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Research Communication

Tocotrienols Inhibit Human Glutathione S-Transferase P1-1

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Summary

Tocopherols and tocotrienols are food ingredients that are believed to have a positive effect on health. The most studied property of both groups of compounds is their antioxidant action. Previously, we found that tocopherols and diverse tocopherol derivatives can inhibit the activity of human glutathione S-transferase P1-1 (GST P1-1). In this study we found that GST P1-1 is also inhibited, in a concentration-dependent manner, by α - and γ -tocotrienol. The concentration giving 50% inhibition of GST P1-1 is $1.8 \pm 0.1 \mu\text{M}$ for α -tocotrienol and $0.7 \pm 0.1 \mu\text{M}$ for γ -tocotrienol. This inhibition of GST P1-1 is noncompetitive with respect to both substrates CDNB and GSH. We also examined the 3D structure of GST P1-1 for a possible tocopherol/tocotrienol binding site. The enzyme contains a very hydrophobic pit-like structure where the phytyl tail of tocopherols and tocotrienols could fit in. Binding of tocopherol and tocotrienol to this hydrophobic region might lead to bending of the 3D structure. In this way tocopherols and tocotrienols can inhibit the activity of the enzyme; this inhibition can have far-reaching implications for humans.

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Keywords Glutathione S-transferase P1-1; human; inhibition; tocotrienol; vitamin E.

INTRODUCTION

Vitamin E is the generic name for both tocopherol and tocotrienol derivatives. Tocopherols and tocotrienols both consist of a common chromanol head (with two rings: one phenolic and one heterocyclic) and a 16-carbon tail attached at the 2-position.

The tocotrienols differ from the tocopherols only in the tail. Tocopherols have a saturated phytyl side chain, whereas the tail of tocotrienols is unsaturated and forms an isoprenoid chain (1–3). Tocotrienols and tocopherols are further divided into several vitamers (α , β , δ , and γ) depending on the number and position of methyl groups on the chroman head (Fig. 1) (1–3). They are both used as food additives. The natural sources of tocopherol mainly consist of nuts and common vegetable oils (i.e., wheat germ, sunflower (4)). Tocotrienols are minor plant constituents, with a relative high abundance in cereal grains (i.e., oat, barley, and rye) and certain vegetable oils (i.e., palm oil and rice bran oil; (4)).

Both tocopherols and tocotrienols are well recognized for their antioxidative effect. It has been stated that, in vitro, the tocotrienols are more potent antioxidants than the tocopherols (3). Aside from their function as antioxidants, tocotrienols are reported to reduce plasma cholesterol levels and lipid- and nonlipid-related risk factors for cardiovascular disease (4).

Tocopherols and diverse tocopherol derivatives can inhibit the activity of glutathione S-transferase P1-1 (GST P1-1; (5, 6)). This detoxification isoenzyme is the most prominent GST in erythrocytes and is also present in human skin (7, 8). In this study we examined the effect of two vitamers of tocotrienol on the activity of GST P1-1.

MATERIALS AND METHODS

Chemicals

Tocotrienols were a gift from BASF (Stuttgart, Germany); 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione S-transferase P1-1 (from human placenta) were obtained from Sigma, St. Louis, Missouri, USA. Reduced glutathione (GSH) was obtained from ICN Biomedicals Inc., Costa Mesa, California, USA. All other chemicals were of analytical grade purity.

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Figure 1. Molecular structure of tocopherols and tocotrienols. The naturally occurring vitamers— α , β , γ , and δ —have methylation patterns as indicated.

Assay of Glutathione S-Transferase Activity

GST activity was measured as described by Mannervik and Guthenberg (8) with slight modifications. In short, the reaction of 1 mM CDNB with 1 mM GSH in 100 mM potassium phosphate, pH 6.5, at 37°C was monitored spectrophotometrically by recording the increase in absorbance at 340 nm. Effects of various concentrations of α - and γ -tocotrienol (final concentration 0.25 μ M–5 μ M) on GST activity were determined. A stock solution of tocotrienol was prepared in ethanol. The final concentration of ethanol in the incubation mixture was 1% v/v; this concentration of ethanol had no effect on GST activity. The mixture of the GST enzyme (0.0095 U/ml in buffer) with tocotrienol was incubated for 2 min at 37°C before activity measurement. A correction for the spontaneous reaction was made for the formation of the conjugate of GSH and CDNB in the absence of enzyme and in the presence of ethanol (1%). Ethanol was added as a control because tocotrienol was dissolved in ethanol. To study the inhibitory mechanism, substrate concentrations (CDNB or GSH) were varied. When CDNB was varied, the GSH concentration was kept at 1 mM and vice versa, the concentration of tocotrienol was constant (1 μ M).

