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Endoperoxide Pathway in Prostaglandin Biosynthesis in the Soft Coral *Gersemia fruticosa*

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Abstract: An acetone powder preparation of the White Sea soft coral *Gersemia fruticosa* converts exogenous arachidonic acid to a prostaglandin-endoperoxide, identified as PGG_2 by chemical and spectral studies. The latter serves as a key intermediate from which, evidently by nonenzymic transformations, all optically active coral prostaglandins are formed.

In spite of widespread occurrence of A-, E- and F-type prostaglandins (PGs) in certain corals, e.g. *Plexaura homomalla* and *Lobophyton depressum*, yet the mechanism of biosynthesis of these compounds in corals has remained unresolved[1]. It has been suggested that the marine pathway must be totally different from the mammalian endoperoxide route and that an unstable allene oxide formed from arachidonic acid via a lipoxygenase pathway could be a key intermediate in the biosynthesis of prostaglandins[2]. However, these hypotheses have not found an experimental confirmation, due mainly to the inability of the coral preparations used to form chiral prostaglandins *in vitro*.

In our earlier studies we discovered that the Arctic soft coral *Gersemia fruticosa* is able to synthesize optically active PGD_2, PGE_2, $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$, as well as 8R-H(P)ETE and 11R-H(P)ETE from tritiated arachidonic acid *in vitro*[3]. The product pattern as well as the fact that in the presence of $SnCl_2$ as a mild reducing agent only $PGF_{2\alpha}$ was formed, encouraged us to assume that the transformation of arachidonic acid into prostaglandins in *G. fruticosa* occurs via a common prostaglandin-endoperoxide intermediate. This report describes the isolation of this unstable intermediate from short incubations of *G. fruticosa*, and the identification of the compound as PGG_2.

G. fruticosa was collected[4] in the Gulf of Kandalaksha, White Sea, thereafter immediately frozen in liquid nitrogen, transported in dry ice and stored below -60°C to preserve the enzymic activity. Short

incubations of the coral acetone powder with tritiated arachidonic acid[5] did not lead to appreciable yields of PGH_2. The main labeled peroxy-compound, less polar than PGH_2, co-chromatographed on TLC with a mammalian PGG_2 standard[6]. No significant enhancement in the production of PGH_2 was detected in the presence of various reducing cosubstrates or stimulators of prostaglandin hydroperoxidase as adrenalin (1mM), L-tryptophan (5mM), phenol (1mM), and hydroquinone (0.7mM). On the contrary, in the presence of L-tryptophan, the highest yield of PGG_2 was obtained. The addition of hemin (1μM) alone or together with the above-mentioned electron donors was also ineffective for the conversion of PGG_2 to PGH_2, indicating the lack of the hydroperoxidase activity in the coral preparation.

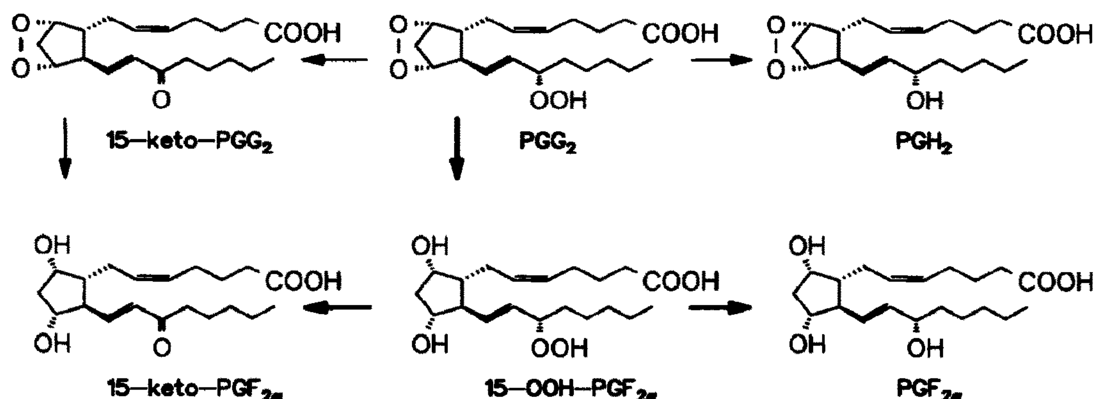
To obtain more material for the final identification of this intermediate, ten scaled-up incubations (1.3 g of acetone powder and 6 mg of unlabeled arachidonic acid each) were carried out under the conditions described in[5]. Normal phase HPLC[7] of the combined extracts gave 4 mg of a pure compound.

