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Received for review August 4, 1988. Accepted December 27, 1988. Supported in part by the National Dairy Board and Wisconsin Milk Marketing Board.

Bleaching of β-Carotene by Trout Gill Lipoxygenase in the Presence of Polyunsaturated Fatty Acid Substrates

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The 12-lipoxygenase (LO) from trout gill effectively bleaches β-carotene in conjunction with the peroxidation of different polyunsaturated fatty acid (PUFA) substrates. The maximum velocity of bleaching differed significantly between LO from gill and the 15-LO from soybean for six PUFA substrates, compared to linoleic acid. The lag times before reaching maximum velocity of bleaching were shorter for trout gill LO than were those catalyzed by soybean LO for six of the eight PUFA substrates tested. This may reflect the presence of oxidative cofactors in the trout gill LO preparation.

Plant lipoxygenases (LO) have been extensively studied with regard to fatty acid peroxidation (Yoon and Klein, 1979; Klein et al., 1984). The enzyme has been implicated as a major contributor to off-flavors in legumes (Kalbrener et al., 1974) and in causing chemical changes in fruits and vegetables (Tressl et al., 1981). LO also plays a role in bread making by increasing mixing tolerance, serving as a bleaching agent, and improving dough rheology (Faubion and Hoseney, 1981). Recently, researchers have prepared LO from fish and other animal sources, in sufficient purity, for studying lipid oxidation rates (German and Kinsella, 1985; Yokoyama et al., 1986). The LO isolated from trout converts polyunsaturated fatty acids (PUFA) from fish muscle into PUFA hydroxyperoxides that can generate flavors and off-flavors (German and Kinsella, 1985; Hsieh and Kinsella, 1989). This LO action may cause discoloration of certain fish (Tsukuda, 1970), conceivably by bleaching of carotenoids following free-radical quenching (Krinsky and Deneke, 1982; Halevy and Sklan, 1987; Kanner et al., 1987). In order to assess this possibility, we compared the relative capacities of the LO isolated from trout gill and soybean to catalyze β-carotene bleaching in the presence of various PUFA substrates of both $n-6$ ($\omega 6$) and $n-3$ ($\omega 3$) families.

MATERIALS AND METHODS

The polyunsaturated fatty acids (PUFA; 99+% purity) linoleic (18:2, $n-6$), eicosadienoic (20:2, $n-6$), homo-γ-linolenic (20:3, $n-6$), eicosatrienoic (20:3, $n-3$), arachidonic (20:4, $n-6$), docosatrienoic (22:3, $n-3$), docosatetraenoic (22:4, $n-6$), and docosahexaenoic acid (22:6, $n-3$) were obtained from Nu-Chek Prep (Elysian, MN). These unsaturated fatty acids were dissolved in ethanol, diluted to a concentration of 25 mM, and stored at −70 °C under a nitrogen atmosphere.

Soybean 15-lipoxygenase (Type II, E.C. 1.13.13) (Sigma Chemical Co., St. Louis, MO) (10 mg) was dissolved in 100 mL of 0.05 M phosphate buffer (pH 7.8) and used as such. Trout gill containing LO (5 mg of protein), prepared by the method of German et al. (1986), was dissolved in 100 mL of 0.05 M phosphate buffer (pH 7.8) and used as such. Both LO preparations readily caused peroxidation of PUFA as assessed by oxygen consumption and autoradiography (Hsieh et al., 1988).

β-Carotene bleaching was assessed with pure β-carotene dispersed via the method of Aziz et al. (1971). Thus, 25 mg of β-carotene was dissolved in 25 mL of chloroform with 1 mL of Tween 80 detergent (ICI America's Inc., Wilmington, DE). Of this solution, 1 mL was evaporated to dryness on an aspirator and reconstituted in 10 mL of distilled water to yield a clear micellar solution (19 mM) of β-carotene.

The reaction mixture of trout gill LO contained 75 μg of trout gill protein dissolved in 1.5 mL of 0.05 M phosphate buffer (pH 7.8), 0.3 mL of emulsion containing β-carotene (30 mM), and different PUFA substrates (180 μM) for a total reaction volume of 1.8 mL.

The reaction mixture using soybean LO contained 150 μg of LO dissolved in 1.5 mL of 0.05 M phosphate buffer (pH 7.8), 0.3 mL of emulsion containing β-carotene (30 mM), and different PUFA substrates (180 μM) for a total reaction volume of 1.8 mL. The time course of bleaching at 25 °C was recorded on a Cary 219 spectrophotometer (Varian, CA) by measuring the decrease in absorbance at 460 nm, the wavelength of maximum absorbance of β-carotene, following initiation of the reaction by addition of the PUFA substrates.

