each in our daily food. The findings of Sainani et al.[34] also support our observations and evidence is thus accumulating that the use of these vegetables or their oils may help one as a precautionary measure against hyperlipidemia which may lead to atherosclerosis and heart diseases.

1 The authors acknowledge with thanks Prof. J.K. Sen Gupta, and the authorities of the University of Maiduguri for the award of a grant to this project. Authors, to whom reprint requests should be addressed.
2 M.M. Janot and J. Laurine, C.r. Acad. Sci. Paris *191*, 1098 (1930).
3 H.D. Brahmachari and K.T. Augusti, J. Pharm. Pharmac. *13*, 128 (1961); *14*, 254 (1962).
4 P. Laland and O.W. Havrivold, Z. Physiol. Chem. *221*, 180 (1933).
5 K.L. Sebastian, N.T. Zacharias, Babu Philip and K.T. Augusti, Indian J. Physiol. Pharmac. *23*, 27 (1979).
6 N.T. Zacharias, K.L. Sebastian, Babu Philip and K.T. Augusti, Indian J. Physiol. Pharmac. *24*, 151 (1980).
7 Molaparast-Shahidsaless, Fathieh, Earlshrago and C.E. Elson, J. Nutr. *109*, 1560 (1979).
8 S.N. Penninton, H.A. Barakat, G.L. Dohn and C.P. Smith, Experientia *34*, 564 (1978).
9 J.T. Anderson, Am. J. clin. Nutr. *20*, 168 (1967).
10 J. Macdonald, Progr. Biochem. Pharmac. *8*, 216 (1973).
11 P.J. Barter, P.J. Nestel and K.F. Carrol, Metabolism *21*, 117 (1972).
12 B.M. Wolfe and S.P. Ahuja, Metabolism *26*, 963 (1977).
13 M.J. Albrink, Diabetes *23*, 913 (1974).
14 L.A. Carlson and L.E. Bottiger, Lancet *1*, 865 (1972).
15 K.H. Temple, Med. Ernähr. *3*, 197 (1962).
16 A.R. Saghir, L.K. Mann, R.A. Bernhard and J.V. Jacobsen, Proc. Am. Soc. hort. Sci. *84*, 386 (1964).
17 K.T. Augusti, V.C.M. Roy and M. Semple, Experientia *30*, 1119 (1974).
18 H. Platenius, Agric. Res. *51*, 847 (1935).
19 T. Shiff, P.S. Roheim and H.A. Eder, J. Lipid Res. *12*, 596 (1971).
20 A.M. Asatoor and E.J. King, Biochem. J. *56*, 44 (1954).
21 J.G. Reinhold, in: Standard methods in clinical chemistry, vol. 1, p. 88. Ed. M. Reiner. Academic Press, New York 1953.
22 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
23 R.M. Burton, in: Methods in enzymology, vol. 3, p. 246. Eds S.P. Colowick and N.O. Kaplan. Academic Press, New York 1957.
24 A. Zlatkis, B. Zak and G.J. Boyle, J. Lab. clin. Med. *41*, 486 (1953).
25 D. Koch-Weser, P.B. Szanto, E. Farber and H. Popper, J. Lab. clin. Med. *36*, 694 (1950).
26 C.J. Cavallito, J.S. Buck and C.M. Suter, J. Am. chem. Soc. *66*, 1952 (1944).
27 I.M. Kolthoff, W. Striks and R.C. Kapoor, J. Am. chem. Soc. *77*, 4733 (1955).
28 J.C. Crawhall and R.W.E. Watts, Am. J. Med. *45*, 736 (1968).
29 K.T. Augusti, Indian J. exp. Biol. *15*, 1223 (1977).
30 S. Black, in: Methods in enzymology, vol. 5, p. 992. Eds S.P. Colowick and N.O. Kaplan. Academic Press, New York 1962.
31 K.T. Augusti, Indian J. exp. Biol. *15*, 489 (1977).
32 K.T. Augusti, Indian J. Biochem. Biophys. *11*, 264 (1974).
33 T.M. Lukes, J. Food Sci. *36*, 662 (1971).
34 G.S. Sainani, Indian J. med. Res. *69*, 776 (1980).

