Rat and Mouse Liver Class *Theta* Glutathione S-Transferases Inactivating Reactive Metabolites Arylmethyl Sulfates

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Introduction

The first success in cDNA cloning of class *theta* glutathione (GSH) S-transferase (GST) catalyzing the inactivation of reactive sulfate esters formed metabolically from carcinogenic arylmethanols, such as 7-hydroxymethyl-12-methylbenz[*a*]anthracene and 5-hydroxymethylchrysene (5-HCR), was achieved with rat liver GST Yrs-Yrs by the authors.¹⁾ Although DNA sequences of structural genes encoding classes *alpha*, *mu*, and *pi* GST subunits have been elucidated, nothing has been known of the genes encoding class *theta* GST subunits. The possible existence of a class *theta* GST rapidly scavenging 5-sulfoxymethylchrysene (SMCR) formed from 5-HCR has been suggested in the mouse liver cytosol as demonstrated in the rat. The present study deals with 1) the DNA sequence of rat gene encoding the GST subunit Yrs and 2) purification and characterization of a class *theta* GST, designated mYrs-mYrs, from mouse liver cytosol.

Methods

A Sprague-Dawley rat liver λ EMBL4 genomic library was screened using GST *theta*-1 cDNA encoding the GST subunit Yrs as a probe. Purification of GST mYrs-mYrs from male ddY mouse (7 weeks) liver cytosol was carried out using 6 steps of column chromatography on DE-52, S-hexyl-GSH Sepharose, PBE-94 for chromatofocusing, Blue Sepharose, GSH Sepharose, and TSKgel G3000SW.

Results and Discussion

A genomic clone with an approximate molecular size of 15 kb was isolated from the library and found to contain 5 exons, spanning approximately 4 kb, for the entire sequence of GST Yrs

mRNA. The exon-intron structure of the GST Yrs gene differed from those of reported rat genes for the classes *alpha*, *mu*, and *pi* GST subunits. The result of comparison of the reported amino acid sequences of the class *theta* GSTs Yrs-Yrs and 5-5 in the rat and T1-1 and T2-2 in the human indicates that the class *theta* GSTs consist of two distinct subfamilies, 1 (GSTs 5-5 and T1-1) and 2 (GSTs Yrs-Yrs and T2-2). Comparing local amino acid sequences encoded by the exons of the Yrs gene with the corresponding sequence regions of the subunits 5, T1, and T2, the C-terminal sequences encoded by exons 4 and 5 were found to be major factors weakening the overall sequence identity between the subfamilies.

A novel homo-dimeric GST mYrs-mYrs, catalyzing the GSH conjugation of the reactive sulfate ester, SMCR, was purified to homogeneity with a 1750-fold purification from mouse liver cytosol. GST mYrs-mYrs showed immuno-cross-reactivity only with anti-serum raised against rat class *theta* GST Yrs-Yrs, and its first 28 N-terminal amino acid sequence was identical with that of the GST Yrs. GST mYrs-mYrs represented more than 95% of the mouse liver cytosolic GST activity to scavenge SMCR and had a much higher GSH peroxidase activity toward fatty acid hydroperoxides than did the other classes of mouse GSTs. These facts indicate that GST mYrs-mYrs belongs to the subfamily 2 of class *theta*.

References

- 1) K. Ogura, T. Nishiyama, T. Okada, J. Kajita, H. Narihata, T. Watabe, A. Hiratsuka and T. Watabe, *Biochem. Biophys. Res. Commun.*, **181**, 1294 (1991).