3D Structure Evaluation

The PDB file for human GSTP1-1 with GSH bound [PDB ID: 7GSS (9)] was downloaded from the PDB database (<http://www.rcsb.org/pdb>) (10) and examined with Rasmol (version 2.6, (<http://www.umass.edu/microbio/rasmol>)). Hydrophobic amino acids were color-coded light gray (green in full color version) using the Rasmol “color hydrophobic light gray

Figure 2. Effect of α -tocotrienol (■) and γ -tocotrienol (□) on the activity of GST P1-1. The IC₅₀ values are $1.8 \pm 0.1 \mu$ M for α -tocotrienol and $0.7 \pm 0.1 \mu$ M for γ -tocotrienol. Each point denotes the mean $\pm$ standard error of the mean of three experiments.

(green in full color version)” setting. The G-site of GSTP1-1 was previously identified by Reinemer et al. (11). The nonhydrophobic amino acids in this region were color-coded black (blue in full color version).

RESULTS

Both α - and γ -tocotrienol inhibited the GST P1-1 activity in a concentration-dependent manner (Fig. 2). The concentration giving 50% inhibition of the GST P1-1 activity (IC₅₀) is $1.8 \pm 0.1 \mu$ M for α -tocotrienol and $0.7 \pm 0.1 \mu$ M for γ -tocotrienol. The nature of the inhibition of γ -tocotrienol was studied by making Lineweaver-Burk plots for both the substrates CDNB and GSH. GST activity was measured with variable concentrations of either CDNB (Fig. 3) or GSH (data not shown) in the

Figure 3. Lineweaver-Burk plot showing noncompetitive inhibition of human GST P1-1 towards CDNB by 1 μ M γ -tocotrienol. The K_m and V_{max} (mean $\pm$ standard error of the mean) for CDNB (●) are, respectively, 1.1 ± 0.1 mM and $20.7 \pm 2.5 (\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1})$. In the presence of γ -tocotrienol (○), these values are 1.3 ± 0.1 mM and $14.6 \pm 1.2 (\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1})$.

Figure 4. Three-dimensional structure of human GST P1-1 with GSH bound. (The figure is shown in full color at <http://taylorandfrancis.metapress.com/link.asp?id=103791>.) GSH is shown as a ball-structure. The hydrophobic parts are light gray (green in full color version) and the hydrophilic parts are gray (white in full color figure). The black part (blue in full color version) is the hydrophilic part of the glutathione binding site. The dark gray part (orange in full color version) depicts the hydrophilic amino acids at the interface between the two monomers of the human GST P1-1. The hydrophobic region to which tocopherols and tocotrienols are proposed to bind is white (purple in full color version).

presence of a fixed concentration ($1 \mu\text{M}$) of γ -tocotrienol. GST showed characteristic Michaelis-Menten behavior towards both substrates (Fig. 3). As depicted in Fig. 3, γ -tocotrienol lowered the V_{max} , but did not change the K_m for CDNB. The same results are obtained for GSH.

Three-dimensional evaluation of the GSTP1-1 protein structure revealed that there are surprisingly few hydrophobic amino acids present in the contact region of the two monomers. The hydrophilic amino acids in the contact region are color-coded dark gray (orange in full color version) in Fig. 4. Further evaluation of possible hydrophobic binding locations for tocopherols and tocotrienols revealed that each monomer contains a region consisting of two α -helix fragments (15–22 and 68–78), with hydrophobic amino acids all on one side and folded towards each other, and a third hydrophobic two-amino acid fragment (150–151) that also folds to the same region (white [purple in full color version] fragment in Fig. 4). This region is very close to the GSH binding site (G-site) (black [blue in full color version] part in Fig. 4 is hydrophilic part of G-site); the minimum

distance between a GSH member atom and the nearest atom in the region is only $6.5 \text{ \AA}$.

DISCUSSION

Comparable to tocopherol and α -tocopherol derivatives, tocotrienols inhibit the activity of GST P1-1 in a concentration-dependent manner. The IC_{50} of the tocotrienols (α -tocotrienol $1.8 \mu\text{M}$ and γ -tocotrienol $0.7 \mu\text{M}$) are in the same range of the IC_{50} of the tocopherols (α -tocopherol $0.7 \mu\text{M}$ and δ -tocopherol $0.8 \mu\text{M}$) (6). So the tocopherols and the tocotrienols have a comparable potency to inhibit the GST P1-1 activity. The antioxidant activity of tocopherols and tocotrienols is also comparable, since the group that displays the central antioxidant activity, i.e., the chromanol head, is identical. Both tocopherols and tocotrienols scavenge the chain propagating peroxy radical in the process of peroxidation of lipids (3). Although it has been reported that, in vitro, the tocotrienols are more potent antioxidants than the tocopherols, tocotrienols have a lower bioavailability after oral ingestion (3).