## An arachidonic acid-specific lipoxygenase from the gorgonian coral *Pseudoplexaura porosa*[1]

D.R. Doerge and M.D. Corbett

*Food Science and Human Nutrition Department, Pesticide Research Laboratory, The University of Florida, Gainesville (Florida 32611, USA), 25 January 1982*

*Summary.* A novel lipoxygenase was partially purified from the gorgonian coral *Pseudoplexaura porosa* and was found to be specific for arachidonic acid. This soluble enzyme catalyzed the formation of 15-hydroperoxy-eicosatetraenoic acid (15-HPETE) from arachidonic acid.

Lipoxygenases [E.C. 1.13.11.12] have been isolated and characterized from both plant and mammalian sources[2-5]. While the role of lipoxygenases in plants remains unexplained, lipoxygenase activity in mammals is usually associated with tissues that actively metabolize prostaglandins, thromboxanes and leukotrienes[4,6]. This is the first description of lipoxygenase activity in an invertebrate species.

Reports of prostaglandins in certain gorgonian corals[7,8] have stimulated speculation regarding the function of prostaglandins in these marine invertebrates[9]. The enzyme, prostaglandin endoperoxide synthetase, has been reported to be present in a large number of marine invertebrates[10], but characterized only in *Plexaura homomalla*[11]. Morse et al.[10] used an arachidonate-dependent epinephrine oxidation assay to test for the presence of enzymes that initiate prostaglandin biosynthesis, and concluded that this enzyme was common to many gorgonians. We found this method to be very unreliable for the detection of prostaglandin synthetase in gorgonian corals and doubt its validity as the sole test for prostaglandin synthetase activity in any tissue[12]. Examination of arachidonic acid metabolism in *Pseudoplexaura porosa* showed the absence of prostaglandin biosynthesis, but did show the presence of an active lipoxygenase. We now report on the specificity of this lipoxygenase towards arachidonic acid.

*Experimental.* Fatty acids of 99% purity and soybean lipoxygenase were purchased from Sigma Chemical Co. Bistrimethylsilyl-trifluoroacetamide (Regisil) was purchased from Regis Chemical Co. TLC was performed on EM silica gel 60 F-254 precoated plates (Brinkmann Instruments) in solvent systems A: benzene/dioxane/acetic acid (95:5:2) and B: hexane/ethyl ether/acetic acid (60:40:1). Lipoxygenase products were visualized with $I_2$ and with a peroxide specific spray reagent[13]. The hydroperoxide, 15-HPETE, was prepared with soybean lipoxygenase and purified by the procedure of Funk et al.[14]. The hydroperoxide product of the *Ps. porosa* lipoxygenase reaction was prepared by incubation of 140 µg of partially purified enzyme with 500 µM arachidonic acid added as a solution in 30 µl of methanol in a total volume of 5.0 ml of 50 mM Tris · HCl, pH 8.0 at 25 °C for 15 min. The solution was acidified to pH 4 with 0.2 M citric acid and extracted 3 times with 10 ml of ice-cold water, dried over sodium sulfate, evaporated to dryness in vacuo, and dissolved in 1.0 ml of absolute ethanol. Aliquots of 5 µl were applied to TLC plates and developed in solvent systems A and B. The single product band migrating with $R_f = 0.16$ in solvent system A and $R_f = 0.26$ in solvent system B was reactive toward the peroxide specific spray reagent[13]. Soybean lipoxygenase and *Ps. porosa* derived hydroperoxides were converted to

TMS-HETE methyl esters by standard methods[14]. The TMS-HETE methyl esters were analyzed with a Finnigan 4021 Gas Chromatograph/Mass Spectrometer.

