Rat glutathione transferase 8-8, an enzyme efficiently detoxifying 4-hydroxyalk-2-enals

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Rat glutathione transferase 8-8 is one of the less abundant cytosolic glutathione transferases, accounting for approx. 1% of the total activity with 1-chloro-2,4-dinitrobenzene in liver. The enzyme is eluted at pH 6.3 upon chromatofocusing and has so far been identified in liver, kidney, lung and testis. Characteristic properties include high relative activity with ethacrynic acid (70% of the specific activity with 1-chloro-2,4-dinitrobenzene) and an apparent subunit M_r of 24500. The most significant property noted is the high catalytic activity in the conjugation of 4-hydroxyalk-2-enals, major products of lipid peroxidation. The catalytic efficiency with these substrates exceeds corresponding values for all known substrates tested with any glutathione transferase, which suggests that transferase 8-8 may have evolved to detoxify 4-hydroxyalk-2-enals.

Glutathione transferase *4-Hydroxyalk-2-enal* *Detoxication* *Lipid peroxidation* *Glutathione conjugation* *Reactive metabolite*

1. INTRODUCTION

Oxidation of organic molecules containing unsaturated carbon–carbon bonds may give rise to electrophilic products such as epoxides, hydroperoxides, and activated alkenes. In most cases these reactive substances are toxic to biological systems; some are mutagenic, teratogenic or carcinogenic. Since glutathione is capable of reacting with electrophiles and thereby deactivating many toxic compounds, we have previously emphasized that a major biological role for the glutathione transferases may be to catalyze the detoxication of products of oxidative metabolism [1].

Lipid peroxidation of biological membranes yields reactive alkenes and aldehydes, including the highly toxic 4-hydroxyalk-2-enals [2]. A major product of peroxidative degradation of arachidonic acid is 4-hydroxynon-2-enals [3,4]. It was recently found that six purified rat glutathione transferases are highly active in the conjugation of glutathione with 4-hydroxyalk-2-enals and that these compounds are among those giving the highest enzymatic reaction rates of the known substrates [1,5]. Rat glutathione transferase 4-4 exhibited the highest activity of the enzyme forms tested. Three distinct forms of mouse glutathione transferase also showed high catalytic activity [1]. Subsequent studies of glutathione transferase 4-4 in rat heart, where it is a dominating enzyme form, have confirmed its importance in the detoxication of 4-hydroxynon-2-enals [6]. It was furthermore demonstrated that the corresponding glutathione *S*-conjugate was released from the heart upon perfusion of the organ with 4-hydroxynon-2-enal.

This paper reports that rat glutathione transferase 8-8 is an enzyme at least one order of magnitude more efficient than transferase 4-4 in the conjugation of 4-hydroxyalk-2-enals. It is suggested that this enzyme may have evolved to effect detoxication of this group of reactive products of oxidative metabolism.

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2. MATERIALS AND METHODS

4-Hydroxyalk-2-enals (*trans* isomers) were generously provided by Dr H. Esterbauer, Institut für Biochemie, Universität Graz, Graz, Austria. The preparation of substrate solutions and the spectrophotometric assay of glutathione conjugation were performed as described in [5].

Glutathione transferase 8-8, earlier referred to as the acidic transferase eluted at pH 6.3 from a chromatofocusing column [7–9] (the consulting group for the nomenclature of new species of glutathione transferase [10] has decided to recommend this designation for the enzyme studied), was purified by affinity chromatography on *S*-hexylglutathione coupled to epoxy-activated Sepharose 6B [11] and fast protein liquid chromatofocusing essentially as described by Guthenberg et al. [8].

3. RESULTS AND DISCUSSION

Glutathione transferase 8-8 is an acidic enzyme form (p*I* = 6.0) isolated from the cytosol fraction of several rat tissues. It has previously been described as a glutathione transferase showing high relative activity with ethacrynic acid as the electrophilic substrate [7–9]. The enzyme used in the experiments reported here was prepared from rat liver, but has also been isolated from kidney [8], lung [7,9] and testis (Guthenberg, C., Ålin, P. and Mannervik, B., unpublished). Some of the characteristic properties of glutathione transferase 8-8 are compiled in table 1.

The apparent subunit M_r of transferase 8-8 as estimated by SDS-polyacrylamide gel electrophoresis is intermediate between the values for subunits 1 (M_r 25000) and 7 (M_r 24000). This molecular property in combination with the acidic isoelectric point and the high relative activity with ethacrynic acid distinguish glutathione transferase 8-8 from other transferases previously isolated from rat tissues [7–9,12]. The activity with ethacrynic acid is 70% of that with 1-chloro-2,4-dinitrobenzene; transferase 7-7, previously the most active enzyme form known, displays a value of 16% [8].

