# The isozyme pattern of glutathione S-transferases in rat heart

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The isozyme pattern of glutathione S-transferases of rat heart differs markedly from that of liver. Heart lacks the majority of basic isozymes recognized in rat liver, and isoelectric focusing of glutathione S-transferases obtained from affinity chromatography on S-hexylglutathione-linked Sepharose 6B revealed that 3 major acidic isozymes with p$I$ values of 6.8, 6.3 and 4.9 are present in heart, at least two of which were not found in liver. In addition, multiple bands were found in the p$I$ range 5.0–5.5 for glutathione S-transferase activity and protein staining.

*Glutathione* S-*transferase*    *Rat heart*    *Isozyme*    *Isoelectric focusing*
*Affinity chromatography*    *Detoxication*

## 1. INTRODUCTION

Glutathione S-transferase activity (EC 2.5.1.18) presents one of the body's most important defense mechanisms against endogenously generated electrophilic intermediates and numerous xenobiotics by preventing these reactive compounds from possible interaction with macromolecules [1]. Principally, the enzyme(s) catalyze the reaction of glutathione with electrophilic compounds to form glutathione thioether conjugates, known as the first step in the formation of mercapturic acids [2]. Multiple forms of glutathione S-transferase have been found and there has been much interest in their purification and characterization, especially those of mammalian liver [3–5].

Recently, we found for the isolated perfused rat heart that it releases substantial amounts of the glutathione S-conjugate, S-(2,4-dinitrophenyl)glutathione, in response to infusion of 1-chloro-2,4-dinitrobenzene [6], strongly indicating the existence of cardiac glutathione S-transferases and of an export system for the glutathione S-conjugates thus formed. The process consisting of S-conjugation and subsequent export of the products may serve a physiologically significant defense in the heart just as it does in liver and lung, since next to the lung and heart is the organ first exposed to reactive compounds which may have escaped from the hepatic detoxication system. Further, as exemplified by cardiomyopathy caused by administration of adriamycin, the heart can become a target organ to some xenobiotics.

Thus, aiming to understand the characteristics of the cardiac detoxication system related with glutathione/glutathione S-transferases, we have undertaken a comparison of glutathione S-transferases in the heart with those in the liver. We here demonstrate that the isozyme pattern of glutathione S-transferases in rat differs conspicuously from that found in liver.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of crude extract

Hearts from male Wistar rats of 250–300 g body weight, fed on stock diet (Altromin), were first perfused with Krebs-Henseleit buffer solution for 5 min under the condition in [6] to remove blood, and stored in liquid nitrogen until required. The hearts, 20 g from 30 rats for each preparation, were pulverized at −70°C and allowed to thaw partially in 60 ml of 10 mM Tris–HCl (pH 7.8, buffer A) containing 1 mM EDTA and 0.25 M sucrose.

The tissue was homogenized by use of a motor-driven Potter-Elvehjem Teflon-glass homogenizer and centrifuged at $10000 \times g$ for 20 min. The cytosol fraction was obtained by further centrifugation of the supernatant fraction at $105000 \times g$ for 40 min. The resultant supernatant fraction was chromatographed on a column of Sephadex G-200 ($5 \times 36$ cm) packed in buffer A containing 1 mM EDTA. The eluted fractions containing glutathione S-transferase activity were pooled.

## 2.2. Ion-exchange chromatography on CM– and DEAE–cellulose columns

Protein in the preparation was precipitated with 660 g/l of ammonium sulfate and collected by centrifugation at $10000 \times g$ for 30 min. The precipitate fraction was dialyzed against 10 mM potassium phosphate (pH 6.7), buffer B), and then applied to a column of CM–cellulose (Whatman CM-52, $1.8 \times 40$ cm) equilibrated with buffer B. Protein was eluted with 400 ml of a linear gradient of KCl (0–200 mM) in the same buffer.

The 'flow-through' fraction which was not bound by CM–cellulose was equilibrated with 10 mM Tris–HCl (pH 8.0, buffer C), and applied to DEAE–cellulose (Whatman DE-52, $1.8 \times 40$ cm) equilibrated with the same buffer. Four hundred ml of a linear gradient of KCl (0–200 mM) in buffer C was applied.

