

Glutathione Transferases in Rat Testis *

CLAES GUTHENBERG, ING-MARI ÅSTRAND, PER ÅLIN and BENGT MANNERVIK

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

Six glutathione transferases (EC 2.5.1.18) in rat liver cytosol have been characterized as binary combinations of four protein subunits A, B, C, and L.¹ These proteins, which have basic isoelectric points, are responsible for the major part of the glutathione transferase activity in rat liver cytosol as measured with the universal electrophilic substrate 1-chloro-2,4-dinitrobenzene (CDNB).^{**} In addition, three minor forms of glutathione transferase with lower isoelectric points exist in rat liver.

In a survey of glutathione transferases in various rat organs it was noted that the testis has high activity and that a major part of the activity resides in an acidic protein apparently not present in the liver.^{2,***} Similar results were independently obtained by other investigators.³ The present communication documents this finding further, and demonstrates that also the pattern of basic glutathione transferases in rat testicular cytosol differs from that found in liver.

Experimental. Homogenates (20 %, w/v) of rat testes (25 g) were prepared in 0.25 M sucrose, by use of a Potter-Elvehjem teflon-glass homogenizer, and centrifuged at 10 000 g for 10 min. The cytosol fraction was obtained by further centrifugation of the supernatant fraction at 105 000 g for 60 min. The cytosol fraction (94 ml) was chromatographed on a column of Sephadex G-25 (4×40 cm) packed in 10 mM Tris/HCl (pH 7.8), and the transferase-containing fractions were applied to an affinity column (2×14 cm) of *S*-hexylglutathione linked to epoxy-activated Sepharose 6B, equilibrated with the same buffer. The column was rinsed with 300 ml of 0.2 M NaCl in 10 mM Tris/HCl (pH 7.8) and the transferases

eluted with 100 ml of 5 mM *S*-hexylglutathione in the NaCl-fortified buffer. The eluted enzyme-containing fractions were pooled, desalted on Sephadex G-25 (equilibrated with 5 mM Tris/HCl, pH 8.0) and concentrated to 5 ml by ultrafiltration. The subsequent chromatofocusing on gel PBE 118 was performed as described in Ref. 1. After finishing the elution with ampholytes, additional transferase activity was eluted with 1 M NaCl. This last fraction was equilibrated with 25 mM Tris/acetic acid (pH 8.3) by chromatography on a column (2×32 cm) of Sephadex G-50 and applied to a second column (1×25 cm) of chromatofocusing gel PBE 94, equilibrated with the same buffer. The adsorbed transferases were eluted with 300 ml of a mixture of Polybuffer 96 (30 %) and Polybuffer 74 (70 %), diluted 1:10 and adjusted to pH 5.0 with acetic acid. Assays of glutathione transferase activities and other experimental procedures were carried out as previously described.^{1,4}

Results and discussion. The cytosol fraction of rat testes was found to have a glutathione transferase activity with CDNB of 1.5 $\mu\text{mol}/\text{min}$ per mg protein. This specific activity is somewhat higher than that of rat liver cytosol.^{4,5} The transferase activity was separated from the bulk protein by use of affinity chromatography, and various isozymes of glutathione transferases were subsequently resolved by chromatofocusing. A pH gradient of 10.8–8.0 eluted 5 isozymes and further elution with 1 M NaCl released approximately 50 % of the activity that had been applied to the column (Fig. 1). The latter fraction of activity could be resolved into at least 5 additional isozymes with lower isoelectric points by chromatofocusing in the pH interval of 8–5.

The isozyme pattern of glutathione transferase in rat testis differs markedly from that of rat liver, the most conspicuous difference being that a major part of the activity is borne by proteins with acidic isoelectric points. The existence of such acidic transferases has earlier been reported,^{2,3} but the resolution of these enzymes into several isozymes has not been reported earlier. The dominating acidic transferase (isoelectric point at pH 5.9) was found to have the highest specific activity (about 100 $\mu\text{mol}/\text{min}$ per mg with CDNB) found for any glutathione transferase from the rat. This enzyme is currently being characterized in greater detail.

The pattern of basic transferases in rat testis differs from that of rat liver in that no significant amount of glutathione transferases L₂ and BL is detectable in the testis. The lack of these isozymes (which would be eluted first from the chromatofocusing column) was demonstrated by analysis of protein subunit sizes, reactions with

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** Abbreviation: CDNB, 1-chloro-2,4-dinitrobenzene.