Table 1

Characteristic properties of rat glutathione transferase 8-8

Apparent subunit M_r	24500
p*I* (at 4°C)	6.0
pH of elution (chromatofocusing)	6.3
Specific activity (μmol·min^{-1}·mg^{-1}) with:	
1-Chloro-2,4-dinitrobenzene	10
Ethacrynic acid	7
4-Hydroxynon-2-enal	170

Glutathione transferase 8-8 was assayed with several 4-hydroxyalk-2-enals under the standard conditions defined in [5], i.e. 0.1 mM alkenal and 0.5 mM glutathione at pH 6.5 and 30°C. Table 1 shows that 4-hydroxynon-2-enal, which is produced in significant amounts from arachidonic acid during lipid peroxidation [3,4], is conjugated at a rate of 170 μmol·min^{-1}·mg^{-1}, a specific activity higher than those obtained with any other substrate previously tested with transferases isolated from rat liver [5,12].

The catalytic efficiency, expressed as k_{cat}/K_m, was determined as previously described [5] with 4-hydroxypent-2-enal, 4-hydroxynon-2-enal, and 4-hydroxydodec-2-enal. In these experiments 2.5 mM glutathione was used in order to approach intracellular levels. Fig.1 shows that the k_{cat}/K_m value increases markedly with increasing length of the hydrocarbon side chain of the alkenal. The k_{cat}/K_m value for 4-hydroxynon-2-enal (4.2 $s^{-1}\cdot\mu M^{-1}$) is 15-fold higher than that obtained with transferase 4-4, previously the most effective enzyme known [5]. The value for 4-hydroxydodec-2-enal obtained with transferase 8-8 is even twice as high.

The dependence of k_{cat}/K_m on pH was determined with 4-hydroxynon-2-enal as substrate. The value of k_{cat}/K_m increased approximately linearly from 0.8 $s^{-1}\cdot\mu M^{-1}$ at pH 5.5 to 8.0 $s^{-1}\cdot\mu M^{-1}$ at pH 7.5. Higher pH values could not be investigated owing to high absorbance of ionized glutathione at 224 nm (the wavelength used to monitor the reaction). Thus, at a physiological pH value the activity of the enzyme is even higher than at the pH of 6.5 used in the standard assay system.

It has been stated that glutathione transferases are poor catalysts, since the specific activities with most substrates are comparatively low [13]. However, the 'natural' electrophilic substrates for

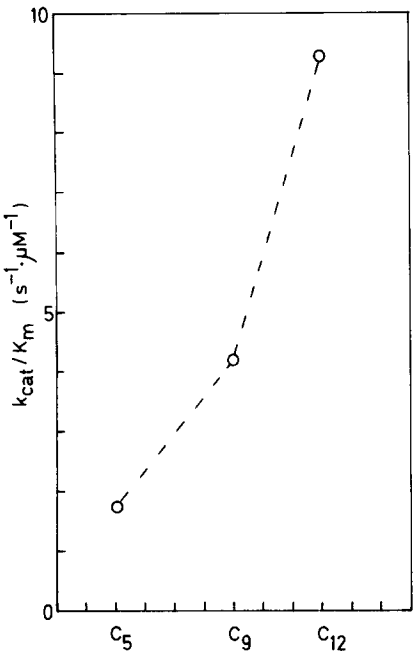

Fig.1. Dependence of catalytic efficiency of rat glutathione transferase 8-8, k_{cat}/K_m, on length of carbon chain in 4-hydroxyalk-2-enals. Values were determined with 2.5 mM glutathione at pH 6.5 and 30°C as previously described [5]. The k_{cat} values were calculated on the basis of M_r = 50000 for the dimeric enzyme.

the enzymes are unknown and high catalytic efficiencies may be obtained with proper substrates. The present investigation shows that the naturally occurring 4-hydroxyalkenals are in fact excellent substrates for rat glutathione transferase 8-8. The value of k_{cat}/K_m for 4-hydroxydodec-2-enal (9.3 $s^{-1}\cdot\mu M^{-1}$) is not far below those cited for the most efficient enzymes [14]. These results suggest that glutathione transferase 8-8 has evolved to effect the detoxication of the highly toxic alkenals. Furthermore, they support the view that a major biological function of glutathione transferases in general may be the conjugation and deactivation of reactive electrophilic products of oxidative metabolism [1,15].

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