Purification of Microsomal Glutathione S-Transferase *

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The glutathione S-transferases play a major role in the detoxication and excretion of xenobiotics.[1] The individual members of this family of enzymes exhibit broad and overlapping substrate specificities towards a variety of mutagenic, carcinogenic and pharmacologically active substances.[2] These substrates resemble one another in that they are all hydrophobic and electrophilic. Many of the reactive intermediates formed by the metabolism of xenobiotics *via* the cytochrome P-450 system are hydrophobic and electrophilic and can thus serve as substrates for the glutathione S-transferases.[3,2]

Soluble glutathione S-transferases have been purified and characterized from a number of different sources.[3] Rat liver cytoplasm contains at least 7 such enzymes and the most throughly studied of the glutathione S-transferases are the three major cytosolic forms from this organ, *i.e.*, glutathione S-transferases A, B, and C.[1,4,5] Recently, rat liver microsomes have also been shown to demonstrate glutathione S-transferase activity, both in our laboratory and by others.[6,7]

In order to characterize this enzyme, compare it to the soluble proteins which catalyze similar reactions and investigate its role in drug metabolism, we have developed a simple procedure for purifying the mixrosomal glutathione S-transferase to near homogeneity in high yield.

Male Sprague-Dawley rats weighing 180–200 g were starved overnight and liver microsomes prepared as described previously,[8] except that the microsomes were washed twice with 0.15 M Tris-HCl, pH 8.0, in order to remove cytosolic contamination.[6] The microsomal fractions from 10 rats were pooled and resuspended in 35 ml 0.25 M sucrose.

20 ml 10 mM N-ethyl maleimide in 10 mM potassium phosphate buffer, pH 7.0, was then added dropwise with gentle stirring to the microsomal suspension at 4 °C over the course of 5 min. This treatment results in a 6–8-fold activation of the microsomal glutathione S-transferase,[9] thereby facilitating the purification and assuring that we isolate the endogenous microsomal enzyme and not cytosolic contaminants in the microsomal fraction. After an additional 2 min, 2 ml 0.1 M glutathione, adjusted to pH 7.0 with KOH, was also added to the incubation mixture, which was then gently stirred for 5 min. Glutathione reacts rapidly with remaining N-ethyl maleimide, thus terminating the activation, as well as preventing the inhibition of the microsomal glutathione S-transferase which occurs when microsomes are incubated with this sulfhydryl reagent for longer periods of time.

Subsequently, the microsomal transferase was solubilized by adding 20 ml 10% Triton X-100 dropwise to the mixture over the course of 10 min and stirring for an additional 20 min. The potassium phosphate concentration of the mixture was then adjusted to 10 mM by the addition of 0.57 ml 1 M potassium phosphate, pH 7.0. All subsequent operations were conducted in the coldroom at 4–8 °C.

The activated and solubilized microsomes were then loaded onto an hydroxyapatite column (4 × 35 cm) equilibrated with 10 mM potassium phosphate, pH 7.0 – 1 mM glutathione – 0.1 mM EDTA – 1% Triton X-100 – 20% glycerol (hereafter referred to as standard buffer). This column was eluted at a rate of 1 ml/min with 5 l of a linear gradient of 0.02–0.3 M potassium phosphate, pH 7.0, in standard buffer. 20 ml fractions were collected and the fractions containing glutathione S-transferase activity towards 1-chloro-2,4-dinitrobenzene[5] (fractions 112–135 eluting between 135 and 165 mM potassium phosphate) were pooled to give a total volume of 480 ml. (At least 0.02% Triton X-100 is required in the assay mixture to obtain full activity of the solubilized microsomal glutathione S-transferase.) The potassium phosphate concentration in this pool was reduced by passage through a Sephadex G-25 fine column (9 × 30 cm) eluted with standard buffer.

The Sephadex G-25 pool, with a total volume of 500 ml, was subsequently applied to a carboxymethyl-Sepharose column (2 × 7 cm) at a rate of 0.3 ml/min. This column was then eluted with 200 ml of a linear gradient of 0–0.2 M KCl in standard buffer at the same rate. 1.8 ml fractions were collected and the top fractions and edge fractions (as shown in Fig. 1) were pooled individually. These pools can be stored at −20 °C for at least half a year without loss of activity.

