Acta Biochimica Polonica

Vol. 53 No. 3/2006, 547–552

on-line at: www.actabp.pl

Regular paper

Tocopherol esters inhibit human glutathione *S*-transferase omega

Adriana Sampayo-Reyes[1]✉ and Robert A. Zakharyan[2]

[1]*División de Farmacología y Toxicología, Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social, Monterrey Nuevo León, México;* [2]*Department of Molecular and Cellular Biology; The University of Arizona, Tucson, Arizona;* ✉*e-mail: adriana.sampayo@biomedicas.net*

Received: 02 February, 2006; revised: 23 June, 2006; accepted: 09 August, 2006
available on-line: 01 October, 2006

Human glutathione *S*-transferase omega 1-1 (*hGSTO1-1*) is a newly identified member of the glutathione *S*-transferase (*GST*) family of genes, which also contains alpha, mu, pi, sigma, theta, and zeta members. hGSTO1-1 catalyzes the reduction of arsenate, monomethylarsenate (MMA(V)), and dimethylarsenate (DMA(V)) and exhibits thioltransferase and dehydroascorbate reductase activities. Recent evidence has show that cytokine release inhibitory drugs, which specifically inhibit interleukin-1b (IL-1b), directly target hGSTO1-1. We found that (+)-α-tocopherol phosphate and (+)-α-tocopherol succinate inhibit hGSTO1-1 in a concentration-dependent manner with IC_{50} values of 2 μM and 4 μM, respectively. A Lineweaver-Burk plot demonstrated the uncompetitive nature of this inhibition. The molecular mechanism behind the inhibition of hGSTO1-1 by α-tocopherol esters (vitamin E) is important for understanding neurodegenerative diseases, which are also influenced by vitamin E.

Keywords: hGSTO1-1, Vitamin E, glutathione *S*-transferase, human, MMA(V) reductase

INTRODUCTION

Glutathione S-transferases (GSTs) are a family of enzymes that catalyze the conjugation of glutathione (GSH) to electrophiles (Hayes *et al.*, 2005). There are seven major types of human cytosolic GSTs: alpha, mu, pi, sigma, theta, zeta, and omega. These enzymes contribute to the biotransformation of a wide range of exogenous and endogenous compounds. hGSTO1-1 is a homodimer that catalyzes the reduction of MMA(V), which is an important step in the biotransformation of inorganic arsenic (Zakharyan *et al.*, 2001). In fact, the known MMA(V) reductase protein is identical to the protein encoded by the gene recently identified as hGSTO1-1 (Board *et al.,* 2000; Zakharyan *et al.,* 2001).

The omega family of human GST (GSTO1-1 and GSTO2-2) has properties unlike those of the other known GSTs (Board *et al.,* 2000). Human GSTO1-1 exhibits both thioltransferase and dehydroascorbate reductase activities and also serves as a reductase for arsenate, MMA(V), and DMA(V) (Aposhian *et al.,* 2004; Zakharyan *et al.,* 1999). The *hGSTO2-2* protein also possesses both MMA(V)- and DMA(V)-reducing activities (Schmuck *et al.,* 2005). In addition, hGSTO1-1 is structurally similar to the CLIC proteins, a family of intracellular chloride channels. Furthermore, hGSTO1-1 modulates the activity of a ryanodine receptor (Dulhunty *et al.,* 2001) and plays a role in a nuclear antioxidant system (Yin *et al.,* 2001). A wide range of human tissues including liver, macrophages, glial cells, and endocrine cells express hGSTO1-1 (Yin *et al.,* 2001). The physiological importance of hGSTO1-1 has not been fully elucidated yet.

Recently, Laliberte *et al.* (2003) reported that cytokine release inhibitory drugs, which specifically inhibit IL-1b, directly target hGSTO1-1. Thus, hGSTO1-1 may be involved in the modulation of the activity of IL-1b. This type of inflammatory response has been suggested as a contributing mechanism to the pathogenesis of both Alzheimer's and Parkinson's disease.