Specimens of *Ps. porosa* were collected at Bache Shoal off Elliot Key, FL and transferred in site-collected sea-water to Miami, FL where they were transferred to a free flowing aquarium for long term holding. Identification was made by microscopic analysis of the $CaCO_3$ spicules and confirmed by Ms M.A. Russell of the University of Miami.

In a typical experiment, 2 g of coral tips were washed with sea-water, blotted dry, minced with scissors and homogenized at 0 °C in 20 ml Tris · HCl, pH 8.0 containing 1 M NaCl with a Tekmar Tissumizer for 2 30-sec periods at maximum speed. The homogenate was centrifuged at 20,000 × g for 20 min at 0 °C to remove the $CaCO_3$ spicules, photosynthetic zooxanthellae[15], and cellular debris. For partial purification, the homogenate was brought to 30% saturation with solid ammonium sulfate at 0 °C and allowed to stand for 30 min. The resultant precipitate was collected by centrifugation at 20,000 × g for 15 min, resuspended in 5.0 ml of 50 mM Tris · HCl, pH 8.0 and recentrifuged to remove any insoluble material. The resultant solution was dialyzed overnight against 1 l of the same buffer at 0 °C. The dialyzed enzyme solution was used for all lipoxygenase assays. The enzyme was found to be unstable and lost about 50% of the original activity in 24 h at 0 °C. Protein concentration was determined by the method of Bradford[16]. The overall purification for the lipoxygenase was 1.6-fold, and the crude enzyme has a sp. act. of 154 nmoles hydroperoxide formation-$min^{-1}$-$mg^{-1}$ protein.

The formation of lipid hydroperoxide was monitored in a Beckman Model 35 spectrophotometer at 234 nm at 25 °C. The reaction was initiated by addition of the fatty acid substrate in 10 µl of methanol to give the indicated concentration. Initial rates were determined from the linear portion of the progress curves, and the concentration of the hydroperoxide product was determined from $\varepsilon_{234} = 2.8 \times 10^4\ M^{-1}\ cm^{-1}$ (Tolberg and Wheeler[17]).

*Results.* Double reciprocal analysis of the rate data for the oxygenation of arachidonic acid by the *Ps. porosa* lipoxygenase by the linear least square method gave $K_m + 3.8$ µM and $V_{max} = 163$ nmoles/min/mg protein (correlation coefficient = 0.99). The table shows the lipid substrate specificity of the *Ps. porosa* lipoxygenase. These data demonstrate the high specificity of this invertebrate lipoxygenase for arachidonic acid. The table also shows that typical lipid substrates for plant lipoxygenases[18] are only weakly metabolized by the *Ps. porosa* lipoxygenase. The product of the *Ps. porosa* lipoxygenase was found to cochromatograph with authentic 15-HPETE in both TLC systems employed. GC/MS analysis of the HPETE methyl ester-trimethylsilyl ethers of the *Ps. porosa* lipoxygenase and soybean lipoxygenase products from arachidonic acid demonstrated that they are identical.

An HPLC assay for prostaglandin biosynthesis has been developed based on the method of Kupfer et al.[19]. In this assay, $PGA_2$ and $PGE_2$ are converted to $PGB_2$ by treatment with ethanolic KOH[20], and the $PGB_2$ is then quantitated by HPLC by monitoring at 280 nm. This procedure permits the detection of at least 10 ng of $PGB_2$. No $PGA_2$, $PGB_2$ or $PGE_2$ was found in homogenates of *Ps. porosa* incubated with arachidonic acid.

