Purification of Rat Liver Microsomal Glutathione Transferase

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Summary: The rat liver microsomal glutathione transferase was activated by N-ethylmaleimide, solubilized by Triton X-100 and purified by chromatography on hydroxyapatite and CM Sephadex C-50. A 28-fold purification resulted in a 38% yield. The purified protein moved as a band with an apparent molecular weight of 14000 on sodium dodecyl sulphate polyacrylamide gel electrophoresis and appeared to be nearly homogeneous. The N-terminal amino acid of the purified enzyme was alanine, identified by the dansyl method. Optimum pH and temperature were 6.8 and 30°C, respectively.

Key words: microsomal glutathione transferase, liver, purification

The glutathione transferases are known to exist in various organs of different species; they play an important role in biotransformation of carcinogens, mutagens, pesticides, and other xenobiotics[11]. They have been extensively studied. Most of them are localized in the cytosol. In recent years, rat liver microsomal glutathione transferase has been shown to be distinct from the cytosolic forms of this enzyme. It has been studied only for a few years, but for the sake of the important toxicological significance[2] thorough investigations have been made in certain fields[9,77], such as activation by sulfhydryl reagents, substrate specificity, some properties of the purified enzyme, amino acid composition, primary structure, and so on.

In our efforts to purify the microsomal glutathione transferase, we elucidated the mechanism of reaction catalyzed, relationship of structure and function, the effects of xenobiotics on the enzyme and its role in metabolism of xenobiotics. This paper presents a report on the purification and identification of this enzyme, and optimum pH and temperature are also reported.

MATERIALS AND METHODS

Chemicals
Glutathione (GSH) was obtained from Kojin Chemical Co., Japan; Triton X-100 from Rohm-Mass Co.; Sephadex G-25 and CM-Sephadex C-50 from Pharmaceutic Co., Sweden; N-ethylmaleimide, recrystallized by benzene in our laboratory, from Shanghai Institute of Biochemistry, Academia Sinica, China; 1-chlor-2, 4-dinitrobenzene (CDNB) from Shanghai Chemical Reagent Co., China. Hydroxyapatite was prepared by Hirano's method[23]. All other chemicals were of reagent grade and purchased from com-
Preparation and washing of microsomes

Male Wistar rats weighing 200 ± 20 g were starved overnight and liver microsomes were prepared as described by Ernster with slight modification; the microsomes were washed twice with 0.15 mol/L Tris–HCl buffer pH 8.0.

Activation and solubilization of microsomes

The microsomes were activated with N–ethylmaleimide and solubilized by Triton X-100 according to the method described by Morgenstern.

Purification of the enzyme

The activated and solubilized microsomes were loaded onto a hydroxyapatite column (4 × 35 cm) equilibrated with 10 mmol/L potassium phosphate buffer pH 7.0, containing 1 mmol/L EDTA, 1 mmol/L GSH, 1% Triton X-100 and 20% glycerol, hereafter referred to as standard buffer. This column was eluted with 4 L of a linear gradient of 0.01–0.3 mol/L KCl in standard buffer. The fractions exhibiting glutathione transferase activity toward CDNB were pooled. The potassium phosphate concentration in this pool was reduced by passage through a Sephadex G-25 fine column (6.5 × 25 cm) and eluted with standard buffer.

The Sephadex G-25 pool was subsequently applied to a carboxymethyl-Sephadex C-50 column (2 × 10 cm), which was then eluted with a linear gradient of 10–200 mmol/L KCl in standard buffer. The fractions with glutathione transferase activity were collected, pooled and stored at −20°C.

Assay of enzyme

Microsomal glutathione transferase activity toward CDNB as the second substrate was assayed spectrophotometrically. Its molar extinction coefficient was 9.6 mmol⁻¹·cm⁻¹. In the assay mixture at least 0.02% Triton X-100 was required to obtain full activity of the solubilized microsomal glutathione transferase.

RESULTS AND DISCUSSION

For purification of microsomal glutathione transferase, the prepared microsomes must be washed twice with 0.15 mol/L Tris–HCl buffer pH 8.0, in order to reduce the contamination by cytosolic glutathione transferase. The glutathione transferase activity in second washing supernatant was less than 5% of microsomal glutathione transferase in precipitant.

The activation of this enzyme by treatment of N–ethylmaleimide resulted in an approximately four-fold increase in the total activity. After solubilization with non-ionic detergent Triton X-100, the preparation tended to be well-distributed. At first it was passed through hydroxyapatite column (fig.1). Although chromatography on hydroxyapatite is not an effective purification step for this enzyme (three-fold), it proved to be an indispensable "clean-up" step before ion exchange chromatography, since the enzyme did not adhere to a CM–Sephadex C-50 column when solubilized microsomes were applied directly to it.

After desalting on a column of Sephadex G-25, the pools were accomplished by chromatography on CM–Sephadex C-50. The glutathione transferase was eluted by 10–20 mmol/L KCl gradient from CM–Sephadex C-50 column as one single peak (fig. 2). As it is shown in table 1, the specific activities of the peak fractions and the