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; GST, glutathione-*S*-transferase; hGSTO1-1, recombinant human glutathione-*S*-transferase class Omega; IPTG, isopropyl-β-D-thiogalactopyranoside; MMA(III), monomethylarsonous acid; MMA(V), monomethylarsonic acid; vitamin E, α-tocopherol.

A wide range of cell culture, animal model, and human epidemiological studies have suggested a role for vitamin E in brain function and in the prevention of neurodegeneration; however, the underlying molecular mechanisms of this activity remain largely unknown (Azzi & Stacker, 2000; Anatol *et al.*, 2004). The antioxidant activity of vitamin E has been investigated extensively although other activities have also been identified (Traber & Packer, 1995).

Numerous compounds have been shown to inhibit GST activity. Specifically, α-tocopherol (vitamin E) has been shown to be a potent inhibitor of GSTP1-1 (van Haaften *et al.*, 2001; 2002). An extended amino terminus and cysteine residues at the active site distinguish hGSTO1-1 physically from the other GSTs, including GSTP1-1. The active site of hGSTO1-1 is more consistent with glutaredoxin activity than with GST activity (Sinning *et al.*, 1993; Dirr *et al.*, 1994). In addition to the structural differences, hGSTO1-1 exhibits different functional properties than other GSTs, including GSTP1-1. GSTP1-1 contributes to resistance against cytostatic drugs and apoptosis (Johansson *et al.*, 2000). Both hGSTO1-1 and GSTO2-2 have been documented to induce inflammation and to contribute to the pathogenesis of Alzheimer's disease and apoptosis (Ogru *et al.*, 2004; Giri *et al.*, 2005; Wang *et al.*, 2005). Thus, despite the marked differences between hGSTO1-1 and GSTP1-1, we examined hGSTO1-1 as a possible target for inhibition by α-tocopherol.

A novel ester (RRR-bis-α-tocopheryl phosphate or RRR-di-α-tocopheryl phosphate) that consists of two α-tocopherol molecules linked by a phosphate group has recently been described. α-Tocopherol phosphate is water soluble unlike other α-tocopheryl esters. In addition, α-tocopheryl phosphate may have anti-inflammatory properties that are protective against atherosclerosis (Gianello *et al.*, 2005). Interest in α-tocopheryl phosphate derivatives has increased after the recent discovery that this compound is present in plant and animal tissues as well as in food stuffs. These findings raised the possibilities that α-tocopheryl phosphate is a reserve form of α-tocopherol and that this compound may be capable of regulatory effects at the cellular level. Here, we describe the interactions between (+)-α-tocopherol phosphate, (+)-α-tocopherol succinate and hGSTO1-1.

MATERIALS AND METHODS

Chemicals. We obtained (+)-α-tocopherol phosphate disodium salt, (+)-α-tocopherol acid succinate, and disodium methyl arsenate (MMA(V)) from Chem Service, Inc. (West Chester, PA, USA). Glutathione *S*-transferase omega (hGSTO1-1) was a generous gift from Professor P. G. Board, NADPH, GSH, GSH reductase, 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid, sodium deoxycholate, isopropyl-β-D-thiogalactopyranoside (IPTG), and Luria Bertani Broth and agar (LB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [^{14}C]MMA(V) (0.55 μCi/nmol) was obtained from Professor E. A. Mash, Jr., University of Arizona (USA). Monoflow-3 scintillation cocktail was obtained from National Diagnostics (Atlanta, GA, USA). Carbon tetrachloride and diethylammonium diethyldithiocarbamate were obtained from Aldrich (Milwaukee, WI, USA). Ni-NTA resin was purchased from Qiagen (Tokyo, Japan). All other chemicals were either analytical grade or the highest quality obtainable. Water was distilled and doubly deionized.