RESULTS AND DISCUSSION

Initially, bleaching activity was calibrated by determining the amounts of the soybean and trout gill lipoxygenase preparations that caused similar maximum velocities of bleaching of β-carotene with linoleic acid as the substrate. Thus, 100 μg of soybean LO and 50 μg of

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Table I

PUFA substrate	lag time, min		V_{max}, AU min^{-1} × 10^3		signif level[a]
	trout gill	soybean	trout gill	soybean	
linoleic acid (18:2, $n-6$)	3.8 ± 0.8	3.7 ± 0.4	39.5 ± 0.5	41.2 ± 1.4	
eicosadienoic acid (20:2, $n-6$)	0.0 ± 0.2	2.8 ± 0.3	24.4 ± 1.7	23.3 ± 2.5	
homo-γ-linolenic acid (20:3, $n-6$)	2.3 ± 0.3	4.5 ± 0.5	73.7 ± 2.4	30.0 ± 0.2	0.0001
eicosatrienoic acid (20:3, $n-3$)	2.8 ± 0.8	2.3 ± 0.1	30.1 ± 2.3	53.5 ± 4.0	0.005
arachidonic acid (20:4, $n-6$)	1.2 ± 0.4	2.5 ± 0.1	116.7 ± 11.7	25.8 ± 0.9	0.0005
docosatrienoic acid (22:3, $n-3$)	0.0 ± 0.1	5.2 ± 0.8	31.2 ± 1.3	37.6 ± 1.0	0.005
docosatetraenoic acid (22:4, $n-6$)	1.8 ● 0.2	2.7 ± 0.4	78.7 ± 1.4	41.5 ± 2.7	0.0001
docosahexaenoic acid (22:6, $n-3$)	1.3 ± 0.2	2.0 ± 0.2	46.0 ± 0.8	32.5 ± 1.5	0.0005

[a] Significantly different V_{max} of bleaching as catalyzed by trout gill and soybean lipoxygenase preparations.

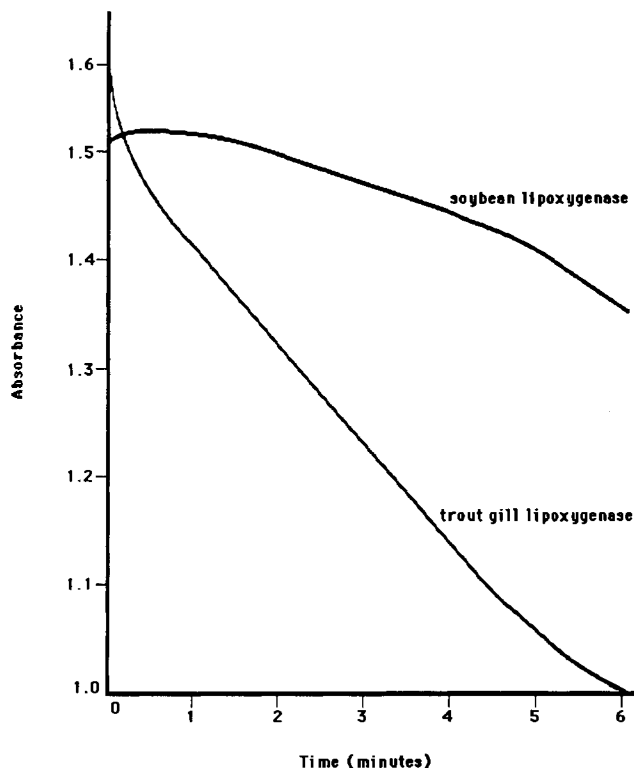

Figure 1. Relative rates of bleaching, i.e. decrease in absorbance of β-carotene (at 460 nm), as catalyzed by trout gill and soybean lipoxygenase preparations in the presence of arachidonic acid. For maximum velocity and lag time numbers, see Table I.

