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A novel selenium-containing glutathione transferase zeta1-1, the activity of which surpasses the level of some native glutathione peroxidases

Keyan Zheng [a], Philip G. Board [b], Xiaofang Fei [c], Ye Sun [a], Shaowu Lv [a], Ganglin Yan [a], Junqiu Liu [d], Jiacong Shen [d], Guimin Luo [a,*]

[a] *Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education, Jilin University, Changchun 130021, People's Republic of China*
[b] *Molecular Genetics Group, John Curtin School of Medical Research, Australian National University, Canberra, Australia*
[c] *College of Life Science, Jilin University, Changchun 130021, People's Republic of China*
[d] *Key Laboratory for Superamolecular Structure and Materials of the Ministry of Education, Jilin University, Changchun 130021, People's Republic of China*

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Abstract

Glutathione peroxidase (GPX) is a critical antioxidant selenoenzyme in organisms that protects cells against oxidative damage by catalyzing the reduction of hydroperoxides by glutathione (GSH). Thus, some GPX mimics have been generated because of their potential therapeutic value. The generation of a semisynthetic selenoenzyme with peroxidase activity, which matches the catalytic efficiencies of naturally evolved GPX, has been a great challenge. Previously, we semisynthesized a GPX mimetic with high catalytic efficiency using a rat theta class glutathione transferase (rGST T2-2) as a scaffold, in which the highly specific GSH-binding site is adjacent to an active site serine residue that can be chemically modified to selenocysteine (Sec). In this study, we have taken advantage of a new scaffold, hGSTZ1-1, in which there are two serine residues in the active site, to achieve both high thiol selectivity and highly catalytic efficiency. The GPX activity of Se-hGSTZ1-1 is about 1.5 times that of rabbit liver GPX, indicating that the selenium content at the active site plays an important role in enhancement of catalytic performance. Kinetic studies revealed that the catalytic mechanism of Se-hGSTZ1-1 belong in a ping–pong mechanism similar to that of the natural GPX.

Keywords: Enzyme mimics; Glutathione peroxidase; Human glutathione transferase zeta1-1; Selenium-containing human glutathione transferase zeta1-1; Chemical mutation

Abbreviations: GPX, glutathione peroxidase; GSH, glutathione; rGST T2-2, rat theta-class glutathione transferase T2-2; hGSTZ1-1, human glutathione transferase zeta1-1; Sec, selenocysteine; PMSF, phenylmethanesulfonyl fluoride; CFA, chlorofluoroacetic acids; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PEG, polyethylene glycol.

* Corresponding author. Tel.: +86 431 88498974; fax: +86 431 88980440.
E-mail address: luogm@jlu.edu.cn (G. Luo).

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1. Introduction

Glutathione peroxidase (GPX, EC.1.11.1.9) is a well-known selenoenzyme that functions as an antioxidant by catalyzing the reduction of harmful hydroperoxides by glutathione (GSH) and protecting cells against oxidative damage. It occurs extensively in cells, blood, and tissues, and its activity has been related to several diseases such as diabetes (Davis, Lavine, Arredondo, McMahon, & Tenner, 2002), Keshan disease (Burke & Opeskin, 2002), angiocardiopathy (Forgione et al., 2002), and cataracts (Babizhayev, 1996). Therefore, GPX is one of the most important antioxidant enzymes in living organisms. However, the therapeutic usage of the native GPX is limited because of its instability. Because it contains selenocysteine (Sec) encoded by the stop codon UGA, it is extremely difficult to prepare by genetic engineering techniques, and its availability is limited. Chemical modification is a good way to simulate the natural enzyme, but most of the artificial GPX mimics such as ebselen (2-phenyl-1, 2-benzisoselenazol-3(2*H*)-one), have low GPX activity because they lack a critical substrate binding site. Chemical modification of a natural enzyme with a GSH-binding site is a novel method to mimic GPX. The key to this approach is to find an ideal scaffold, which must possess two properties: a special GSH-binding site and a potential catalytic group close to the GSH-binding site. In previous studies, we successfully semisynthesized a GPX mimic by the use of recombinant rat theta-class glutathione transferase T2-2 (rGST T2-2) as a scaffold and chemical modification of a serine in the active site. The modified Se-rGST T2-2 has a similar activity to the natural GPX (Ren et al., 2002). The rGST T2-2 has a natural GSH-binding site and contains a serine close to the substrate-binding site. We have now found a better scaffold, human glutathione transferase zeta1-1 (hGSTZ1-1), which has a natural GSH-binding site and contains two serine residues in the active site.

Glutathione transferases (GSTs, EC.2.5.1.18) are a family of multifunctional proteins capable of detoxifying endogenous and xenobiotic electrophiles by addition of GSH to the electrophiles (Armstrong, 1997; Dirr, Reinemer, & Huber, 1994; Vanhaelen, Francis, & Haubruge, 2004; Zielinski & Pörtner, 2000). The human zeta class GST, hGSTZ1-1 was discovered by a bioinformatics approach and identified in human expressed sequence tag databases (Board, Baker, Chelvanayagam, & Jermiin, 1997; Board et al., 2001). It shows a little activity with conventional GST substrates (1-chloro-2, 4-dinitrobenzene; organic hydroperoxides), and it was found to catalyze the transformation of some kinds of α-haloacids and the cistrans isomerization of maleylacetoacetate to fumarylacetoacetate (Board & Anders, 2005; Tong, Board, & Anders, 1998a, 1998b). The crystal structure of hGSTZ1-1 shows that its active site is located in a deep crevice between the N- and C-terminal domains and contains three very highly conserved residues (Ser^{14}–Ser^{15}–Cys^{16}) to form the characteristic zeta class SSC motif (Board et al., 1997). Ser 14 and Ser 15 are located close to the thiol of GSH bound in the active site and they can easily be chemically modified to selenocysteine (Sec) after activation with phenylmethyl sulfonylfluoride (PMSF). The active site of hGSTZ1-1 is very accessible and allows ready access of both GSH and H_2O_2.

Here we report the chemical conversion of hGSTZ1-1 into a highly active GPX that exceeds the catalytic efficiency of naturally occurring rabbit liver GPX.

2. Materials and methods

2.1. Materials

Ethyl chlorofluoroacetate was obtained from Fluka Chemical Corp. Reduced glutathione, NADPH, glutathione reductase and phenylmethanesulfonyl fluoride (PMSF) were products of Sigma. All the other chemicals were obtained from Beijin Chemical Factory, China, and were of analytical grade. Chlorofluoroacetic acids (CFA) were prepared as described previously (Tong et al., 1998a,b) and the products were 99% pure by ^{19}F NMR spectroscopy.

2.2. Expression and purification of hGSTZ1-1 and its mutants

hGSTZ1-1 and its mutants were expressed in *Escherichia coli* as His_6-tagged proteins and purified by nickel–agarose column chromatography as described previously (Blackburn, Tzeng, Anders, & Board, 2000). The purity of the final purified expression product was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was determined by the method of Bradford with Bio-Rad dye-binding reagent and with bovine serum albumin as the standard (Bradford, 1976).

2.3. Preparation of selenoenzyme

The selenoenzyme was prepared by a previously described method (Sun et al., 2004) with the following modifications. A volume of 73 μl of PMSF solution (10 mg/ml acetonitrile) was added to a 10 ml solution containing 100 μg/ml hGSTZ1-1 (or its mutant), 50 mM