Recombinant hGSTO1-1 protein expression. Recombinant histidine-tagged hGSTO1-1 proteins were expressed using pQE30 constructs in *Escherichia coli* M15 cells containing the Rep4 plasmid (Qiagen) and purified by nickel agarose affinity chromatography. Briefly, a seed culture of the transformed cells was grown in 10 mL LB medium containing 100 μg/mL ampicillin and 25 μg/mL kanamycin. The culture was grown overnight at 37°C with vigorous shaking. The following day, a 1 mL aliquot of the overnight culture was added to new media (500 mL) and grown to an OD_{600} of 0.5–0.7. Gene expression was induced by the addition of IPTG to a final concentration of 1 mM. The cultures were then grown for an additional 4 h. The culture was then centrifuged at 4000 × *g*, and the pellet was stored at –70°C until use. The expressed 6×-His-tagged protein (hGSTO1-1) was purified with Ni-NTA resin (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. The enzyme was eluted in 250 mM imidazole, 50 mM sodium phosphate, and 300 mM NaCl, pH 8.0. The purified protein was dialyzed against 20 mM Tris/HCl, 60 mM NaCl, and 5 mM dithiothreitol, pH 8.0, before storage at –70°C (Board *et al.*, 2000).

MMA(V) reductase assay. The reaction mixture contained 0.1 M Tris/HCl (pH 8.0), 5 mM GSH, 22 nmol and 3.4 × 10^5 c.p.m. of [^{14}C]MMA(V), and 4 μg hGSTO1-1 in a final volume of 250 μL. The reaction mixture was incubated at 37°C for 60 min. Samples were then placed on ice, and 0.5 mL of carbon tetrachloride containing 10^{-2} M diethylammonium diethyldithiocarbamate (DDDC) was added. The mixture was shaken vigorously for 15 min at room temperature. After centrifugation at 3000 × *g*, the organic phase was separated and MMA(III) was back-extracted from the organic phase with 0.5 mL of 0.1 M sodium hydroxide. After centrifugation at 3000 × *g*, the aqueous phase was separated and transferred into scintillation vials containing 120 μL of 0.5 M HCl. National Diagnostics Monoflow-3 scintillation

cocktail (5 mL) was added, and the contents of the vials were counted in a Beckman model LS7800 liquid scintillation counter (Zakharyan & Aposhian, 1999).

MMA(V) reductase assay (spectrophotometric method). Reduction of MMA(V) with hGSTO1-1 was measured in the presence of glutathione and NADPH at 340 nm. MMA(V) reduction was assayed with 5 mM GSH, 18 mM MMA(V), 0.25 mM NADPH, 0.8 U GSH reductase, and 8 μg hGSTO1-1 in 0.1 M Tris buffer, pH 8. The reduction was monitored spectrophotometrically by recording the decrease in absorbance at 340 nm. Effects of various concentrations of (+)-α-tocopherol acid succinate (final concentration 0.5 μM to 16 μM) on hGSTO1-1 activity were determined. A stock solution of (+)-α-tocopherol acid succinate was prepared in ethanol. The final concentration of ethanol in the incubation mixture (1%, v/v) had no effect on GSTO1-1 activity. The reaction mixture was incubated for 2 min at 37°C before activity measurement.

Inhibition of MMA(V) reductase activity by sodium deoxycholate, ethacrynic acid, and 1-chloro-2,4-dinitrobenzene (CDNB). MMA(V) reductase activity was assayed with 0.6 μCi [^{14}C]MMA(V), 20 mM MMA(V), 5 mM GSH, 4 μg hGSTO1-1 and increasing concentrations of the inhibitors according to previously described methods (Zakharyan & Aposhian, 1999).

Protein assay. Protein concentrations were determined according to the Bradford method (Bradford, 1976) using bovine serum albumin as the standard.

Other methods. Kinetic parameters were calculated by linear regression of the Lineweaver-Burk plot of the reciprocal initial rate *versus* the reciprocal concentration. The results are given as mean ± S.E.M. and represent three independent experiments. The results represent three independent preparations of hGSTO1-1 protein.

RESULTS

The reduction of MMA(V) by hGSTO1-1 was inhibited by α-tocopherol phosphate in a concentration-dependent manner (Fig. 1). The IC_{50} for (+)-α-tocopherol phosphate was in the range of 2 ± 0.072 μM. Following the addition of 8 μM tocopherol phosphate, no GST activity was detected. The GST activity was measured with increasing concentrations of MMA(V) in the presence of a fixed concentration (2 μM) of (+)-α-tocopherol phosphate. hGSTO1-1 showed characteristic Michaelis-Menten behavior with respect to MMA(V) reduction (Fig. 2).

