FEBS Letters 410 (1997) 210–212 FEBS 18739

Reduction of thymine hydroperoxide by phospholipid hydroperoxide glutathione peroxidase and glutathione transferases

Yongping Bao[a], Per Jemth[b], Bengt Mannervik[b], Gary Williamson[a,*]

[a]*Department of Biochemistry, Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UA, UK*
[b]*Department of Biochemistry, Uppsala University, Biochemical Center, Box 576, S-75123 Uppsala, Sweden*

Received 16 April 1997; revised version received 2 May 1997

Abstract Thymine hydroperoxide (5-hydroperoxymethyluracil), a model compound representing products of oxidative damage to DNA, is a substrate for glutathione peroxidase and some isoforms of glutathione transferase. In this paper, we show that selenium-dependent human phospholipid hydroperoxide glutathione peroxidase (Se-PHGPx) exhibits about four orders of magnitude higher activity on thymine hydroperoxide than that of other human enzymes such as selenium-dependent glutathione peroxidase and various representatives of glutathione transferases. The results indicate that Se-PHGPx may be an important enzyme in repairing oxidatively damaged DNA.

Key words: Phospholipid hydroperoxide glutathione peroxidase; Thymine hydroperoxide; Glutathione transferase; Glutathione peroxidase; Selenium

1. Introduction

Cellular oxidative stress can result in severe metabolic dysfunctions, including peroxidation of membrane lipids and DNA damage [1]. In DNA, thymine residues have the highest electron affinity and therefore are the most likely sites of free radical damage, giving rise to thymine hydroperoxide (Thy-OOH) [2,3]. A number of protective enzymes including GPx and some glutathione transferases (GSTs) can detoxify Thy-OOH and DNA hydroperoxides by reducing them to their corresponding alcohols in the presence of glutathione (GSH) [4–8]. In this paper, we demonstrate that Se-PHGPx is about four orders of magnitude more active in the reduction of Thy-OOH than are other glutathione-dependent enzymes.

2. Materials and methods

Thymine hydroperoxide was prepared using H_2O_2 according to [9]. The hydroperoxy group was measured by an iodometric assay [10] and the extinction coefficient at 353 nm of thymine hydroperoxide is 6.8×10^3 M $^{-1}$ cm^{-1}. The glutathione peroxidase activities of Se-PHGPx and GSTs were measured by a coupled assay based on the oxidation of NADPH by glutathione reductase [11] using 0.1 mM Thy-OOH in 0.1 M Tris buffer (pH 7.4) 2 mM EDTA, 1 mM NaN_3 and 3 mM GSH. To check that the stoichiometry of reduction of thymine hydroperoxide was equivalent to the oxidation of NADPH in this assay, the reaction was performed under the same conditions and the amount of NADPH oxidised was noted to be 0.05 mmol. The reaction mixture was then applied to an ODS2 column (25×1 cm, Capital HPLC, UK) connected to an HPLC run isocratically in water. The thymine hydroperoxide and thymine hydroxide elute at the same retention time, but the column separates other reaction components. The fractions were collected from the HPLC and assayed using the iodometric assay [10]. The decrease in the iodometric value relative to standards of thymine hydroperoxide was 0.05 mmol, which is identical to the oxidation of NADPH. This experiment confirms that the reduction of thymine hydroperoxide correlates stoichiometrically with the oxidation of NADPH.

Human liver PHGPx was purified using ammonium sulphate precipitation, affinity chromatography on bromosulphophthalein-glutathione-agarose, gel filtration and anion exchange chromatography according to [12]. Human glutathione peroxidase was purchased from Sigma (80 Units/mg protein, one unit catalyzes the oxidation by H_2O_2 of 1.0 μmol of reduced glutathione to oxidized glutathione per min at pH 7.0 at 25°C). V_{max} and K_m calculated by methods of Wilkinson [13].

Purification of human GSTs A1-1, P1-1 and M1-1 (allelic variant b) and the rat enzyme rGST T2-2 have been described previously [14–17]. GST A2-2 [K. Svensson, M. Widersten and B. Mannervik, unpublished work] and GST A4-4 [I. Hubatsch, M. Ridderström, and B. Mannervik, unpublished work] were expressed and purified essentially in the same way as GST A1-1, while GST T1-1 [P. Jemth and B. Mannervik, unpublished work] was obtained in a fashion similar to that used for rGST T2-2. Briefly, cloned cDNAs encoding the different GSTs were expressed heterologously in *E. coli* by growing bacteria carrying expression vectors in 500 ml 2TY medium (16 g tryptone, 10 g yeast extract, 5 g NaCl and 50 μg ampicillin per litre). Expression of recombinant protein was induced by adding 0.2 mM isopropyl β-D-thiogalactopyranoside to the medium at OD_{600}=0.2–0.3. The cultures were then incubated at 37°C in a rotary shaker overnight, and the cells were harvested by centrifugation and disrupted by sonication. GST isoenzymes of classes Alpha, Mu and Pi were purified on S-hexylglutathione [18] or GSH [19] affinity columns prepared from epoxy-activated Sepharose 6B (Pharmacia Biotech, Uppsala, Sweden). Class Theta GSTs were purified by ion-exchange chromatography on DEAE-Sepharose 6B and immobilized ferric ion affinity chromatography [17] using Chelating Sepharose FF (Pharmacia Biotech). The human GST T1-1 was also subjected to dye affinity chromatography on Matrex Orange A gel (Amicon, Beverly, MA, USA). Protein concentrations were determined spectrophotometrically at 280 nm using extinction coefficients obtained by amino acid analysis of the different isoenzymes.

