Inhibition of Oxidative and Antioxidative Enzymes by Trans-Resveratrol

X. Fan and J.P. Mattheis

ABSTRACT: Trans-resveratrol, a phytoalexin produced by a variety of plants, has been shown to inhibit oxidative enzymes in an animal cell system. Its effect on several oxidative and antioxidative enzymes from plants was investigated using in vitro assays. Trans-resveratrol inhibited superoxide dismutase, lipoxygenase, catalase, peroxidase, polyphenol oxidase, and 1-aminocyclopropane-1-carboxylic acid oxidase with apparent K_i's of 10, 90, 100, 255, 305, and 350 μM, respectively. Trans-resveratrol inhibited lipoxygenase activity more effectively than other lipoxygenase inhibitors, including propyl gallate, ibuprofen, ursoic acid, acetylsalicylic acid, and salicylhydroxamic acid.

Key Words: trans-resveratrol, superoxide dismutase, lipoxygenase, catalase, peroxidase, polyphenol oxidase, and 1-aminocyclopropane-1-carboxylic acid oxidase.

Introduction

OXIDATIVE ENZYMES ARE OF INCREASING INTEREST TO THE plant scientist as well as the food industry because of their effects on plant metabolism and the nutritional benefits they impart to plant foods. Polyphenol oxidase (PPO) is responsible for enzymatic browning in a wide range of vegetable, fruit, and juices (Mayer and Harel 1979). Peroxidases may be responsible for the generation of free radicals, and may have a role in lignin synthesis, hormone metabolism, and response to stress (Gaspar and others 1982). Another enzyme, lipoxygenase, catalyzes the oxidation of polyunsaturated fatty acids to produce hydroperoxides which can be broken down by other enzymes (Siedow 1991). Lipoxygenase may also catalyze the oxidation of carotenoids, including β-carotene. Lipoxygenase has a profound influence on color, flavor, texture, and nutritive properties of food (Robinson and others 1995). Another important enzyme in plant systems, 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase, catalyzes the synthesis of the plant hormone ethylene (Yang and Hoffman 1984). Enzymes, such as catalase and superoxide dismutase (SOD), may serve to inactivate oxygen species and function as antioxidants in biological systems (Larson 1988).

Active oxygen species are generated during normal aerobic metabolism. These free radicals can cause deleterious oxidation of lipids, proteins and nucleic acids that seriously perturb normal cell metabolism. Plants and animals posses numerous antioxidant systems for protection against these active oxygen species. Trans-resveratrol, a natural compound, has been found in a variety of plants; for example, grapes and peanuts (Gorham 1980). Trans-resveratrol has been suggested to reduce heart disease and to have anti-cancer activity. These effects could be due to trans-resveratrol's ability to inhibit low-density lipoprotein oxidation (Frankel and others 1993), block platelet aggregation (Kimura and others 1985, Chung and others 1992) and eicosanoid synthesis (Pace-Asciak and others 1995). Trans-resveratrol also acts as an antioxidant and inhibits cyclooxygenase and hydperoxidase activities in an animal cell system (Jang and others 1997). Trans-resveratrol is a phytoalexin produced during environmental stress or pathogenic attack (Langcake and Pryce 1976). However, the physiological function of trans-resveratrol in plants is not well defined. In this study, the effect of trans-resveratrol on several oxidative and antioxidative enzymes found in plants was investigated.

Materials and Methods

Chemicals.

All chemicals were purchased from Sigma (St Louis, Mo., U.S.A.). A stock solution of trans-resveratrol (10 mM) was prepared in ethanol. Trans-resveratrol in the assay buffer was diluted from this stock solution. There were 3 replicates for all enzyme assays.

Preparation and assay of enzymes.

Lipoxygenase. Lipoxygenase from soybean was purchased from Sigma and assayed as described by the supplier, with modification using linoleic acid as the substrate. Activity was measured by incubating the enzyme solution with 17.8 μM linoleic acid in 0.2 M Na-borate (pH 9.0) containing 5% ethanol. The absorbance at 236 nm was monitored for 5 min with a spectrophotometer (HP 9451A, Hewlett Packard, Avondale, Pa., U.S.A.) at 22 °C. One unit of activity was defined as an absorbance increase of 0.001 per min. The effect of trans-resveratrol on lipoxygenase was compared with that of other lipoxygenase inhibitors, propyl gallate, ibuprofen, ursoic acid, acetylsalicylic acid, and salicylhydroxamic acid. All inhibitors were present in the assay at a concentration of 267 μM.