The Lineweaver-Burk plot shows that the inhibition of hGSTO1-1 with MMA(V) as a substrate

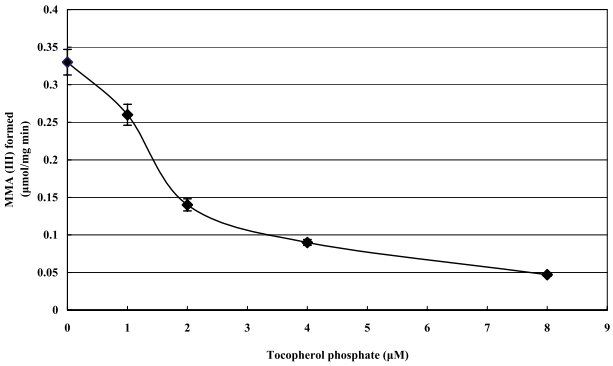

Figure 1. Effect of tocopherol phosphate on hGSTO1-1 activity.
The hGSTO1-1 activity was measured under the following conditions: 0.6 μCi MMA(V), 20 mM cold MMA(V), 5 mM GSH, 2.5 μg hGSTO1-1 on 0.1 Tris/HCl, pH 8. The IC_{50} value for tocopherol phosphate is 2 ± 0.072 μM. Each point denotes the mean of three independent experiments (± S.E.M.).

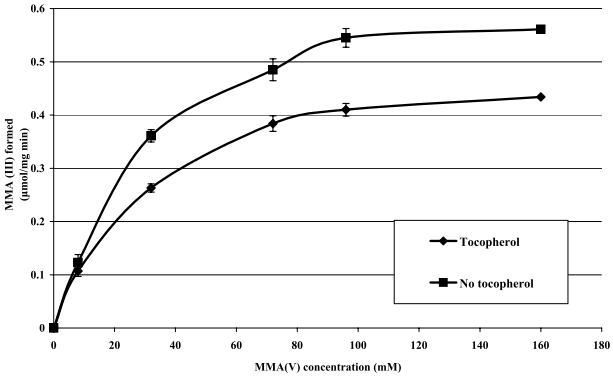

Figure 2. Dependence of hGSTO1-1 activity on tocopherol phosphate concentration.
The hGSTO1-1 activity was measured under the following conditions: 0.6 μCi MMA(V), 8–160 mM cold MMA(V), 5 mM GSH, 2.5 μg hGSTO1-1. In one set of experiments, 2 μM tocopherol phosphate in 0.1 M Tris/HCl, pH 8, was present. Each point denotes the mean of three independent experiments (± S.E.M.).

by 2 μM of (+)-α-tocopherol phosphate is uncompetitive (Fig. 3). The regression line represents a K_m of about 42.18 ± 3.4 × 10^{-3} M with a V_{max}= 0.78 ± 0.039 μmol/mg min for MMA(V) alone while the K_m is 32.17 ± 1.91 × 10^{-3} M with a V_{max}= 0.53 ± 0.023 μmol/mg min in the presence of 2 μM tocopherol phosphate.

Additionally, other compounds were tested for inhibitory effects on hGSTO1-1 (Table 1). Both tocopherols were very potent inhibitors of hGSTO1-

Table 1. Inhibitors of hGSTO1-1

Inhibitors	(IC_{50}) ± S.E.M.
CDNB	900 ± 75 μM
Deoxycholate	1020 ± 82 μM
Ethacrynic acid	25 ± 1 μM
Tocopherol phosphate	2 ± 0.072 μM
Tocopherol succinate	4 ± 0.13 μM