3. Results and discussion

Table 1 shows that Se-PHGPx and different GSTs exhibited glutathione peroxidase activities towards Thy-OOH. Among the human enzymes, GST M1-1, GST A4-4 and Se-GPx exhibited no detectable activity. GST T2-2 from rat was the most active isoenzyme of GST on Thy-OOH. Another rat class Theta enzyme, rGST T1-1, has been shown to have some activity with DNA hydroperoxide [8], and the corresponding human enzyme hGST T1-1 also had a low activity (Table 1). However, Se-PHGPx is by far the most active enzyme on Thy-OOH. Michaelis-Menten kinetics were followed with $K_m = 0.17 \pm 0.02$ mM and $V_{max} = 1200 \pm 108$ μmol/min per mg protein. Comparison of the specific activities of the

*Corresponding author. Fax: (44) 1603-507-723.

Abbreviations: Se-PHGPx, selenium-dependent phospholipid hydroperoxide glutathione peroxidase; hGST, human glutathione transferase; rGST, rat glutathione transferase; Thy-OOH, thymine hydroperoxide; Se-GPx, selenium-dependent glutathione peroxidase; GSH, glutathione

PII S0014-5793(97)00591-7

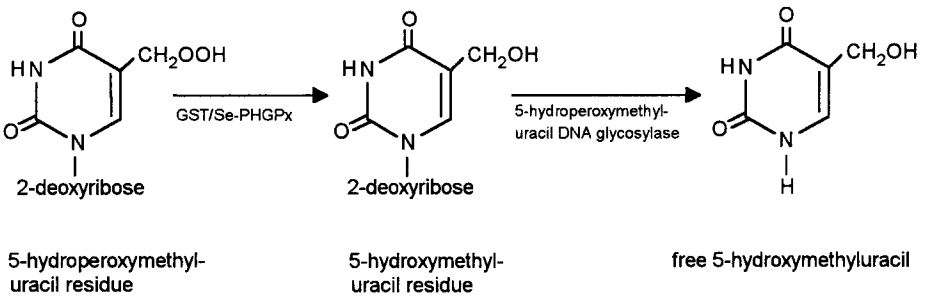

Fig. 1. Repair of oxidatively damaged DNA hydroperoxide: reduction catalyzed by GST/Se-PHGPx and hydrolysis catalyzed by 5-hydroperoxymethyluracil DNA glycosylase. It is possible that the reduction of the hydroperoxide may occur before or after hydrolysis.

most active GST (rGST T2-2) with Se-PHGPx, shows that Se-PHGPx is ~4000-fold more active than rGST T2-2. Meyer and co-workers reported a contamination in their GPx preparation with a specific activity of up to 1000 μmol/min per mg protein on DNA hydroperoxide [8], which we can now suggest to have been Se-PHGPx. In view of the present results, it is also possible that the activity reported by Christophersen [4] should be ascribed to Se-PHGPx, an enzyme not yet discovered at the time.

It is assumed that the first radical attack, in the presence of oxygen, on the 5-methyl group of a thymine residue in DNA gives a 5-hydroperoxymethyluracil residue [7]. Subsequently it is reduced by glutathione-dependent enzymes, and then excised by 5-hydroperoxymethyluracil DNA glycosylase (Fig. 1).

Se-PHGPx can also reduce phospholipid hydroperoxides, cholesterol hydroperoxides and oxidized low-density lipoproteins [20,21] and recently we have shown that Se-PHGPx plays a major role in the metabolism of hydroperoxy phospholipids in cells [22]. Lipid hydroperoxides and aldehydes formed during lipid peroxidation can react with DNA to cause strand breakage and adduct formation [23–25], so that Se-PHGPx diminishes the formation of lipid alkoxyl and peroxyl radicals through the reduction of lipid hydroperoxides to the corresponding less reactive lipid hydroxides. Therefore Se-PHGPx may protect against DNA damage at two levels: prevention of the formation of DNA damage by removal of lipid hydroperoxides and by directly repairing oxidatively damaged DNA.

Se-PHGPx is present in almost all human organs and tissues [26], but testes contain the highest levels [27,28]. In rat testis nuclei, PHGPx is bound to chromatin [29]. The location and large amount of PHGPx in testes suggest that Se-PHGPx may play a very important role in maintaining the highly fidelity of DNA replication in the reproductive organs.

In certain other organs, GSTs are very abundant. Although GSTs have relatively low activities on Thy-OOH, the abundance of many GST isoforms in many tissues (up to 5% of the total soluble protein in liver [30]) suggests the role of GSTs in protection against DNA damage may be important in tissues where Se-PHGPx is low.