PPO. ‘Fuji’ apple peel (10g) was homogenized for 3 min with 50 ml cold acetone (−20 °C). The homogenate was filtered through a glass fiber filter (Whatman GF/C) in a Buchner funnel, and the residue washed with cold acetone until a colorless filtrate was obtained. The acetone powder was dried under vacuum. PPO was extracted by suspending 2 g of the acetone powder in 90 ml 50 mM Na-phosphate (pH 7.0) with 1 M KCl. After agitation on ice for 30 min, the mixture was filtered through Miracloth (Calbiochem, San Diego, Calif., U.S.A.), and centrifuged at 28,000g for 30 min. The supernatant was used as the enzyme source.
natant was used for further purification. A 30 to 80% 
(NH₄)₂SO₄ precipitation was dissolved in 20 ml 50 mM Na-
phosphate buffer (pH 6.5), and dialyzed overnight against 0.4 
M (NH₄)₂SO₄ and 0.4 M KCl at 4 °C. After centrifugation 
(28,000g, 10 min), the supernatant was purified by hydropho-
bic chromatography according to Trejo-Gonzalez and Soto-
Valdez (1991). The supernatant was applied onto a Phenol 
Sepharose CL4B column (28 x 80 cm; Pharmacia, Piscat-
away, N.J., U.S.A.) that was equilibrated with a 50 mM Na-
phosphate buffer (pH 6.8) with 0.4 M (NH₄)₂SO₄ and 0.1M 
KCl. PPO was eluted with a linear gradient of 0.4 M to 0 M 
(NH₄)₂SO₄ and 0.1 M to 0 M KCl at 1 ml/min. All fractions (5 
ml each) containing PPO activity were combined. PPO was 
assayed according to Sciancalepore and Longone (1984). The 
reaction mixture contained 1.5 ml 0.1 M Na-citrate (pH 5.0), 
1 ml 0.02 M catechol in the same buffer, and 0.5 ml enzyme 
preparation. The increase in A 420 was recorded every 10 sec 
up to 5 min from the time the enzyme extract was added. 
One unit of enzyme activity was defined as an absorbance in-
crease of 0.001 per min at 22 °C.

Peroxidase. Peroxidase was extracted and purified similar 
to PPO. Total peroxidase activity was assayed by incubating 
20 ml of extract with 1 ml of 100 mM guaiacol, 0.3 ml of 1% 
H₂O₂, and 1 ml of 20 mM Na-phosphate buffer (pH 6.5) (Mill-
ner and others 1987). The reaction was initiated by the addi-
tion of H₂O₂ and the increase in A 470 was determined for 5 
min at 22 °C. One unit of enzyme activity was defined as an 
absorbance increase of 0.001 per min.

ACC oxidase. Extraction and assay of ACC oxidase from ‘Fuji’ apples were as described previously (Fan and others 
1996). Briefly, cortex tissue was homogenized with 400 mM 
K-phosphate (pH 7.2) containing 10 mM NaS₂O₅, 4 mM 2-
mercaptoethanol, and 3 mM Na-ascorbate. The homogenate 
was filtered and centrifuged at 28,000 g. The pellet was resus-
pended in 200 mM MOPS (pH 7.2), 1 mM dithiothreitol, 3 
mM Na-ascorbate, 10% glycerol (W/V), and 0.2% Triton X-
100 (V/V). The mixture was centrifuged and the supernatant 
was used to assay for ACC oxidase activity. The reaction mix-
ture contained 100 mM MOPS (pH 7.2), 20 μM FeSO₄, 1 mM 
ACC, 1mM Na-ascorbate, 6% (V/V) CO₂ (in the gas phase) 
and 10% (W/V) glycerol in a sealed test tube. The reaction 
was initiated by addition of 100 μl enzyme extract. After 30 
min incubation with shaking at 25 °C, headspace ethylene 
was measured by a gas chromatography.

SOD. SOD was extracted from ‘Fuji’ apples. Cortex tissue 
(20 g) was homogenized with 40 ml extraction buffer (0.1 M

Figure 1—Effect of trans-resveratrol on lipoxygenase ac-
tivity in vitro. Each data point is the mean of 3 replicates.

Figure 2—Lineweaver-Burk plot of lipoxygenase activity 
versus linoleic acid concentration at: o, 0; +, 100; Δ,133 μM trans-resveratrol. Each data point is the mean of 3 
replicates.