## 2.3. Affinity chromatography and isoelectric focusing

An S-hexylglutathione-linked Sepharose 6B affinity column was prepared as in [7]. The active fraction obtained from Sephadex G-200 was applied to the affinity column ($2.5 \times 4$ cm) equilibrated with buffer A. The column was rinsed with 0.2 M NaCl in buffer A. The adsorbed glutathione S-transferases were eluted with 5 mM S-hexylglutathione in the salt-fortified buffer. After desalting, the enzyme-containing effluent was concentrated to 1 ml (1.0–1.5 mg protein/ml) by ultrafiltration. The isoelectric focusing of the enzyme preparation was performed on ampholine PAG plates (pH 3.5–9.5, LKB, 1804-101) at 4°C using an LKB 2217 Ultrophor electrofocusing unit. The pH values were determined relative to the marker proteins of the calibration kit (pI Standards, Pharmacia). Densitometric scanning of Coomassie blue G 250-strained gels was performed in a Beckman

ACTA III spectrophotometer with a scanning adaptor.

## 2.4. Enzyme activities

Glutathione S-transferase activity was assayed as in [3] by using 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB). Glutathione peroxidase activity was assayed as in [8] by using t-butyl hydroperoxide and hydrogen peroxide.

## 2.5. Chemical and biochemicals

These were obtained from Boehringer (Mannheim), Merck (Darmstadt) and Sigma (München). t-Butyl hydroperoxide was a gift from Peroxid Chemie (Müchen).

## 3. RESULTS AND DISCUSSION

### 3.1. Ion-exchange chromatography of cardiac glutathione S-transferases

Basic glutathione S-transferases in rat liver have been resolved by CM–cellulose column chromatography into 6 apparently different forms designated AA, A, B, C, D and E [3]. One acidic form, which is not bound by CM–cellulose, has been named M [8]. Besides catalysing the glutathione conjugation reaction, the basic forms are known to bear functions as ligandin and non-selenium-dependent glutathione peroxidase. Ligandin has been found identical with transferase B ([9] cf. [10]) and the glutathione peroxidase activity has been ascribed to transferase B and AA [11].

Regarding cardiac glutathione S-transferases, properties are known only to a limited extent. The activity of S-transferases in rat heart was estimated to be $10 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g heart}^{-1}$ towards CDNB [6], corresponding to about 10% of the hepatic level [12]. Nevertheless, rat heart contains only 0.06% of ligandin as compared to liver [13]. No activity of non-selenium-dependent glutathione peroxidase has been found in rat heart cytosol [8]. From this, it can be deduced that forms B and AA of hepatic S-transferase are poor in rat heart, which raises the question of the nature of the major forms in cardiac glutathione S-transferases.

Fig.1 shows elution profiles of glutathione S-transferase activity of heart and liver on CM–cellulose column chromatography. In the control
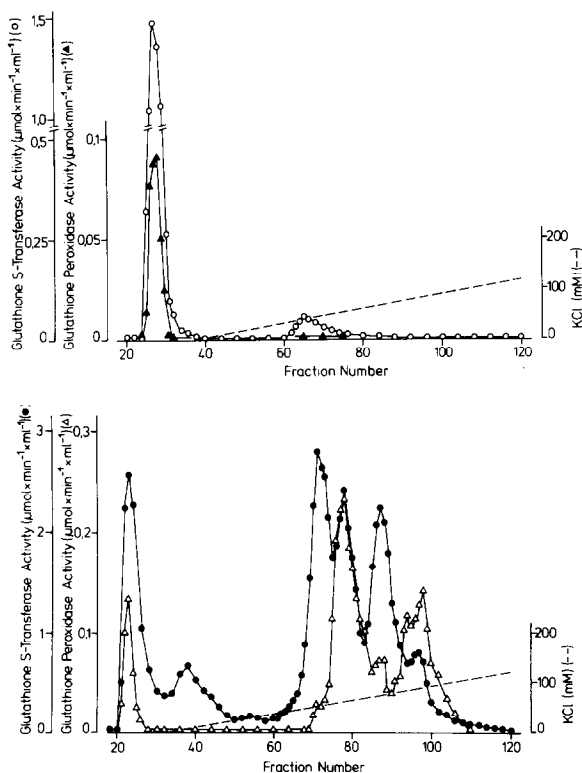
Fig.1. Cation-exchange chromatography on a CM–cellulose column of the enzyme preparations from heart (A) and liver (B). Liver enzyme preparation was obtained from 5 g of liver as described in section 2 (○,●) Glutathione S-transferase activity towards CDNB, (△,▲) glutathione peroxidase activity towards t-butyl hydroperoxide.



