
Modulation of Lipoxygenase Activity by Bacterial Hopanoids

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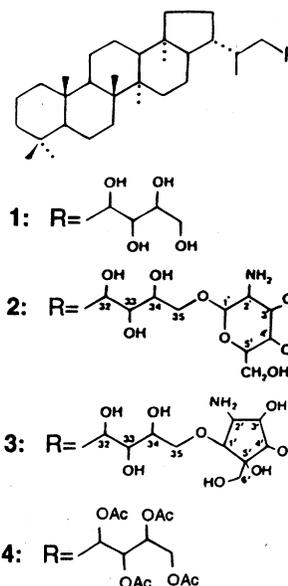
Tetrahydroxybacteriohopane (1), a bacterial hopanoid, inhibited soybean 15-lipoxygenase with an IC₅₀ of about 10 μM. After per-*O*-acetylation of 1 no inhibition of the 15-lipoxygenase was observed. Two other bacterial hopanoids, tetrahydroxybacteriohopane glucosamine (2) and tetrahydroxybacteriohopane ether (3), stimulated the activity of soybean 15-lipoxygenase. The activities of two other arachidonic acid-metabolizing enzymes, human 5-lipoxygenase and prostaglandin H synthase, were unaffected by 1.

Hopanoids are a group of pentacyclic triterpenes that occur in numerous species of bacteria and blue-green algae.¹ Because of the similarity in size and shape of hopanoids to sterols such as cholesterol, their role as stabilizers of bacterial membranes and "prokaryotic sterol equivalents" has been proposed.² The only biological and/or pharmacological activity that has been reported for hopanoids is that certain hopanoids exhibit anticancer activity.^{3,4}

Three hopanoids from *Zymomonas mobilis* (1–3) were purified as previously reported^{5,6} and evaluated for their ability to inhibit three arachidonic acid-metabolizing enzymes (Table 1). Most nonsteroidal antiinflammatory drugs (NSAIDs) inhibit all three of these enzymes.⁷ Soybean 15-lipoxygenase (LO) was inhibited by one of the hopanoids, tetrahydroxybacteriohopane (THBH) (1), with an IC₅₀ of about 10 μM. The per-*O*-acetate of THBH (4) did not inhibit soybean 15-LO. Two other hopanoids, THBH-glucosamine (2) and THBH-ether (3), were also evaluated for their effect on soybean 15-LO. These compounds did not inhibit soybean 15-LO, however, they actually stimulated the enzyme activity.

The two other arachidonic acid-metabolizing enzymes, human 5-LO and prostaglandin H synthase, were not significantly inhibited by 25 or 50 μM of 1. Because of the lack of inhibition of these enzymes by 1, compounds 2, 3, and 4 were not evaluated for their effect on these two enzymes.

Because most NSAIDs inhibit 5-LO, 15-LO, and prostaglandin H synthase,⁷ our finding that 1 is a natural product that selectively inhibits 15-LO without inhibiting either 5-LO or prostaglandin H synthase indicates that 1 potentially has unique pharmacological properties. Compounds that specifically inhibit 15-LOs have been sought after for use as drugs for treating atherogenesis and asthma.⁷ Further studies on the properties of 1 inhibition of 15-LO and the lipoxygenase stimulatory hopanoids may be useful for delineating molecular requirements for modulators (stimulants and inhibitors) of lipoxygenase activity and could lead to new paradigms for treatment of specific inflammatory diseases.



Experimental Section

General Experimental Procedures. The bacterium *Zymomonas mobilis* was grown anaerobically in a 40-L fermentor and harvested as previously described.⁶ Cells were extracted with CHCl₃-MeOH, and the three major hopanoids (1–3) were purified (purity was monitored via HPLC analyses) using solid-phase extraction and semi-preparative HPLC methods as previously described.⁶ Tetrahydroxybacteriohopane (1) was per-*O*-acetylated by removing the solvent and shaking about 1 mg of sample with a mixture of 1 mL of Ac₂O and 1 mL of pyridine, at room temperature for 18 h. The Ac₂O and pyridine were removed via a stream of nitrogen, yielding acetate 4.

Assay of Soybean 15-Lipoxygenase. Soybean 15-lipoxygenase (Type V) was obtained from Sigma Chemical Co. The reaction mixture (1.8 mL), consisting of 0.1 M borate buffer, pH 9.0; 5 μL (0.5 μg, 315 units) of soybean 15-lipoxygenase dissolved in borate buffer; and 25 μL of inhibitor dissolved in EtOH, was preincubated for 3 min. The reaction was started by injecting 100 μL of substrate mixture (prepared fresh daily and consisting of 1.0 mg arachidonic acid sonicated in 2.3 mL 0.1 M borate buffer, pH 9.0), yielding a final concentration of 50.9 μM arachidonic acid in the reaction vessel. The rate of disappearance of dissolved O₂ was monitored with a Clark Oxygen Electrode (Yellow