*Ps. porosa* lipoxygenase substrate specificity. All assays were performed as described in the experimental section at the indicated fatty acid concentrations

| Fatty acid | Lipoxygenase rate (nmole/min/mg protein) |
|---|---|
| 250 µM linoleic acid | 5 |
| 250 µM α-linoleic acid | 9 |
| 250 µM γ-linoleic acid | 3 |
| 50 µM arachidonic acid | 84 |

*Discussion.* This report describes the properties of a lipoxygenase from the gorgonian coral *Ps. porosa*, and is the first reported isolation of a lipoxygenase from an invertebrate source. This enzyme is specific for arachidonic acid, and the only observed product from arachidonic acid is 15-HPETE. Plant lipoxygenases, most notably soybean lipoxygenase, have a broad specificity for polyunsaturated fatty acid substrates[18]. Thus, the *Ps. porosa* lipoxygenase more closely resembles mammalian lipoxygenases in its substrate specificity. Lipoxygenases have been isolated from mammalian sources that metabolize arachidonic acid exclusively and to give different isomers[3-5]. Mammalian lipoxygenases are very often highly unstable, and the *Ps. porosa* lipoxygenase was also found to be very unstable during isolation.

Since prostaglandin synthetase is not present in *Ps. porosa*, but is known in at least one other gorgonian species, the presence of an arachidonic acid-specific lipoxygenase presents an evolutionary curiosity. A closer inspection of the phylogenetic distribution of lipoxygenases and prostaglandin synthetases among such invertebrates could be most informative with regards to the evolution of these enzymatic activities.

1 This investigation was supported by PHS grant No. CA32395 awarded by the National Cancer Institute and by PHS Research Career Development Award No. ES00120 to MDC awarded by the National Institute of Environmental Health Sciences, DHHS. The authors acknowledge Ms M.A. Russell and Dr D.G. Anderson of the University of Miami for assistance in the collection of gorgonian corals. Preliminary work was conducted at the University of Miami School of Marine and Atmospheric Sciences. This is journal series No. 3638 from the Florida Agricultural Experiment Station.

2 M. Hamberg, B. Samuelsson, I. Bjorkhem and H. Danielson, in: molecular mechanisms of oxygen activation, p. 30. Ed. O. Hayashi. 1974.
3 D.H. Nugteren, Biochim. biophys. Acta *380*, 299 (1975).
4 M. Hamberg and B. Samuelsson, Proc. natl Acad. Sci. USA *71*, 3400 (1974).
5 S. Narumiya, J.A. Salmon, F.H. Cottee, B.C. Weatherly and R.J. Flower, J. biol. Chem. *256*, 9583 (1981).
6 J.A. Lindgren, G. Hansson and B. Samuelsson, FEBS Lett. *128*, 329 (1981).
7 A.J. Weinheimer and R.L. Spraggins, Tetrahedron Lett. *59*, 5185 (1969).
8 Y. Komoda, T. Kanayaso and M. Ishikawa, Chem. Pharm. Bull. *27*, 2491 (1979).
9 F.M. Bayer and A.J. Weinheimer, in: Prostaglandins from *Plexaura homomalla;* ecology, utilization and conservation of a major medical resource, p. 137. Eds F.M. Bayer and A.J. Weinheimer. 1974.
10 D.E. Morse, M. Kayne, M. Tidyman and S. Anderson, Biol. Bull. *154*, 440 (1978).
11 E.J. Corey, W.N. Washburn and J.C. Chen. J. Am. chem. Soc. *95*, 2054 (1973).
12 D.R. Doerge and M.D. Corbett, Chem.-Biol. Interact., in press (1982).
13 E. Stahl ed., Thin Layer Chromatography, 2nd edn, p. 870. 1966.
14 M.O. Funk, R. Issac and N.A. Porter, Lipids *11*, 113 (1976).
15 J.R. Rice, G. Papastephanou and D.G. Anderson, Biol. Bull. *138*, 334 (1970).
16 M.M. Bradford, Analyt. Biochem. *72*, 248 (1976).
17 W.E. Tolberg and D.H. Wheeler, J. Am. chem. Soc. *35*, 385 (1958).
18 M. Hamberg and B. Samuelsson, J. biol. Chem. *242*, 5329 (1967).
19 D. Kupfer, J. Navarro, G.K. Miranda and A.D. Theoharides, Archs Biochem. Biophys. *205*, 297 (1980).
20 M. Bygdeman and B. Samuelsson, Clinica chim. Acta *10*, 566 (1974).