Thymine hydroperoxide, a substrate for rat Se-dependent glutathione peroxidase and glutathione transferase isoenzymes

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The thymine hydroperoxide, 5-hydroperoxymethyluracil, is a substrate for Se-dependent glutathione (GSH) peroxidase and the Se-independent GSH peroxidase activity associated with the GSH transferase fraction. These enzymes may contribute to repair mechanisms for damage caused by oxygen radicals. GSH transferases 1-1, 2-2, 3-3, 4-4, 6-6 and 7-7 [(1984) Biochem. Pharmacol. 33, 2539–2540] are shown to differ considerably in their ability to utilize this substrate. For example, high activity is found in GSH transferase 6-6 which is the major isoenzyme in spermatogenic tubules where DNA synthesis is so active and faithful DNA replication so important. The activity of the purified GSH transferase isoenzymes towards 5-hydroperoxymethyluracil is comparable with their activity towards other endogenous substrates related to cellular peroxidation such as linoleate hydroperoxide and 4-hydroxynon-2-enal or biologically important xenobiotic metabolites such as benzo(*a*)pyrene-7,8-diol-9,10-oxide.

Glutathione peroxidase *Glutathione transferase* *Thymine hydroperoxide* *DNA* *Oxygen radical*

1. INTRODUCTION

Oxygen centred free radicals peroxidize both cellular lipids and DNA [1–4]: this damage appears to be mitigated by GSH-dependent enzymes [5–8]. For example, in the case of lipids it has been shown that both Se-dependent GSH peroxidase and GSH transferase isoenzymes inhibit microsomal lipid peroxidation [7], the substrates in both cases being free fatty acid hydroperoxides rapidly released from peroxidized phospholipids by phospholipase A_2 [7,8]. A recent study with GSH transferase isoenzymes shows that they differ considerably in their ability to utilize fatty acid hydroperoxides as substrates and that their ability to inhibit lipid peroxidation is proportional to their enzymic activity [9]. In the case of DNA, γ-radiation of aqueous solutions gives rise to pyrimidine hydroperoxide moieties [10] and incubation of peroxidized DNA or thymine hydroperoxide with GHS and rat liver soluble supernatant fraction causes oxidation of GSH implying that these peroxides are substrates for GSH peroxidase activity [5].

Here, we show that 5-hydroperoxymethyluracil (Thy^{α} OOH) is a substrate for both an Se-dependent GSH peroxidase fraction and purified GSH transferase isoenzymes. The means whereby these enzymes might participate in the repair of DNA after oxygen centred free radical damage is discussed and the activity of Se-dependent peroxidases and GSH transferases towards Thy^{α} OOH is compared with that towards other substrates of biological importance.

2. MATERIALS AND METHODS

Rats of the Wistar strain were inbred in the Courtauld Institute of Biochemistry. Most chemicals were from commercial sources and of analytical grade. 5-Hydroperoxymethyluracil was made according to [11]. GSH peroxidase activity

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Table 1

GSH peroxidase activities of purified GSH transferases and Se-dependent GSH peroxidase

Enzyme	Specific activity (μmol/min per mg protein)			
	Thy$^{\alpha}$ OOH	Lin OOH	HO-nonenal	BPDE
Se-dependent GSH peroxidase fraction	0.18	0.20	nil	nil
GSH transferase 1-1	0.13	2.80	2.60	0.10
GSH transferase 2-2	1.01	1.80	0.67	0.08
GSH transferase 3-3	0.49	0.20	2.70	0.03
GSH transferase 4-4	0.27	0.20	6.90	0.36
GSH transferase 6-6	1.27	0.06	n.d.	0.19
GSH transferase 7-7	1.25	1.50	n.d.	0.33

Data for Lin OOH, HO-nonenal and BPDE are from [9], [17] and [19] respectively; n.d., not determined

was assayed [12] using 0.5 mM Thy$^{\alpha}$ OOH and GSH transferase activities were measured according to [13]. GSH transferase isoenzymes were isolated from rat liver, kidney and testis according to [14–16]. Se-dependent and Se-independent GSH peroxidase activities in rat liver soluble supernatant fraction were separated by chromatography on Sephadex G-100.

3. RESULTS AND DISCUSSION

Table 1 shows that crude Se-dependent GSH peroxidase and each of the purified GSH transferase isoenzymes tested had GSH peroxidase activity towards Thy$^{\alpha}$ OOH and each GSH transferase isoenzyme differs in its ability to utilize Thy$^{\alpha}$ OOH. If GSH peroxidase activity towards Thy$^{\alpha}$ OOH is compared with that towards linoleate hydroperoxide (Lin OOH) it is seen that while the activity of Se-dependent GSH peroxidase is similar towards both types of substrate, the GSH transferase isoenzymes show marked differences. Thus, GSH transferase 6-6 is the most active towards Thy$^{\alpha}$ OOH but the least towards Lin OOH. Conversely, GSH transferase 1-1 is the least active towards Thy$^{\alpha}$ OOH but the most active towards Lin OOH. On the other hand, GSH transferases 2-2 and 7-7 have a similar high activity towards both substrates and GSH transferase 4-4 a similar low activity. The rates of reduction of the above hydroperoxides are comparable with the rates of conjugation of electrophilic substrates of biological importance, e.g. 4-hydroxynon-2-enal (HO-nonenal) and benzo(*a*)pyrene-7,8-diol-9,10-oxide (BPDE) [17–19] (see table 1).

Some if not all of these GSH peroxidases may participate in the repair of peroxidation damage to DNA by reducing hydroperoxypyrimidines to hydroxypyrimidines, and hydroxyhydroperoxydihydropyrimidines to glycols. One of the possible succeeding steps would then be excision of the resulting pyrimidine alcohols and glycols by DNA glycosylases. The isolation of a 5-hydroxymethyluracil DNA glycosylase from mouse cells [20] which could carry out such a step gives support to this hypothesis as does also the observation that a 5-hydroxymethyluracil and 5,6-dihydroxy-5,6-dihydrothymine, in amounts in excess of those which could be derived from the diet or gut flora, are excreted in rat and human urine [21,22]. It may be relevant in this respect that GSH transferase 6-6 is most abundant in spermatogenic tubules [23] which are principally concerned with DNA replication and the formation of spermatozoa.

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