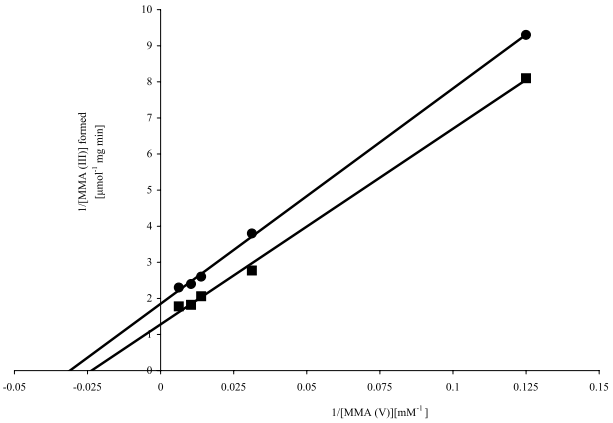

Figure 3. Lineweaver-Burk plot of inhibition of hGSTO1-1 by 2 μM tocopherol phosphate.
A K_m of 42.18 ± 3.4 × 10^{-3} M with a V_{max} of 0.78 ± 0.039 μmol/mg min was obtained by linear regression for MMA(V) alone (■). A K_m of 32.17 ± 1.91 × 10^{-3} M with V_{max} of 0.53 ± 0.023 μmol/mg min was obtained by linear regression following the addtion of 2 μM tocopherol phosphate (●). Each point denotes the mean of three independent experiments (± S.E.M.).

1 with similar potency. On the other hand, CDNB, ethacrynic acid, and deoxycholate, which is a nonsubstrate inhibitor of GSTs, failed to significantly inhibit hGSTO1-1.

DISCUSSION

The previously reported inhibitors of GST activity exert their action by binding to the hydrophobic pocket of the active site of GST (H-site) (Burg *et al.,* 2002). Others have reported that RRR-α-tocopherol, the naturally occurring form of vitamin E, probably binds to a lipophilic pit-like structure in GST. Binding of RRR-α-tocopherol to this site induces a conformational change of the GST molecule and thus diminishes the activity of the enzyme (van Haaften *et al.,* 2002). Conformational changes could be caused by an increase in protein volume and/or structural modifications of the lipophilic regions between the two monomers of GST. Binding of a compound to this region could, in fact, change the activity of the enzyme (van Haaften *et al.,* 2002). In our experiments, the addition of 2 μM (+)-α-tocopherol to a solution of purified hGSTO1-1 resulted in a dramatic inhibition of the enzyme (the IC_{50} value for (+)-α-tocopherol phosphate was 2 μM). Thus, (+)-α-tocopherol phosphate is a potent inhibitor of human GSTO1-1. Similar effects were observed for (+)-α-tocopherol succinate. While the effects of tocopherol phosphate and tocopherol succinate on hGSTO1-1 were specific, other compounds such as CDNB, deoxycholate, which is a nonsubstrate inhibitor of GSTs, and ethacrynic acid were inhibitory with IC_{50} values of 900 ± 75, 1020 ± 82 and 25 ± 1 μM, respectively.

A novel natural form of vitamin E, tocopheryl phosphate, has been detected in biological tissues as well as in a variety of foods (Gianello *et al.,* 2005). This molecule is a water-soluble tocopherol ester and thus may be valuable in cellular functions (Gianello *et al.,* 2005). Particularly, α-tocopherol phosphate was shown to exert a protective effect against ultraviolet-induced skin damage. This protection was greater than that seen with α-tocopherol acetate (Nakayama *et al.,* 2003).

Studies encompassing cell culture, animal model, and human epidemiological approaches have indicated a role for vitamin E both in normal brain function and in the prevention of neurodegeneration; however, the underlying molecular mechanisms of this association are not understood (Rota *et al.,* 2005). In a placebo-controlled trial, administration of vitamin E (2000 IU/day for 2 years) slowed (by 53%) the functional deterioration in patients with moderate Alzheimer's disease. These findings suggest that the use of α-tocopherol may actually delay clinically important functional deterioration in patients with Alzheimer's disease (Sano *et al.,* 1997). The prevention of Alzheimer's disease progression has been linked to vitamin E from food sources but not from supplements (Clare *et al.,* 2005). One potential explanation for this difference stems from the fact that vitamin E supplements consist of α-tocopherol alone while naturally occurring vitamin E consists of four different tocopherol forms (α, β, γ, and δ) and four corresponding tocotrienols. Thus, the authors of the above mentioned study propose that the effects of vitamin E on Alzheimer's disease are not due to α-tocopherol alone but to either another tocopherol form or a combination of tocopherol forms (Clare *et al.,* 2005).