The inhibition of GST P1-1 by tocotrienols is, similar to the inhibition by tocopherols (5), noncompetitive with respect to both substrates CDNB and GSH. We have previously suggested that the probable mechanism of GST inhibition by tocopherols and tocopherol derivatives is the induction of conformational changes of the enzyme. These conformational changes could be caused by volume increases and structural modifications of lipophilic regions that are present in the interface between the two monomers of the GST enzyme (12).

The three-dimensional evaluation of the GST structure showed that binding of tocopherols or tocotrienols in the contact region of the two monomers is unlikely because a large hydrophilic fragment of amino acids is present in this region (dark gray [orange in full color version] part in Fig. 4). However, a very hydrophobic pit-like structure, where the phytol tail of tocopherols and tocotrienols could fit in, is present just below the G-site. It is likely that binding of hydrophobic tocopherol or tocotrienol occurs preferentially in this hydrophobic region.

Several groups found that GST P1-1 can bind various hydrophobic compounds that can induce significant conformational changes of the protein. This resulted in a marked reduction of enzyme activity. The amino acid sequences involved in the binding of these hydrophobic compounds are residues 141–156 (13–15). It appeared that binding of a hydrophobic compound (e.g., 1-amino-8-naphthalene sulfonic acid) results in noncompetitive inhibition (toward substrates, GSH and CDNB) of the GST activity (15). The pit-like structure described in this study (Fig. 4) contains an overlapping region (residues 150–151) with the hydrophobic binding region described in earlier studies (residues 141–156). Our three-dimensional structure analyses showed that sequences 150–151 within the sequences 141–156 are the most important for the binding of tocopherols/tocotrienols. These two amino acids are hydrophobic and are the only amino acids that have the right orientation

in the sequence 141–156 to interact with lipophilic compounds. Together with the amino acids in sequences 15–22 and 68–78, the amino acids in sequence 150–151 form the pit-like structure in GST P1-1, where the tail of tocopherol and tocotrienol fits in. Binding of tocopherols and tocotrienols to this region might lead to the bending of the 3D structure by pushing the three hydrophobic regions apart, resulting in distorting the G-site and possibly the hydrophobic-site (H-site).

Although the tocopherols and tocotrienols share the same mechanism of inhibition of GST P1-1, their retention in the body is different. The α -tocopherol transfer protein (α -TTP) binds to α -tocopherol with high affinity and specificity. This protein enhances transfer of α -tocopherol between membranes and determines the level of plasma and tissue α -tocopherol in the different organs. The affinity of α -tocotrienol for α -TTP is only 12% of that of α -tocopherol (16). Due to this lower affinity of α -tocotrienol compared to α -tocopherol and due to lower intake of α -tocotrienol compared to α -tocopherol, the concentration of α -tocopherol in most organs is much higher than the concentration of α -tocotrienol (17).

Less than 2% of the total amount of vitamin E in most vital tissues consists of tocotrienol. Surprisingly, dietary tocotrienols accumulate specifically in the skin (18) because up to 13% of total vitamin E in skin consists of tocotrienol (17). Vitamin E in the skin has multiple important functions besides the antioxidant function. For example, skin has been suggested to be an important storage site for vitamin E (19) and vitamin E has a regulatory role in maintaining the barrier function of the skin (17). It is known that the skin also contains a relatively high amount of GST P1-1 (20). GST P1-1 detoxifies a wide variety of xenobiotics including (+)-anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene ((+)-anti-BPDE) (21). This compound is a well-known mutagenic, tumorigenic, and carcinogenic compound. Tocotrienols may reduce the defense against this noxious compound. This can have far-reaching consequences in human health.