*** Data presented at the Meeting of the Swedish Biochemical Society in Stockholm, 26–27th November, 1981.

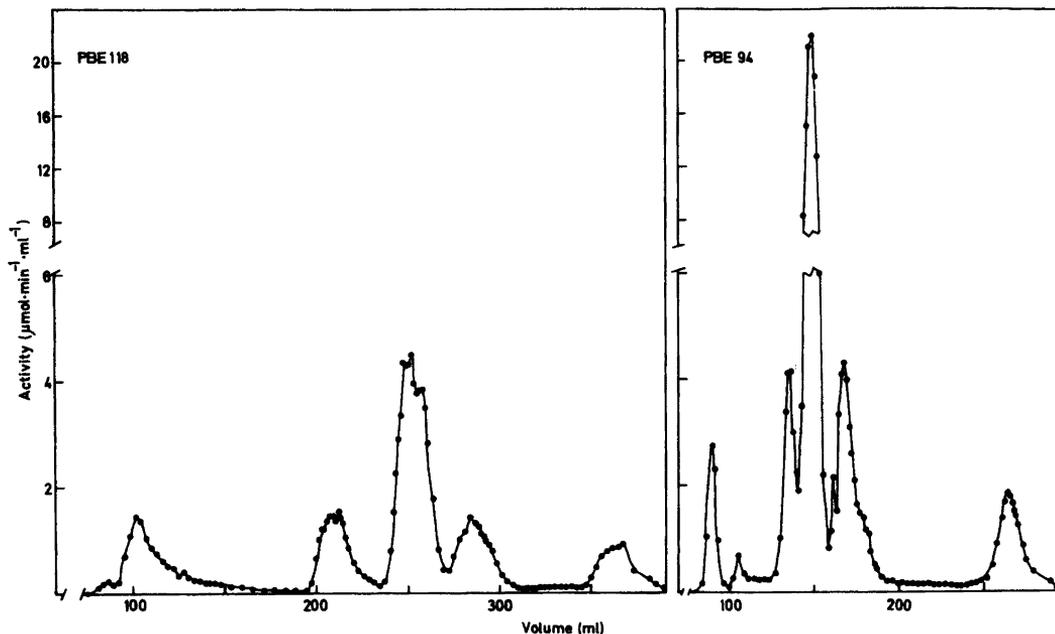


Fig. 1. Separation of glutathione transferase from rat testicular cytosol by chromatofocusing. The left part of the elution profile was obtained by chromatofocusing on gel PBE 118 and the right part by chromatofocusing on gel PBE 94. Transferase activity was measured at pH 6.5 and 30 °C with 1 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene as substrates.

antibodies, and substrates specificities of the enzymes eluted between pH 10.8 and 8.0. The first enzyme to appear was glutathione transferase B₂, and the fifth (as in liver samples) was transferase C₂. The identification of the isozymes in the intermediate peaks of activity has not been completed, but their properties suggest a relationship to protein subunit A (*cf.* Ref. 1), in that antibodies reacting with this subunit gave precipitin reactions with these isozymes. Significant enzymatic activity with 1,2-dichloro-4-nitrobenzene, which is highest for subunit A,¹ also indicated such a relationship.

Thus, this communication demonstrates that rat testis cytosol not only has high glutathione transferase activity, but that the isozyme pattern differs in significant respects from that of liver.

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1. Mannervik, B. and Jensson, H. *J. Biol. Chem.* 257 (1982) 9909.

2. Mannervik, B., Guthenberg, C., Jensson, H., Warholm, M. and Ålin, P. In Larson, A., Orrenius, S., Holmgren, A. and Mannervik, B., Eds., *Functions of Glutathione – Biochemical, Physiological, Toxicological and Clinical Aspects*, Raven, New York 1983, pp. 75–88.
3. Dierickx, P. J. and De Beer, J. O. *Biochemistry Internat.* 3 (1981) 565.
4. Guthenberg, C., Morgenstern, R., DePierre, J. W. and Mannervik, B. *Biochim. Biophys. Acta* 631 (1980) 1.
5. Kraus, P. and Kloft, H.-D. *Enzyme* 25 (1980) 158.

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