The nervous, immune, and endocrine systems are closely linked due, in part, to the presence of molecules such as the interleukins. IL-1 and IL-6, for example, are involved in the inflammatory responses as well as the immune responses that occur in Alzheimer's disease (Serrano-Sanchez & Diaz-Arnesto, 2001). Inhibitors of inflammation may be important for preventing progression of Alzheimer's disease and other neurodegenerative diseases and thus require more in-depth investigations (Laliberte *et al.,* 2003). Studies have suggested that IL-1b is overexpressed in the brains of both Alzheimer's and Parkinson's patients (Laliberte *et al.,* 2003). Furthermore, hGSTO1-1 and possibly hGSTO2-2 have been correlated with an inflammatory response and the age-at onset for Alzheimer's disease (Laliberte *et al.,* 2003). Cytokine release in-

hibitory drugs, which inhibit IL-1b directly, target hGSTO1-1. A possible mechanism for the effect of vitamin E on the delay of clinically important deterioration in Alzheimer's patients may be the direct inhibition of hGSTO1-1 and the subsequent inhibition of the activity of IL-1b, which is one of the key signalling that modulate inflammation. Therefore, vitamin E, which we have shown here to be a potent inhibitor of hGSTO1-1, should be evaluated for its role in inflammatory inhibition in neurodegenerative diseases.

REFERENCES

Anatol K, Svetlana S (2004) Vitamin E in neurodegenerative disorders Alzheimer's disease. *Ann NY Acad Sci* **1031**: 249–262.

Aposhian HV, Zakharyan RA, Avram MD, Sampayo-Reyes A, Wollenberg ML (2004) A review of the enzymology of arsenic metabolism and a new potential role of hydrogen peroxide in the detoxication of the trivalent arsenic species. *Toxicol Appl Pharmacol* **198**: 327–335.

Azzi A, Stocker A (2000) Vitamin E: non-antioxidant roles. *Prog Lipid Res* **39**: 231–255.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254.

Board PG, Coggan M, Chelvanayagam G, Easteal S, Jermiin LS, Schulte GK, Danley DE, Hoth LR, Griffor MC, Kamath AV, Rosner MH, Chrunyk BA, Perregaux DE, Gabel CA, Geoghegan KF, Pandit J (2000) Identification, characterization, and crystal structure of the Omega class glutathione transferases. *J Biol Chem* **275**: 24798–24806.

Burg D, Hameetman L, Filippov DV, van der Marel GA, Mulder GJ (2002) Inhibition of glutathione *S*-transferase in rat hepatocytes by a glycine-tetrazole modified *S*-alkyl-GSH analogue. *Bioorg Med Chem Lett* **12**: 1579–1582.

Clare MM, Evans DA, Tangney CC, Bienias JL, Wilson RS, Aggarwal NT, Scherr PA (2005) Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change 1–3. *Am J Clin Nutr* **81**: 508–514.

Dirr H, Reinemer P, Huber R (1994) X-ray crystal structures of cytosolic glutathione *S*-transferases. Implications for protein architecture, substrate recognition and catalytic function. *Eur J Biochem* **220**: 645–661.

Dulhunty A, Gage P, Curtis S, Chelvanayagam G, Board P (2001) The glutathione transferase structural family includes a nuclear chloride channel and a ryanodine receptor calcium release channel modulator. *J Biol Chem* **276**: 3319–3323.

Gianello R, Libinaki R, Azzi A, Gavin PD, Negis Y, Zingg JM, Holt P, Keah HH, Griffey A, Smallridge A, West SM, Ogru E (2005) Alpha-tocopheryl phosphate: a novel, natural form of vitamin E. *Free Radic Biol Med* **39**: 970–976.

Giri U, Terry NH, Kala SV, Lieberman MW, Story MD (2005) Elimination of the differential chemoresistance between the murine B-cell lymphoma LY-ar and LY-as cell lines after arsenic (As2o3) exposure via overexpression of GSTO1(p28) *Cancer Chemother Pharmacol* **55**: 511–521.