REFERENCES

- Kamal-Eldin, A., and Appelqvist, L. A. (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* **31**, 671–701.
- Komiyama, K., Iizuka, K., Yamaoka, M., Watanabe, H., Tsuchiya, N., and Umezawa, I. (1989) Studies on the biological activity of tocotrienols. *Chem. Pharm. Bull.* **37**, 1369–1371.
- Packer, L., Weber, S. U., and Rimbach, G. (2001) Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. *J. Nutr.* **131**, 369s–373s.
- Theriault, A., Chao, J. T., Wang, Q., Gapor, A., and Adeli, K. (1999) Tocotrienol: a review of its therapeutic potential. *Clin. Biochem.* **32**, 309–319.
- Haaften van, R. I. M., Evelo, C. T. A., Haenen, G. R. M. M., and Bast, A. (2001) Alpha-tocopherol inhibits human glutathione S-transferase pi. *Biochem. Biophys. Res.* **280**, 631–633.
- Haaften van, R. I. M., Evelo, C. T. A., Penders, J., Eijnwachter, M. P. F., Haenen, G. R. M. M., and Bast, A. (2001) Inhibition of human glutathione S-transferase P1-1 by tocopherols and alpha-tocopherol derivatives. *Biochim. Biophys. Acta* **1548**, 23–28.
- Guthenberg, C., and Mannervik, B. (1981) Glutathione S-transferase (transferase pi) from human placenta is identical or closely related to glutathione S-transferase (transferase ρ) from erythrocytes. *Biochim. Biophys. Acta* **661**, 255–260.
- Mannervik, B., and Guthenberg, C. (1981) Glutathione transferase (human placenta). *Method Enzymol.* **77**, 231–235.
- Oakley, A. J., Bello, M. L., Battistoni, A., Ricci, G., Rossjohn, J., Villar, H. O., and Parker, M. W. (1997) The structures of human glutathione transferase P1-1 in complex with glutathione and various inhibitors at high resolution. *J. Mol. Biol.* **274**, 84–100.
- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., and Bourne, P. E. (2000) The Protein Data Bank. *Nucleic Acids Res.* **28**, 235–242.
- Reinemer, P., Dirr, H. W., Ladenstein, R., Huber, R., Lo-Bello, M., Federici, G., and Parker, M. W. (1992) Three-dimensional structure of class pi glutathione S-transferase from human placenta in complex with S-hexylglutathione at 2.8 Å resolution. *J. Mol. Biol.* **227**, 214–226.
- Ricci, G., Lo-Bello, M., Caccurri, A. M., Pastore, A., Nuccetelli, M., Parker, M. W., and Federici, G. (1995) Site-directed mutagenesis of human glutathione transferase P1-1. Mutation of Cys-47 induces a positive cooperativity in glutathione transferase P1-1. *J. Biol. Chem.* **270**, 1243–1248.
- Nishihira, J., Ishibashi, T., Sakai, M., Tsuda, S., and Hikichi, K. (1993) Identification of the hydrophobic ligand-binding region in recombinant glutathione S-transferase P and its binding effect on the conformational state of the enzyme. *Arch. Biochem. Biophys.* **302**, 128–133.
- Nishihira, J., Ishibashi, T., Sakai, M., Nishi, S., and Kumazaki, T. (1993) Identification of the fatty acid binding site on glutathione S-transferase P by immobilization to fatty acid-linked sepharose. *Biochem. Biophys. Res. Commun.* **190**, 823–831.
- Nishihira, J., Ishibashi, T., Sakai, M., Nishi, S., Kondo, H., and Makita, A. (1992) Identification of the fatty acid binding site on glutathione S-transferase P. *Biochem. Biophys. Res. Commun.* **189**, 197–205.
- Hosomi, A., Arita, M., Sato, Y., Kiyose, C., Ueda, T., Igarashi, O., Arai, H., and Inoue, K. (1997) Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Lett.* **409**, 105–108.
- Podda, M., Weber, C., Traber, M. G., and Packer, L. (1996) Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinol, and ubiquinones. *J. Lipid. Res.* **37**, 893–901.
- Ikeda, S., Niwa, T., and Yamashita, K. (2000) Selective uptake of dietary tocotrienols into rat skin. *J. Nutr. Sci. Vitaminol.* **46**, 141–143.
- Shiratori, T. (1974) Uptake, storage and excretion of chylomicron-bound 3H-alpha-tocopherol by the skin of the rat. *Life Sci.* **14**, 929–935.
- Singhal, S. S., Saxena, M., Awasthi, S., Mukhtar, H., Zaidi, S. I., Ahmad, H., and Awasthi, Y. C. (1993) Glutathione S-transferases of human skin: qualitative and quantitative differences in men and women. *Biochim. Biophys. Acta* **1163**, 266–272.
- Mannervik, B. (1985) The isoenzymes of glutathione transferase. *Adv. Enzymol. RAMB* **57**, 357–417.