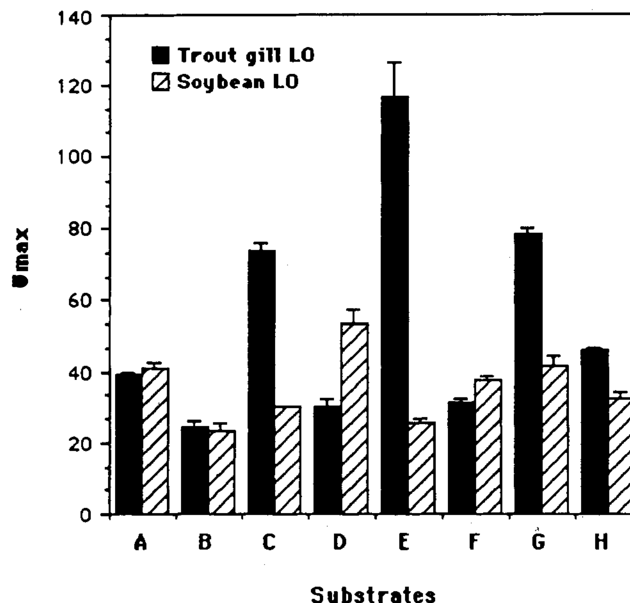

Figure 2. Relative maximum velocities (V_{max}) of β-carotene bleaching by trout gill and soybean lipoxygenase (LO) preparations in the presence of various polyunsaturated fatty acid (PUFA) substrates, as quantified by the change in absorbance units over time (AU min^{-1}), at 460 nm. PUFA substrates were as follows: A, linoleic acid (18:2, $n-6$); B, eicosadienoic acid (20:2, $n-6$); C, homo-γ-linolenic acid (20:3, $n-6$); D, eicosatrienoic acid (20:3, $n-3$); E, arachidonic acid (20:4, $n-6$); F, docosatrienoic acid (22:3, $n-3$); G, docosatetraenoic acid (22:4, $n-6$); H, docosahexaenoic acid (22:6, $n-3$).

trout gill protein (crude LO)/mL of buffer gave maximum velocities of approximately 0.040 AU min^{-1} (Table I). The trout gill preparation containing LO, which actively catalyzes the peroxidation of PUFA (German et al., 1986), concurrently caused the rapid bleaching of β-carotene in the presence of PUFA substrates (Figure 1). The maximum rates of bleaching for each PUFA substrate were compared to the maximum rates obtained for linoleic acid, as catalyzed by the two LO preparations. The maximum rates of bleaching with gill LO varied with the type of PUFA provided as substrate (Figure 2). Against linoleic acid as a reference, maximum bleaching was significantly greater with gill LO than that obtained with soybean LO in the presence of homo-γ-linolenic ($p = 0.0001$), arachidonic ($p = 0.0005$), docosatetraenoic ($p = 0.0001$), and docosahexaenoic acids ($p = 0.0005$) (Table I). Eicosatrienoic acid (20:3, $n-3$), a structural isomer of homo-γ-linolenic acid (20:3, $n-6$), and docosatrienoic acid caused greater maximum velocities of bleaching by the soybean LO compared to trout LO ($p = 0.005$; Table I).

There was a lag time before maximum velocity was attained, and it was shorter for the gill LO, as compared to the soybean LO, for six of the eight PUFA substrates (Table I). The presence of oxidative cofactors in the gill LO preparation (Kanner et al., 1987) may have accelerated the rates of PUFA oxidation.

These observations are consistent with the report of Tsukuda (1970), speculating that LO is involved in carotenoid discoloration, and with the report of Barimalaa and Gordon (1988), which showed that soybean LO cooxidizes β-carotene in the presence of linoleic acid. β-Carotene does not appear to be a conventional antioxidant (Burton and Ingold, 1984), but it can quench singlet oxygen and react with radical intermediates to inhibit lipid oxidation (Krinsky and Deneke, 1982). However, β-carotene in a micellar dispersion, as in this experiment, does not per se quench singlet oxygen (Kanner and Kinsella, 1983a). Therefore, in this system β-carotene is most likely reacting with radical intermediates formed in the LO reaction, as evidenced by β-carotene bleaching. β-Carotene bleaching probably occurs via a radical–diene interaction (Kanner and Kinsella, 1983b; Kanner et al., 1987) that eliminates the conjugated diene system.

Fish muscle tissue, such as that of salmon and trout, contains carotenoids. It is possible that a release of 12-LO from skin tissue of these fish during handling may contribute to their discoloration postmortem. This possibility requires further study.

ACKNOWLEDGMENT

Supported in part by the New York State Sea Grant Program. The suggestions of Dr. Bruce German are gratefully acknowledged.

Registry No. A, 60-33-3; B, 5598-38-9; C, 1783-84-2; D, 17046-59-2; E, 506-32-1; F, 28845-86-5; G, 28874-58-0; H, 6217-54-5; 15-LO, 82249-77-2; LO, 82391-43-3; β-carotene, 7235-40-7.

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Received for review September 30, 1988. Accepted January 30, 1989.