Hayes D, Flanagan JU, Jowsey IR (2005) Glutathione transferases. *Annu Rev Pharmacol Toxicol* **45**: 51–88.

Johansson A, Ridderström M, Mannervik B (2000) The human glutathione transferase P1-1 specific inhibitor ter 117 designed for overcoming cytostatic-drug resistance is also a strong inhibitor of glyoxalase I. *Mol Pharmacol* **57**: 619–624.

Laliberte RE, Perregaux DG, Hoth LR, Rosner PJ, Jordan CK, Peese KM, Eggler JF, Dombroski MA, Geoghegan KF, Gabel CA (2003) Glutathione S-transferase omega 1-1 is a target of cytokine release inhibitory drugs and may be responsible for their effect on interleukin-1β posttranslational processing. *J Biol Chem* **278**: 16567–16578.

Nakayama S, Katoh EM, Tsuzuki T, Kobayashi S (2003) Protective effect of alpha-tocopherol-6-O-phosphate against ultraviolet B-induced damage in cultured mouse skin. *J Invest Dermatol* **121**: 406–411.

Ogru E, Libinaki R, Gianello R, West S, Munteanu A, Zingg J, Azzi A (2004) Modulation of cell proliferation and gene expression by α-tocopheryl phosphates relevance to atherosclerosis and inflammation. *Ann NY Acad Sci* **1031**: 405–411.

Rota C, Rimbach G, Minihane AM, Stoecklin E, Barella L (2005) Dietary vitamin E modulates differential gene expression in the rat hippocampus: potential implications for its neuroprotective properties. *Nutr Neurosci* **1**: 21–29.

Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundam M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, Thal LJ (1997) A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's disease cooperative study. *N Engl J Med* **336**: 1216–1222.

Schmuck EM, Board PG, Whitbread AK, Tetlow N, Cavanaugh JA, Blackburn AC, Masoumi A (2005) Characterization of the monomethylarsonate reductase and dehydroascorbate reductase activities of Omega class glutathione transferase variants: implications for arsenic metabolism and the age-at-onset of Alzheimer's and Parkinson's diseases. *Pharmacogenet Genomics* **15**: 493–501.

Serrano-Sanchez T, Díaz-Armesto I (2001) Participation of interleukin 1 and interleukin 6 in the aetiopathogenesis of Alzheimer's disease. *Rev Neurol* **7**: 648–652.

Sinning I, Kleywegt GJ, Cowan SW, Reinemer P, Dirr HW, Huber R, Gilliland GL, Armstrong RN, Ji X, Board PG, Olin B, Mannervik B, Jones TA (1993) Structure determination and refinement of human alpha class glutathione transferase A1-1, and a comparison with the Mu and Pi class enzymes. *J Mol Biol* **232**: 192–212.

Traber MG, Packer L (1995) Vitamin E: beyond antioxidant function. *Am J Clin Nutr* **62** (Suppl): 1501S–1509S.

van Haaften RI, Evelo CT, Haenen GR, Bast A (2001) Alfa-tocopherol inhibits human glutathione *S*-transferase. *Biochem Biophys Res Commun* **280**: 631–633.

van Haaften RI, Haenen GR, Evelo CT, Bast A (2002) Tocotrienols inhibit human glutathione S-transferase P1-1. *IUBMB Life* **54**: 81–84.

Wang L, Xu J, Ji C, Gu S, Lv Y, Li S, Xu Y, Xie Y, Mao Y (2005) Cloning, expression and characterization of human glutathione S-transferase Omega-2. *Int J Mol Med* **16**: 19–27.

Yin ZL, Dahlstrom JE, Le Couteur DG, Board PG (2001) Immunohistochemistry of omega class glutathione *S*-transferase in human tissues. *J Histochem Cytochem* **49**: 983–988.

Zakharyan RA, Aposhian HV (1999) Enzymatic reduction of arsenic compounds in mammalian systems: the rate-limiting enzyme of rabbit liver arsenic biotransformation is MMA(V) reductase. *Chem Res Toxicol* **12**: 1278–1283.

Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, Liebler DC, Aposhian HV (2001) Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. *Chem Res Toxicol* **14**: 1051–1057.