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In vitro Biosynthesis of Prostaglandins in the White Sea Soft Coral *Gersemia fruticosa*: Formation of Optically Active PGD_2, PGE_2, $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ from Arachidonic Acid

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Abstract: The White Sea soft coral *Gersemia fruticosa* has been found to convert exogenous arachidonic acid to optically active prostaglandins D_2, E_2, $F_{2\alpha}$ and 15-keto-$F_{2\alpha}$, all of which were also isolated as endogenous constituents from the same coral extracts. This is the first report of *in vitro* biosynthesis of "primary mammalian" prostaglandins in coral.

Key words: Natural prostaglandins, Biosynthesis, Soft coral, *Gersemia fruticosa*

Since the discovery of prostaglandin A_2 (PGA_2) methyl ester acetate in the Caribbean coral *Plexaura homomalla*[1], an intriguing unresolved issue regarding prostaglandin biosynthesis pathway has remained. Natural A-, E- and F- type prostaglandins, as well as the clavulones detected in different corals[2], have not been established by *in vitro* experiments with fresh coral preparations to form from radiolabeled arachidonic acid. Unfortunately, the only reported salt dependent biosynthesis of PGA_2 from free arachidonic acid in *Plexaura homomalla*[3] has not found later confirmation[4]. On the other hand, the high level of 8R-lipoxygenase and allene oxide synthetase activity in certain corals suggests the biosynthesis of marine eicosanoids to proceed via the following route: arachidonic acid → 8R-HPETE → allene oxide → pentadienyl cation → cyclic compounds[4,5,6]. The fact that the major *in vitro* metabolites of arachidonic acid, 8R-H(P)ETE as well as racemic preclavulone-A, are not endogenous constituents of corals is in favour of another idea that arachidonic acid in free acid form is not the natural substrate for prostaglandin synthesis in corals and this may occur in lipids[4]. The detection of 8-HETE and 8-HEPE in the extract from the *Gersemia rubiformis* collected in Kamchatka coastal waters[7] encouraged us to pay more attention to "cold sea" soft corals.

In this report we demonstrate the rather unexpected ability of the soft coral *Gersemia fruticosa* to convert exogenous arachidonic acid into typical "mammalian" prostaglandins. Of special interest is the comparison of radiolabeled *in vitro* metabolites with the endogenous eicosanoids identified in the coral extracts.

The endogenous arachidonic acid metabolites[8] were purified by repeated column and HPLC separation of the material obtained by the subsequent extraction of freeze dried corals (1.5 kg)[9] with diethyl ether and methanol. Various monohydroxy acids, mainly 11- and 8-HETE, were identified in the ether extract by GC-MS and ^{13}C-NMR spectroscopy. The ^{13}C-NMR study of 11-HETE and its Mosher's esters[10] established the absolute stereochemistry at C-11 to be R. Surprisingly, all primary prostaglandins, PGD_2 (2 mg), PGE_2 (14 mg), 15-keto-$PGF_{2\alpha}$ (13 mg) and $PGF_{2\alpha}$ (91 mg) were isolated in free acid form by HPLC from methanol extracts. The ^{13}C-NMR and GC-MS spectra and optical rotation data of all the above prostaglandins were identical to those of "mammalian" standards[11].

No trace of PGA_2 was detected in the extracts. Also by HPLC, no detectable amount of $PGF_{2\alpha}$ and PGB_2 was found in the samples obtained after the alkaline hydrolysis of non-acidic lipid fractions of the coral. Consequently, at least under the conditions of the collection and storage of the coral samples, prostaglandins E_2, A_2, B_2 and $F_{2\alpha}$ were not found to occur in acetate and methyl ester form or to be bound to coral lipids.

The enzyme preparations for biosynthetic studies were prepared as acetone powder[4]. In general, the acetone powder (0.5-1.0g) was blended with 15 ml of 50 mM Tris-HCl buffer, pH 8.0. The reaction was started with addition of ^{14}C- or ^{3}H- labeled arachidonic acid (0.2-0.5 mM, 0.2-0.3 mCi/mmol). The incubation was carried out at room temperature for 30 min. The proteins were precipitated with cold acetone. After removing acetone, the residue was acidified and extracted repeatedly with ethyl acetate. The total recovery of radioactivity was about 60%. The products were separated by TLC or HPLC and analyzed by measurement of radioactivity using authentic standards.

When identical oxygenations were carried out using a heat inactivated coral enzyme, no detectable radioactive products were obtained. Control experiments without the addition of arachidonic acid to the acetone powder showed no detectable prostaglandins by HPLC analysis.

The final identification of metabolites was made by scaling up the incubations (15g of acetone powder and 100 mg of unlabeled arachidonic acid) to obtain a sufficient amount of pure material for chemical transformations, HPLC, mass spectra, UV, ^{13}C-NMR and optical rotation measurements in comparison with authentic standards.

Table. *In vitro* Biosynthesis of Eicosanoids From Radiolabeled Arachidonic Acid with Acetone Powder of *Gersemia fruticosa*

Incubation time, min	Addition M	Recovered, % AA	Eicosanoids formed, % H(P)ETEs	DiH(P)ETEs	PGD_2	PGE_2	15-keto-$PGF_{2\alpha}$	$PGF_{2\alpha}$
10		73	10	3	2	<1	1	<1
20		48	18	11	3	2	3	3
40		32	24	13	5	4	5	6
30	$SnCl_2$, 10^{-2}	30	28	7	1	-	-	18
30	L-670,630, 10^{-5}	71	9	5	-	-	-	-
30	Indomethacin, 10^{-6}	34	23	13	4	3	5	5

The results in Table show that major incubation products are monohydroperoxy acids (mainly 8R-HPETE), dihydroperoxy acids (structures not determined) and prostaglandins D_2, E_2 and $F_{2\alpha}$, as well as its metabolite 15-keto-$PGF_{2\alpha}$. It is interesting to mention that in the presence of $SnCl_2$ a considerable amount of 11-HETE is accumulated in the incubation mixture and the ratio of 11-HETE/8-HETE is shifted to 1/4. The prostaglandins mentioned above were found to form in almost equal amounts with a total yield of about 20%. The addition of $SnCl_2$ to the incubation mixture led to the preferable formation of $PGF_{2\alpha}$ instead of other prostaglandins. This finding indicates that *in vitro* synthesis of natural prostaglandins in coral may occur analogously to that in mammals via a common endoperoxide intermediate (PGH_2) derived from arachidonic acid by cyclooxygenase.

However, this hypothesis was not supported by the fact that the biosynthesis of prostaglandins in the coral was not markedly inhibited by the presence of a potent cyclooxygenase inhibitor, indomethacin. On the other hand, after the preincubation of the coral acetone powder in the presence of a selective lipoxygenase inhibitor, L-670,630[12], prostaglandin biosynthesis was completely inhibited.

In summary, our discovery that all the main endogenous metabolites of arachidonic acid (8R-H(P)ETE, 11R-H(P)ETE, PGD_2, PGE_2, $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$) identified in the extracts of *Gersemia fruticosa* are derived from free arachidonic acid by the *in vitro* incubations with the coral preparations, opens a good opportunity to elucidate the natural pathway of prostaglandin formation in coral. Experiments to distinguish the enzymic reactions involved in the cascade of prostaglandin biosynthesis in *Gersemia fruticosa* are in progress.

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