Fig.2. Anion-exchange chromatography on a DEAE–cellulose column of the gluthathione S-transferase fraction not adsorbed by the CM–cellulose column. Data show glutathione S-transferase activity towards CDNB.

experiment with the liver cytosol fraction, 6 major peaks of S-transferase activity were resolved (fig.1B). On the basis of substrate specificity towards CDNB, DCNB and t-butyl hydroperoxide, the last 4 peaks of activity were attributed to transferases C, B, A and AA [3]. Transferase activity in the first peak related with the acidic form was estimated to be 20% of the total activity. In contrast to the hepatic S-transferases, the predominant part of the cardiac S-transferase activity was eluted in flow-through fractions before the application of the salt gradient (fig.1A). One relatively small peak of the activity was found during elution by the linear gradient of KCl (0–200 mM) with no glutathione peroxidase activity. Even when the KCl concentration was increased up to 500 mM after the salt gradient, no extra activity was eluted.
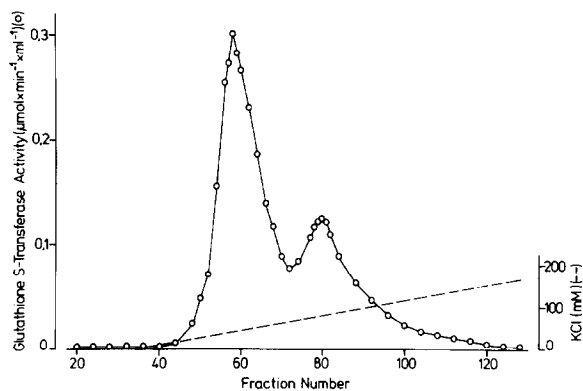
The activity peak in the flow-through fractions amounting to 85% of the total activity was subjected to anion-exchange chromatography on a DEAE–cellulose column. None of the transferase activity but glutathione peroxidase activity was eluted prior to the salt gradient. As shown in fig.2, during elution by the linear gradient of 0–200 mM KCl two peaks of transferase activity were observed. By isoelectric focusing it was found that the activity in the first peak fractions was associated with two proteins with p$I$ of 6.8 and 6.3 (not shown).

The results demonstrate that a major part of the activity of glutathione S-transferases in rat heart is carried by acidic proteins, and not by basic ones as in liver.

