subtracted.

the main difference being that inactivation was more complete (discussed below).

Glutathione transferase activity in DEPC-treated proteoliposomes, in which enzymic activity had been totally inhibited, could be partly restored (10–20 % of the original activity) by reversing the modification with 50 mM-hydroxylamine, pH 7.0, and subsequently dialysing against 0.1 M-potassium phosphate (pH 7.0)/2 mM-GSH (Miles, 1977).

An alternative approach to measuring inactivation was examining the effect of DEPC on the ability of the enzyme to form the Meisenheimer complex between TNB and GSH [as described for cytosolic transferases by Graminski *et al.* (1989)]. Fig. 4 shows that complex formation was almost completely inhibited by pretreatment with DEPC. When the complex was formed before the addition of DEPC, the enzyme was partly protected from the reagent (absorbance decrease was only 30 % during a 10 min incubation; results not shown).

Modification by TNBS and pyridoxal 5'-phosphate

The NEM-treated enzyme showed a high degree of sensitivity towards TNBS and activity was rapidly lost. Inclusion of *S*-hexylglutathione in the incubation system resulted in a slightly slower inactivation rate (50 % inhibition after 1 min). When unactivated enzyme in proteoliposomes was used, the situation was more complicated. An activation phase (1.9 times more active relative to control after 1 min) was followed by an inactivation phase (results not shown). The activation can be explained by the known reactivity of TNBS towards thiol groups (Glazer *et al.*, 1975).

Pyridoxal 5'-phosphate, which does not react with thiol groups (Glazer *et al.*, 1975), significantly decreased the activity of the liposomal enzyme in both the unactivated (results not shown) and activated states (Fig. 5). A certain degree of protection was noticed when including *S*-hexylglutathione in the system (Fig. 5).

Amino acid analyses performed on the reduced pyridoxal 5'-phosphate-protein complex (5 min incubation with 1 mM-pyridoxal 5'-phosphate) revealed that two or fewer lysine residues out of a total of seven were modified in the NEM-treated liposomal enzyme, and that one or fewer were modified in the untreated microsomal glutathione transferase in liposomes (results not shown). It is difficult to reach more exact conclusions from amino acid analysis, since large numbers are compared.

Fig. 5. Inactivation of NEM-activated (3-fold) microsomal glutathione transferase in liposomes by 1 mM-pyridoxal 5'-phosphate

The proteoliposomes (0.28 mg/ml) were incubated as described in the Materials and methods section in the absence (□) or in the presence (■) of 400 μ M-*S*-hexylglutathione. A semilogarithmic curve of accessible activity (10 % activity remaining after 20 min was subtracted from earlier time points) is shown in the inset. The units on the axes of the inset are the same as in the main Figure.

Fig. 6. Inactivation of NEM-activated (10-fold) microsomal glutathione transferase in liposomes by 5 mM-PG

The proteoliposomes (0.45 mg/ml) were incubated as described in the Materials and methods section in the absence (□) or in the presence (■) of 400 μ M-*S*-hexylglutathione. A semilogarithmic curve of accessible activity (11 % activity remaining after 10 min was subtracted from earlier time points) is shown in the inset. The units on the axes of the inset are the same as in the main Figure.

Modification by PG and 2,3-butanedione

The liposomal enzyme was partly inactivated by 5 mM-PG at 20 °C and partly protected against inactivation by 400 μ M-*S*-hexylglutathione (results not shown). The NEM-activated microsomal glutathione transferase again behaved similarly, but was more sensitive than the unactivated enzyme (Fig. 6).

Butane-2,3-dione, which also modifies arginine residues, was used to confirm the results obtained with PG. The enzyme is much less sensitive to this reagent. Even at a high concentration of 20 mM and after 150 min of incubation at room temperature, 50 % and 35 % of the activity remained with the NEM-treated and untreated proteoliposomes respectively as compared with controls.

Amino acid analysis of PG-treated enzyme in liposomes (activated and unactivated) did not reveal any significant decrease in free arginine residues compared with the control. Taking the variation of the different analyses into account, fewer than two arginine residues of the total eleven could have been modified (results not shown).

Modification by TNM

The NEM-modified enzyme (where reaction of TNM with the thiol group is not possible) was sensitive to TNM (50 % inhibition after approx. 20 s). Inclusion of *S*-hexylglutathione afforded some protection against inactivation.

Amino acid analysis of TNM-treated enzyme (2 mM-TNM for 5 min) resulted in the modification of 0.5–1.5 tyrosine residues out of a total of seven (two different experiments).

Modification by PMSF

No decrease in activity was seen when 1 mM-PMSF was added to activated or unactivated microsomal glutathione transferase in liposomes (30 min).

Modification by EDC

No decrease in activity was seen when 20 mM-EDC was added to unactivated microsomal glutathione transferase in liposomes. A 30 % decrease after 90 min (relative to control) was noted with NEM-activated proteoliposomes.

DISCUSSION

The inactivation of microsomal glutathione transferase by DEPC suggests that histidine residues are essential for enzyme activity. The spectral data support this conclusion and also exclude the possibility of reaction of this reagent with tyrosine. All three histidine residues in the enzyme are accessible to DEPC with differing reactivities. Plots of the log(% remaining activity) against time are approximately linear (Figs. 1 and 3). This indicates that modification of one histidine residue is responsible for the observed loss in catalytic activity. The difficulty in obtaining activity data at early time points makes this conclusion tentative. However, the fact that inactivation is almost completed within the time in which the most reactive histidine is modified favours the conclusion that one histidine residue is essential. The lack of total restoration of activity of the DEPC-treated enzyme by hydroxylamine could be due simply to destruction of the enzyme during hydroxylamine treatment and dialysis, or could be explained by the reaction of two DEPC molecules with one histidine residue (Miles, 1977).

