Separation of Glutathione *S*-Transferases from Rat Liver Cytosol by Chromatofocusing*

HELGI JENSSON, PER ÅLIN and BENGT MANNERVIK

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Glutathione *S*-transferases (EC 2.5.1.18) are a group of proteins, which play an important role in detoxification of electrophilic compounds.[1] They are present in all mammals investigated and high specific activities have been found in the liver of rodents.[2] Five forms of glutathione *S*-transferase, with isoelectric points in the range of pH 7 – 10, have been purified to homogeneity from rat liver cytosol. Conventional preparative isoelectric focusing has not given satisfactory separation of these forms. In the present study glutathione *S*-transferases from rat liver cytosol were separated and purified by use of affinity chromatography on *S*-hexylglutathione bound to epoxy-activated Sepharose 6B and by chromatofocusing. The chromatofocusing revealed 7 peaks of activity towards the substrate CDNB.**

Experimental. Rat liver cytosol was obtained as earlier described.[3] The microsome-free supernatant fraction (78 ml) was chromatographed on an *S*-hexylglutathione Sepharose affinity column, prepared as earlier described.[4] The affinity matrix (2 × 12 cm) was equilibrated with 10 mM Tris-HCl (pH 7.8). After application of the sample, the column was washed with the same buffer fortified with 0.2 M NaCl until protein was not further eluted. The GSH *S*-transferases were eluted with 5 mM *S*-hexylglutathione dissolved in 10 mM Tris-HCl (pH 7.8) containing 0.2 M NaCl. The pooled fractions from the affinity chromatography (74 ml) were desalted on a Sephadex G-25 column (4 × 30 cm) equilibrated with 5 mM Tris-HCl, pH 8.0. The desalted solution (130 ml) was concentrated to 4.5 ml by ultrafiltration and then applied on a column of chromatofocusing gel PBE 118 (1 × 30 cm) equilibrated with 25 mM triethylamine-HCl (pH 11) according to the instructions of the manufacturer

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**Abbreviations used: CDNB: 1-Chloro-2,4-dinitrobenzene; SDS: Sodium dodecylsulfate.

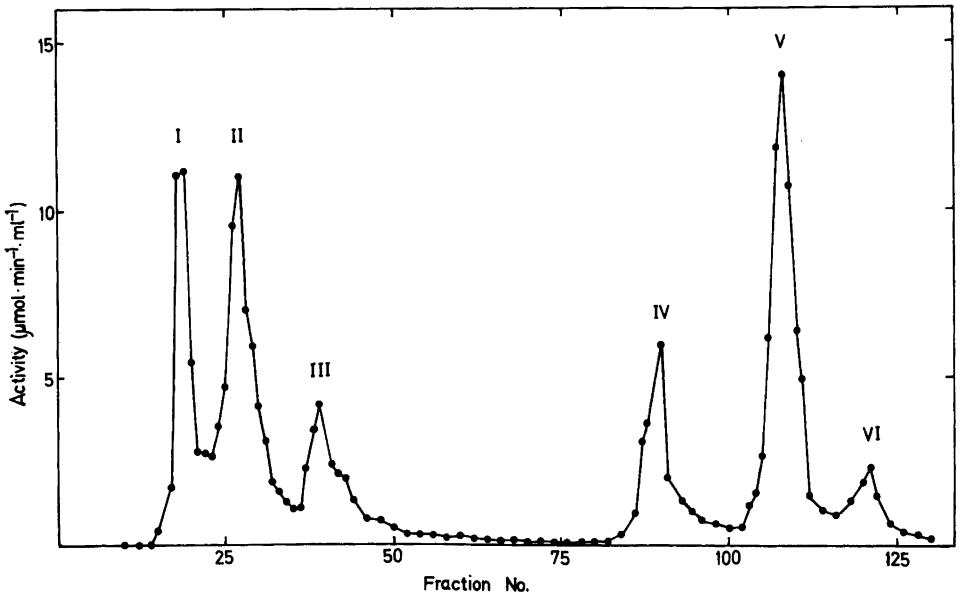

Fig. 1. Separation of glutathione *S*-transferases from rat liver cytosol by chromatofocusing. A sample partially purified by affinity chromatography was chromatographed on a column (1 × 30 cm) of chromatofocusing gel PBE 118. The enzymes were eluted with a pH gradient, pH 11 – 8, and enzymatic activity (●) was measured with CDNB as electrophilic substrate.

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Table 1. Separation of glutathione *S*-transferases.

Fraction	Volume (ml)	Total act.[a] (μmol/min)	Specific act. (μmol/mg min)	Yield (%)
Supernatant	78.0	859.6	1.15	100
S-Hexylglutathione Sepharose 6B	74.0	–	–[b]	–
Sephadex G-25	130.0	548.2	15.6	63.7
Chromatofocusing PBE 118, pH 10.5–8				
Peak I	5.0	44.5	30.9	
Peak II	9.0	72.6	25.1	
Peak III	9.0	27.6	17.7	
Peak IV	13.0	51.2	38.6	36.1
Peak V	13.0	92.2	30.0	
Peak VI	8.0	15.1	43.0	
Peak VII	5.0	6.8	3.0	

[a] Activity measured with CDNB as the electrophilic substrate. [b] *S*-Hexyl glutathione inhibits activity and interferes with protein measurements.

(Pharmacia Fine Chemicals, Uppsala). The enzymes were eluted with 380 ml of Pharmalyte, pH 8–10.5, diluted 1:80 and adjusted to pH 8 with HCl. Fractions of 2 ml were collected. Glutathione *S*-transferase activity was measured with CDNB as the electrophilic substrate as earlier described.[5] Protein concentrations were determined by the method of Lowry *et al.*[6] SDS slab gel electrophoresis was performed essentially as described by Laemmli.[7] Antibodies against glutathione *S*-transferases A and B were raised in rabbits using standard techniques of immunization. The antigens were mixed with Freund's complete adjuvant.

Results and discussion. Chromatofocusing resulted in seven clearly separated peaks of activity towards the substrate CDNB. Six of them were eluted with Pharmalyte buffer as shown in Fig. 1; an additional peak, peak VII, was eluted with 1 M NaCl. Table 1 summarizes the separation of the transferases. When tested by the double diffusion method of Ouchterlony against antibodies to glutathione *S*-transferases B and A, peaks I–III reacted with antibodies to transferase B and IV–VI with antibodies to transferase A, but not *vice versa*. Peak VII gave no precipitate with any of the two antisera. The transferases in peaks I, II, IV, V and VI were homogeneous as judged from SDS slab gel electrophoresis, but peak III contained one major band and one minor band. Peak VII was contaminated with a colored protein (hemoglobin?). The molecular weights of the subunits were estimated as 25 000 and 22 500 for the transferases in peaks I–III and to 23 500 for the transferases in peaks IV–VI. In view of these results the peaks I and II probably contained two different forms of glutathione *S*-transferase B and peaks IV and V correspond to transferases A and C, respectively. The identities of the three remaining peaks are unclear, although the data presented here suggest that peak III is related to transferase B and peak VI to transferases A and C. Peak VII seems not to be related to any of these transferases. Further characterization is required to definitely establish the identity of the peaks. The present paper describes a rapid and efficient procedure to prepare the various glutathione *S*-transferases in pure state. The novel procedure may facilitate further characterization of the molecular properties of and clarification of the relationship between the different forms.

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