### 3.2. Isoelectric focusing of cardiac glutathione S-transferases

To gain further insight into the overall composition of the glutathione S-transferase isozymes in rat heart, the method of isoelectric focusing was employed, which facilitates a comparison of the enzyme activity with the stained protein. For this purpose, S-transferases were immediately isolated from the enzyme preparation obtained from Sephadex G-200 by use of an S-hexylglutathione-linked Sepharose 6B affinity column. In a control experiment, the liver preparation showed 6 distinguishable bands at pH 6.8–9.5 with respect to stained protein (fig.3A) and to S-transferase
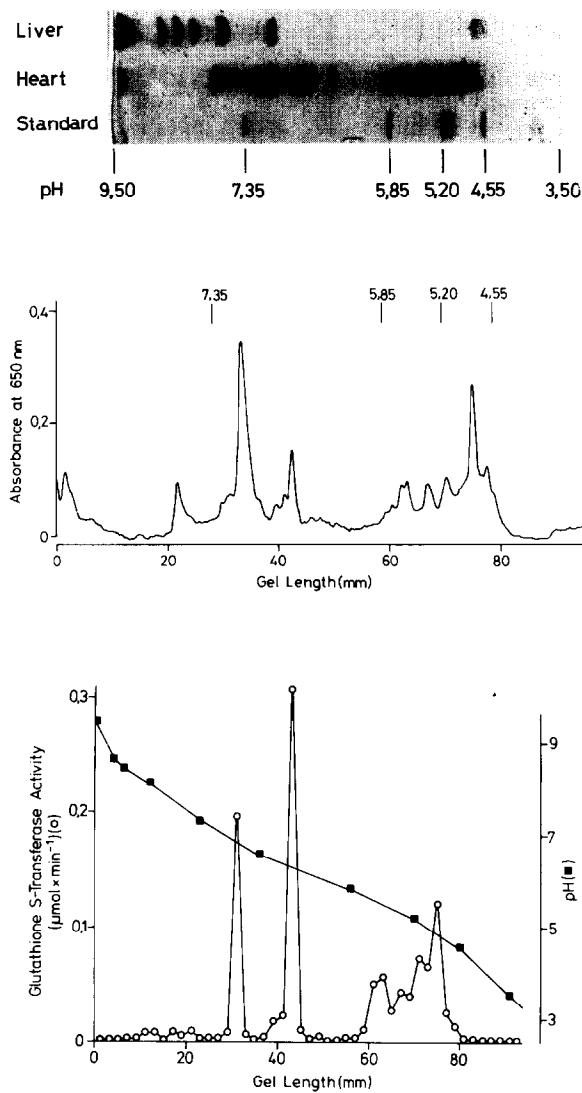
Fig.3. Isoelectric focusing of the glutathione $S$-transfer-ase preparations. (A) Isoelectric focusing of the enzyme preparations from heart and liver obtained by $S$-hexyl-glutathione-linked Separose 6B affinity column chroma-tography. For each isoelectric focusing experiment 100–120 μg of protein was applied. (B) Densitometric scan of the gel shown in A for the heart enzyme prepara-tion. (C) Distribution of glutathione $S$-transferase activity on the gel for the heart enzyme preparation. The gel was cut into sections every 2 mm and the enzyme was extracted in 0.5 ml of 0.5 M potassium phosphate buffer (pH 6.5) containing 1 mM EDTA. The enzyme activity in the extract was measured with CDNB (○). The pH values (■) were determined relative to the marker proteins.

activity (not shown). Because of the upper limit (pH 9.5) of the pH range in the gel, it should be taken into account that the most basic protein band consists of the 3 isozymes $L_2$, BL (transferase B) and $B_2$ (transferase AA), according to the nomenclature in [4]. The next 3 basic proteins are regarded as isozymes $A_2$ (transferase A), AC (transferase C) and $C_2$, respectively (in order of de-creasing p$I$ value), as referred to p$I$ values of heptic $S$-transferases [1,4].

It is evident that the glutathione $S$-transferases in rat heart lack those major basic isozymes in the liver. Most protein bands were found in the acidic pH range 6.8–4.8 (fig.3), supporting the result of the ion-exchange chromatography. Comparison of the distribution of the stained protein (fig.3B) with the $S$-transferase activity on the gel (fig.3C) makes it clear that high enzyme activities are ex-hibited by 3 major proteins with p$I$ of 6.8, 6.3 and 4.9, at least two of which were not found in the liver. The multiple bands in the pH range 5.5–5.0 were also found to have $S$-transferase activity. The hepatic isozyme with p$I$ 6.8 shown in fig.3A cor-responds presumably to transferase M [3] or X [14]. Identification of the proteins with p$I$ 6.8 found in heart and liver preparations will be the next important step.

### 3.3. Concluding remarks

This study demonstrates a distinct difference in the isozyme composition of glutathione $S$-transfer-ases in rat heart and liver, i.e., the cardiac $S$-trans-ferases consist predominantly of acidic proteins, many of which are absent in the liver. Recently, a 22-kDa subunit was found in the acidic glutathione $S$-transferase fractions purified from rat heart, kidney, lung, spleen and testis, which is not expres-sed in the liver [15]. The direct correlation between that subunit and the acidic proteins found here is at present obscure. On the other hand, not only the heart but also rat testis was found to contain a substantial amount of acidic forms of $S$-transfer-ases as compared with basic ones [16]. The acidic $S$-transferases may take a biologically significant share which differs from that for the basic enzymes in those organs. Purification and detailed charac-terization of the acidic isozymes of glutathione $S$-transferases in rat heart will be reported else-where.

## ACKNOWLEDGEMENTS

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