Protection by *S*-hexylglutathione, which is a product analogue and an inhibitor of the enzyme (Mosialou & Morgenstern, 1990), argues for the involvement of a histidine residue in the active site of the enzyme. This conclusion is further supported by the ability of DEPC to abolish TNB-GSH Meisenheimer complex formation by the enzyme and by the ability of this complex to protect the active site (Fig. 4).

Pyridoxal 5'-phosphate (Fig. 5) and TNBS, which both react with amino groups, were found to inactivate the microsomal glutathione transferase. Some protection by *S*-hexylglutathione was found (more pronounced at early time points), indicating modification of an active-site residue(s). The low protection afforded by *S*-hexylglutathione could be due to reaction of the free amino group in *S*-hexylglutathione with the reagents, converting it into a less strongly binding analogue (which agrees with the observation that protection is relatively lower at later time points). The specificity of these reagents, taken together with the amino acid analysis data and the log(% activity) versus time plots, suggest the involvement of one or possibly two lysine residues in catalysis by the microsomal glutathione transferase. The *N*-terminal amino group is a less likely possibility in this respect, since it is most probably located on the luminal surface (observations from topology studies; C. Andersson & R. Morgenstern, unpublished work) of the endoplasmic reticulum, whereas the active site is exposed to the cytosolic side of the membrane (Morgenstern *et al.*, 1980).

The involvement of arginine residues in catalysis is suggested by the sensitivity of the enzyme to PG (Fig. 6). This is, however, contradicted by our observation that 2,3-butanedione at high concentrations inactivates the enzyme at a much slower rate. Although it has been argued that PG could have access to arginine residues in more hydrophobic surroundings than does 2,3-butanedione (Nibhanupudy *et al.*, 1988), the former reagent is also known to react with amino groups (Glazer *et al.*, 1975). Therefore a definite assignment of arginine as essential for microsomal glutathione transferase activity is not possible at present. Amino acid analysis indicates that less than two (if any) arginine residues are reactive towards PG. However, the facts that the inactivation by PG is apparently first order for the activated form of the enzyme (straight log % versus time curve; Fig. 6) and that protection by *S*-hexylglutathione is pronounced (both observations differing from those with the amino-specific reagents) support the presence of an active-site arginine. Here we also observe the largest difference between unactivated and activated enzyme, the latter being more sensitive, indicating the

possibility of a different functional role for arginine in the activated enzyme.

Amino acid analysis showed that TNM reacts with 1.5–0.5 tyrosine residue(s) (two experiments). However, there is no possibility at present of ascribing any involvement of tyrosine in the active site of the enzyme, because of the known reactivity of TNM with histidine residues (Glazer *et al.*, 1975).

Neither PMSF nor EDC affected the activity of microsomal glutathione transferase (unactivated form), and EDC only very slowly decreased the activity of the NEM-treated enzyme. This argues against the involvement of serine and carboxyl residues in enzyme function.

Common to all chemical modification experiments performed here is the use of proteoliposomes (which stabilize the enzyme in the absence of glutathione, a prerequisite for the protection experiments). This might complicate the results, because of the liposomal permeability barrier and unknown inside-outside distribution of the enzyme. Therefore the membranes were freeze-thawed three times to eliminate permeability barriers as a control in the experiments with hydrophilic reagents, and no notable differences were observed. We have also noticed a slight aggregation of the microsomal glutathione transferase in proteoliposomes as analysed by SDS/PAGE (C. Andersson & R. Morgenstern, unpublished work), which might explain the consistently greater degree of inactivation in activated proteoliposomes. The argument is that what is accessible to NEM activation is also accessible to the inactivating reagent. It is fortunate that proteoliposomes could be used in respect to the effect of detergents. Triton X-100 protects the enzyme from modification by DEPC (lower rate), PG and pyridoxal 5'-phosphate (almost completely). This interaction is not simply due to detergent, since Lubrol PX does not afford protection (against DEPC). Lubrol PX, on the other hand, inhibited the protection against inactivation afforded by *S*-hexylglutathione. Thus it appears that Triton X-100 displays a strong interaction with the microsomal glutathione transferase which stabilizes the enzyme. This is probably the reason for the successful application of this detergent during purification (Morgenstern *et al.*, 1982).

The free-floating equivalents (cytosolic glutathione transferases) of the microsomal glutathione transferase have been shown to require histidine (Awasthi *et al.*, 1987) and arginine (Schasteen *et al.*, 1983) residues for activity. Tentative assignments regarding thiol, amino and guanidino groups have also been made (Mannervik & Danielsson, 1988, and references therein). Clearly, in the case of microsomal glutathione transferase the cysteine thiol group is not required for activity (Morgenstern & DePierre, 1983), whereas histidine, amino groups (lysine or the amino function of the *N*-terminal alanine) and (possibly) arginine are involved in the activity. Thus no obvious difference (apart from the consequences of the reaction of cysteine) exist between cytosolic and microsomal glutathione transferase in this respect.

In conclusion, it appears that the postulated glutathione transferase reaction mechanism, in which a base (histidine?) facilitates the deprotonation of the glutathione thiol group and arginine/lysine residues bind to the carboxyl group(s) of glutathione (Mannervik & Danielsson, 1988), could also apply to the microsomal enzyme.

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