Table 1
Glutathione peroxidase activities of GPx, GSTs and PHGPx on Thy-OOH

Enzyme	Specific activity (μmol/min per mg protein)
Se-PHGPx	461 ± 24 [a]
Se-GPx	n.d.[b]
hGST A1-1	0.007 ± 0.001
hGST A2-2	0.008 ± 0.003
hGST A4-4	n.d.
hGST M1-1	n.d.
hGST P1-1	0.011 ± 0.001
hGST T1-1	0.009 ± 0.003
rGST T2-2	0.120 ± 0.022

[a] 800 μmol/min per mg protein using 0.5 mM Thy-OOH.
[b] 0.18 μmol/min per mg protein from Ref. [5] using 0.5 mM Thy-OOH.
n.d., not detectable (< 0.002 μmol/min per mg protein).

Acknowledgements: We wish to thank the Biotechnology and Biological Sciences Research Council (BBSRC) UK, and the Swedish Natural Science Research Council for funding the project. We are grateful to B. Olin, A-S. Johansson, I. Hubatsch and M. Widersten for providing some of the purified GSTs.

References

[1] Halliwell, B. and Aruoma, O.I. (1991) FEBS Lett. 281, 9–19.
[2] Cullis, P.M., Jones, G.D.D., Symons, M.C.R. and Lea, J.S. (1987) Nature 330, 773–774.
[3] Schulte-Frohlinde, D., von Sonntag, C. (1985) in: Oxidative Stress (Sies, H. Ed.), pp. 11–36, Academic Press, London.
[4] Christophersen, B.O. (1969) Biochim. Biophys. Acta 186, 387–389.
[5] Tan, K.H., Meyer, D.J., Coles, B. and Ketterer, B. (1986) FEBS Lett. 207, 231–233.
[6] Tan, K.H., Meyer, D.J., Gillies, N. and Ketterer, B. (1988) Biochem. J. 254, 841–845.
[7] Ketterer, B. and Meyer, D.J. (1989) Mutation Res. 214, 33–40.
[8] Meyer, D.J., Coles, B., Pemble, S.E., Gilmore, K.S., Fraser, G.M. and Ketterer, B. (1991) Biochem. J. 274, 409–414.
[9] Hahn, B.S. and Wang, S.Y. (1976) J. Org. Chem. 41, 567–568.
[10] Buege, J.A. and Aust, S.D. (1978) Methods Enzymol. 52, 302–310.
[11] Maiorino, M., Gregolin, C. and Ursini, F. (1990) Methods Enzymol. 186, 448–457.
[12] Chambers, S.J., Lambert, N. and Williamson, G. (1994) Int. J. Biochem. 26, 1279–1286.
[13] Wilkinson, G.N. (1961) Biochem. J. 80, 324–332.
[14] Stenberg, G., Björnestedt, R. and Mannervik, B. (1992) Protein Expr. Purif. 3, 80–84.
[15] Kolm, R.H., Stenberg, G., Widersten, M. and Mannervik, B. (1995) Protein Expr. Purif. 6, 265–271.
[16] Widersten, M., Huang, M. and Mannervik, B. (1996) Protein Expression Purif. 7, 367–372.
[17] Jemth, P., Stenberg, G., Chaga, G. and Mannervik, B. (1996) Biochem. J. 316, 131–136.
[18] Mannervik, B. and Guthenberg, C. (1981) Methods Enzymol. 77, 231–235.
[19] Simons, P.C. and Vander Jagt, D.L. (1981) Methods Enzymol. 77, 235–237.

[20] Thomas, J.P., Maiorino, M., Ursini, F. and Girotti, A.W. (1990) J. Biol. Chem. 265, 454–461.
[21] Thomas, J.P., Geiger, P.G., Maiorino, M., Ursini, F. and Girotti, A.W. (1990) Biochim. Biophys. Acta 1045, 252–260.
[22] Bao, Y.-P. and Williamson, G. (1996) J. Lipid Res. 37, 2351–2360.
[23] Wang, M.-Y. and Liehr, J.G. (1995) Arch. Biochem. Biophys. 316, 38–46.
[24] Yang, M.-H. and Schaich, K.M. (1996) Free Radic. Biol. Med. 20, 225–236.
[25] Chung, F.-L., Chen, H.-J.C. and Nath, R.G. (1996) Carcinogenesis 17, 2105–2111.
[26] Roveri, A., Maiorino, M. and Ursini, F. (1994) Methods Enzymol. 233, 202–212.
[27] Roveri, A., Casasco, A., Maiorino, M., Dalan, P., Calligaro, A. and Ursini, F. (1992) J. Biol. Chem. 267, 6142–6146.
[28] Roveri, A., Maiorino, M., Nisii, C. and Ursini, F. (1990) Biochim. Biophys. Acta 1028, 211–221.
[29] Godeas, C., Tramer, F., Micali, F., Roveri, A., Maiorino, M., Nisii, C., Sandri, G. and Panfili, E. (1996) Biochem. Mol. Med. 59, 118–124.
[30] Jakoby, W.B. (1978) Adv. Enzymol. Relat. Areas Mol. Biol. 46, 383–414.