Table 1. Effect of Tetrahydroxybacteriohopane (THBH), THBH analogues, and Known Inhibitors, on Arachidonic Acid Metabolizing Enzymes

enzyme	inhibitor	concentration (μM)	enzyme activity (relative \pm s.d.)
soybean 15-LOX	none		100 ^a
	THBH ^b (1)	6.75	68 \pm 5
		12.5	48 \pm 9
		25	15 \pm 6
		50	4 \pm 3
		100	11 \pm 2
	THBH-glucosamine (2)	25	172 \pm 24
		50	188 \pm 14
	THBH-ether (3)	25	151 \pm 10
		50	250 \pm 38
	per-O-acetate of THBH (4)	25	89 \pm 11
		50	114 \pm 17
25		34 \pm 4	
human 5-LO	phenidone	25	34 \pm 4
	none		100 ^c
	THBH	25	98 \pm 2
		50	97 \pm 1
prostaglandin H synthase	phenidone	25	55 \pm 2
	none		100 ^d
	THBH	25	102 \pm 3
		50	103 \pm 2
	indomethacin	2.4	7 \pm 2

^a Enzyme activity in the absence of inhibitor was 20.8 $\mu\text{mole O}_2$ consumed/min/mg of protein. ^b For abbreviations see text. ^c Enzyme activity in the absence of inhibitor was 0.387 $\mu\text{mole O}_2$ consumed/min/mg of protein. ^d Enzyme activity in the absence of inhibitor was 2.78 $\mu\text{mole O}_2$ consumed/min/mg of protein.

Springs Instruments, Yellow Springs, OH) with the temperature controlled at 25 °C, using a method similar to that reported previously for the assay of 5-LO.⁸ The O_2 concentration in an air-saturated aqueous solution at 25 °C was calculated to be 0.24 mM. The assay system was checked by measuring the inhibition of phenidone, a known inhibitor of soybean 15-LO. Each potential inhibitor was tested at each of the listed concentrations at least three times, and enzyme activities presented are the means \pm standard deviations, reported as relative activities (calculated assuming that the activity in the minus-inhibitor control = 100). The actual activities of the controls (in units of $\mu\text{mole O}_2$ consumed/min/mg of protein) are also presented as a footnote to Table 1.

Assay of Recombinant Human 5-Lipoxygenase. Recombinant human 5-lipoxygenase was obtained from Oxford Biomedical Research Co. (Oxford, MI). The reaction mixture (1.80 mL) consisted of 0.25 mM of CaCl_2 , 0.13 mM of ATP, 7 μL of phosphatidylcholine (12 $\mu\text{g}/2$ mL EtOH), 25 μL of inhibitor dissolved in EtOH, and Tris-HCl buffer (50 mM, pH 7.4) and was preincubated for 3 min. The reaction was started by injecting 5 μL of arachidonic acid (3.8 mg/5 mL EtOH) yielding a final arachidonic acid concentration of 4.5 μM in the reaction mixture. The rate of disappearance of dissolved O_2 was monitored with an oxygen electrode at 37 °C, as described above. The O_2 concentration in an air-saturated aqueous solution at 37 °C was calculated to be 0.185 mM. The assay system was checked by measuring the inhibition of phenidone, a known inhibitor of human 5-LO.

Assay of Prostaglandin H Synthase (Cyclooxygenase).

Prostaglandin H synthase was obtained from Oxford Biomedical Research Co. (Oxford, MI). The reaction mixture (1.80 mL) consisted of 0.5 mM of phenol, 1 μM of hematin, enzyme (3.75 μg in 5 μL), 25 μL of inhibitor dissolved in EtOH, and potassium phosphate buffer (0.1 M, pH 7.8) and was preincubated for 3 min. The reaction was started by injecting 15 μL of arachidonic acid dissolved in EtOH, yielding a final concentration of 100 μM of arachidonic acid in the reaction vessel. The disappearance of dissolved O_2 at 37 °C was monitored as described above. The assay system was checked by measuring the inhibition of indomethacin, a known inhibitor of prostaglandin H synthase.

References and Notes

- (1) Rohmer, M.; Bouvier-Nave, P.; Ourisson, G. *J. Gen. Microbiol.* **1984**, *130*, 1137–1150.
- (2) Sahn, H.; Bringer-Meyer, S.; Sprenger, G. In *The Prokaryotes*, 2nd ed.; Balows, A., Truper, H. G., Sworkin, M., Harder, W., Schleifer, K.-H., Eds.; Springer-Verlag: New York, 1992; Vol. 3, pp 2287–2301.
- (3) Nagumo, A.; Takanashi, K.; Hojo, H.; Suzuki, Y. *Toxicol. Lett.* **1991**, *58*, 309–313.
- (4) Chen, Z.; Washio, T.; Sato, M.; Suzuki, Y. *Biol. Pharm. Bull.* **1995**, *18*, 421–423.
- (5) Moreau, R. A.; Powell, M. J.; Osman, S. F.; Whitaker, B. D.; Fett, W. F.; Roth, L.; O'Brien, D. *J. Anal. Biochem.* **1995**, *224*, 293–301.
- (6) Roth, L.; Moreau, R. A.; Powell, M. J.; O'Brien, D. *J. Anal. Biochem.* **1995**, *224*, 302–308.
- (7) Parthasarathy, S.; Rankin, S. M. *Prog. Lipid. Res.* **1992**, *31*, 127–143.
- (8) Breton, J.; Keller, P.; Chabot-Fletcher M.; Hillegass, L.; DeWolf, W., Jr.; Griswold, D. *Prostaglandins, Leucotrienes, Essent Fatty Acids* **1993**, *49*, 929–937.