Protein was determined by the method of Peterson[10] including the TCA-deoxycholate precipitation step in order to avoid interference by the detergent and glutathione present. Bovine serum albumin was used as the standard. SDS-polyacrylamide gel electrophoresis was carried out

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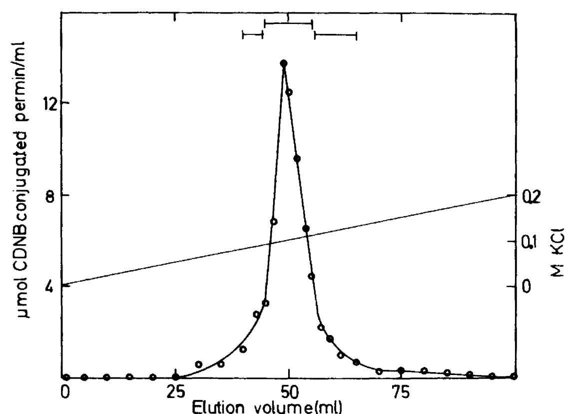

Fig. 1. Elution of the microsomal glutathione *S*-transferase from CM-Sepharose. The details of this purification step are present in the text. 5 – 25 µl aliquots of the different fractions were assayed for glutathione *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene. The fractions which were pooled and used for subsequent analysis are indicated by the bars at the top of the figure.

with 15 % acrylamide in 1 mm thick slab gels with a discontinuous buffer system as described by Laemmli.[11]

After activation with *N*-ethyl maleimide and solubilization with Triton X-100 a 3-fold purification of the microsomal glutathione *S*-transferase could be achieved by chromatography on hydroxyapatite (Table 1). After desalting on a column of Sephadex G-25 fine, a further 11 – 12-fold purification was accomplished by chromatography on CM-Sepharose (Table 1). The glutathione *S*-transferase activity was eluted by a KCl gradient from the CM-Sepharose column as one symmetric peak (Fig. 1).

As also can be seen from Table 1, the specific activities of the peak fractions and the pooled side fractions were equal. In addition both of these preparations were found to be more than 95 % homogeneous (as approximated by scanning the gels with filters open between 440 – 630 nm) and the molecular weight approximately 14 000 by SDS-polyacrylamide gel electrophoresis (not shown). Thus, the peak and side fractions could be pooled.

Since of 30 – 40-fold purification sufficed to yield a nearly homogeneous protein, it can be concluded that the microsomal glutathione *S*-transferase accounts for approximately 2.5 – 3 % of the total

Table 1. Purification scheme for microsomal glutathione *S*-transferase.

Step	Volume (ml)	Activity/ml (µmol/min ml)	Total activity (µmol/min)	Protein (mg/ml)	Total protein (mg)	Specific activity (µmol/min mg)	Purification factor	Recovery %
Microsomes	35	2.8	98	23.8	833	0.12		
Activated solubilized	78	9.2	718	10.7	835	0.86		100
Hydroxy apatite pool	480	0.79	379	0.32	154	2.46	2.9	53
G-25 pool	500	0.60	300	0.26	130	2.31	2.7	42
CM-Sepharose	500	0.13	65	0.22	110	0.59		9
flow through	500	0.13	65	0.22	110	0.59		9
CM-Sepharose peak pool[a]	20	8.2	164	0.27	5.4	30.3	35	23
CM-Sepharose side pool[a]	26	3.7	96.2	0.12	3.1	30.9	36	13

[a] The pools are indicated in Fig. 1.

microsomal protein. The overall recovery of activity was about 37% (peak plus side fractions). This corresponded to a yield of 8.5 mg protein from 10 rats.

This preparation is highly suitable for investigations on the nature of the microsomal glutathione *S*-transferase. We are in the process of determining, among other things, the minimum molecular weight of this enzyme; the size and composition of the complexes it forms with Triton X-100; its amino acid composition, isoelectric point, pH optimum, substrate specificity, *etc.* We are also trying to isolate the unactivated form of the microsomal glutathione *S*-transferase in order to compare its properties to those of the *N*-ethyl maleimide-activated form and to study the process of activation. Of great interest is the possibility that the activity of the microsomal glutathione *S*-transferase could be regulated *in vivo* through a key sulfhydryl group.

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