Figure 3—Comparison of trans-resveratrol (RE) inhibitory 
effect on lipoxygenase activity with propyl gallate (PG), 
ibuprofen (IP), ursolic acid (UA), acetylsalicylic acid (AS), 
and salicylhydroxamic acid (SA). All inhibitors were present 
in the assay buffer at 267 μM. Each data point is the mean 
of 3 replicates.
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K-phosphate pH, 7.8, 0.1 mM EDTA, and 1% PVPP for 5 min. The homogenate was filtered through Miracloth (Calbiochem, San Diego, Calif., U.S.A.), then centrifuged for 10 min at 12,000 g. The supernatant was used for SOD assays according to Giannopolitis and Ries (1977). The reaction mixture included 1 ml water, 1 ml of 0.05 M Na₂CO₃ (pH 10.2) buffer containing 13 mM methionine, 1.3 μM riboflavin and 63 μM nitro blue tetrazolium, and 1 ml enzyme extract in a final volume of 3 ml. An identical tube without the enzyme extract served as a blank. Reactions were carried out for 30 min under illumination at 22 °C. The A₅₆₀ nm was measured and 1 unit of SOD activity was defined as the amount of enzyme which inhibited the reaction rate by 50%.

Catalase. Catalase was purchased from Sigma and assayed as described by the supplier. The reaction mixture contained 25 mM K-phosphate (pH 6.8) and 10 mM H₂O₂. Absorbance at 240 nm was monitored for 1 min after addition of enzyme at 22 °C. One unit was defined as the amount of enzyme decomposing 1 μM H₂O₂ per min.

Results and Discussion

TRAN RESVERATROL INHIBITED LIPOXYGENASE IN A CONCENTRATION-DEPENDENT MANNER, inhibition increased with trans-resveratrol concentration (Figure 1). At 90 μM trans-resveratrol, lipoxygenase activity was half that of the controls. The inhibition of lipoxygenase activity by trans-resveratrol appeared to be uncompetitive with respect to linoleic acid (Figure 2). Trans-resveratrol was the most effective

![Figure 5—Effect of trans-resveratrol on superoxide dismutase (SOD) activity in vitro. Each data point is the mean of 3 replicates.](image)

![Figure 6—Effect of trans-resveratrol on catalase activity in vitro. Each data point is the mean of 3 replicates.](image)
among the lipoxygenase inhibitors tested (Figure 3).

Trans-resveratrol inhibited PPO (Figure 4A) and peroxidase (Figure 4B) with apparent $K_i$ of 305 $\mu$M and 255 $\mu$M, respectively. The relationship between PPO activity and concentration of trans-resveratrol appeared to be linear. SOD activity was inhibited by trans-resveratrol with an apparent $K_i$ of 10 $\mu$M (Figure 5). Catalase was inhibited by trans-resveratrol with a $K_i$ of 100 $\mu$M, but activity was detectable even at high trans-resveratrol concentrations (Figure 6). ACC oxidase was also inhibited by trans-resveratrol with an apparent $K_i$ of 350 $\mu$M, but the enzyme was not totally inhibited even at 3.2 mM (Figure 7).

Many oxidase enzymes require a metal co-factor which is believed to undergo redox changes during the course of catalysis. Lipoxygenase is an Fe containing dioxygenase (Siedow 1991), and there are 3 types of SOD classified by their metal cofactor: Cu/Zn, Mn or Fe (Bannister and others 1987). ACC oxidase requires Fe as a cofactor (Smith and others 1992). PPO may have Cu (Mayer and Harel 1979) and peroxidase uses Fe as co-factors (Gaspar and others 1982). Trans-resveratrol is a phenolic compound containing 2 –OH groups. Any molecule with an unshared electron pair can coordinate to form complexes with metal ions. Trans-resveratrol may act as a metal chelator and eliminate the availability of metal to participate in enzyme activity.

Trans-resveratrol may also serve as a substrate for the oxidase enzymes. Peroxidase is capable of oxidizing trans-resveratrol and forms a compound with a light brown color (Moraes and others 1997). Lipoxygenase may also use trans-resveratrol as a substrate as a light-brown compound formed during our assays (data not shown). Trans-resveratrol may also form a complex with the lipoxygenase enzyme. Peroxidase is capable of oxidizing trans-resveratrol to the linoleic acid solution. Trans-resveratrol may also form a complex with the lipoxygenase enzymatic enzyme partially purified from melon. Peroxidase 31:1485-1494. Trejo-Gonzalez A, Soto-Valdez H. 1991. Partial characterization of polyphenol oxidase extracted from ' Anna' apple. J Amer Soc Hort Sci 116(4):672–675.

Figure 7—Effect of trans-resveratrol on 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity in vitro. Each data point is the mean of 3 replicates.

References


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Author Fan is with the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19098. Author Matthes is with the U.S. Department of Agriculture, Agricultural Research Service, Tree Fruit Research Laboratory, 1104 North Western Avenue, Wenatchee WA 98801. Direct inquiries to author Matthes (E-mail: matthes@tfrl.ars.usda.gov).