The structure of the isolated compound was first established by chemical modifications[8] and subsequent TLC analyses in comparison with authentic standards[6,9]. Treatment of this compound with $SnCl_2$ gave $PGF_{2\alpha}$, indicating the presence of the endoperoxy bridge between C-9 and C-11. The formation of 15-keto-$PGF_{2\alpha}$ as the major product after the dehydration with lead tetraacetate and subsequent reduction with triphenylphosphine gave a strong evidence for the existence of a hydroperoxy group at C-15.

The final confirmation of the structure of PGG_2 was obtained from ^{13}C NMR study. A comparison of the ^{13}C chemical shifts of PGG_2[10] and PGH_2[11] gave the following values of the peroxidation effects: +14.1 ppm (C-15); -4.5 ppm (C-16); +4.7 ppm (C-13); -3.8 ppm (C-14). In all other positions the differences are insignificant. This is in good accordance with characteristic carbon chemical shift values for 15-hydroperoxy-5,8,11Z,13E-eicosatetraenoic acid (15-HPETE) as an allylic secondary hydroperoxide, and the corresponding hydroxy acid (15-HPETE)[12], where, in addition to a 13.9 ppm low field shift of carbinol carbon and 4.7 ppm high field shift on saturated methylene carbon (C-16), a strong change in the polarization of an allylic double bond is observed: α-carbon to CHO (C-14) is shifted to the high field by 4.1 ppm. Thus, the ^{13}C NMR spectrum fully confirms the structure of PGG_2.

To answer the question of whether further conversion of PGG_2 to prostaglandin end-products (i.e. transformation of a 15-hydroperoxy- and 9,11-endoperoxy group to a hydroxy- or keto group) in the coral is enzymic or chemical, incubations of PGG_2 in pH 8.5 Tris-buffer in the presence of active and heat-denaturated coral preparations were carried out. The similar product patterns obtained in both the cases after a 10 min incubation give evidence of a nonenzymic character of conversions. The domination of PGF compounds (15-hydroperoxy-$PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$ and $PGF_{2\alpha}$, about 20% of each) among the decomposition products instead of E- and D-prostaglandins, typical products of the isomerization of PG-endoperoxides in a buffer, is indicative of the presence of endogenous reducing substances which are capable of reducing the endoperoxides to PGF compounds in crude coral preparations[13]. Also, it seems likely that 15-keto-$PGF_{2\alpha}$ is generated nonenzymically from PGG_2 via 15-hydroperoxy-$PGF_{2\alpha}$ or 15-keto-PGG_2[14]. The former route is

favored by a finding that a significant amount of 15-hydroperoxy-$PGF_{2\alpha}$ accumulates during the decomposition of PGG_2 in the presence of either an active or boiled coral enzyme.



The results obtained in this investigation indicate that the prostaglandins in *G. fruticosa* are formed via a PG-endoperoxide pathway. Two interesting points should be mentioned regarding the microsomal enzyme catalyzing the transformation of arachidonic acid to PGG_2: (i) the conversion was not markedly inhibited by indomethacin[3], and (ii) the lack of the prostaglandin-hydroperoxidase activity. The differential inhibition of cyclooxygenase (COX) isozymes COX-1 and COX-2 has been reported recently[15]. Also, it is shown that the replacement of heme prosthetic group with manganese(III)protoporphyrin IX gives an enzyme with a very low peroxidase activity[16]. The purification and characterization of the coral enzyme will illuminate the question of whether a novel COX-isozyme is involved in the marine pathway or, alternatively, whether PGG_2 is formed in the coral by a lipoxygenase pathway. An interesting hypothesis that PGG_2 would be formed via 15-peroxy arachidonate was recently proposed by Corey[17]. Another intriguing question remains regarding the generality of this pathway in other corals including *P. homomalla*.

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4. Samples of *G. fruticosa (Octocorallia, Alcyonacea, Nephtheidae)* were collected by divers at a depth of 25-30 m and water temperature about +5°C in August 1993.
5. 0.5 g of the coral acetone powder, obtained from 50 g of *G. fruticosa* using the method of Brash and co-workers (Brash, A.R.; Baertschi, S.W.; Ingram, C.D.; Harris, T.M. *J. Biol. Chem.* **1987**, 262, 15829), was preincubated in 30 ml of 0.05 M Tris buffer (Ph 8.5) containing 5 Mm L-tryptophan at room temperature for 5 min. After the addition of 60 μM of tritiated arachidonic acid (0.3 mCi/mmol), the incubation mixture was stirred for 1 min, immediately acidified to pH 3 with citric acid and extracted twice by wortexing and subsequent centrifugation with 25 mL of cooled hexane-diethyl ether (1:4). The combined top layers were dried over anhydrous Na_2SO_4. After evaporation of solvents at 0°C the residue was dissolved in ethyl acetate-hexane and passed through a silica gel cartridge by gradient elution with 10, 15, 20 and 25 % of ethyl acetate in hexane. PGG_2 was eluted with a 20 % eluent.
6. Authentic PGG_2 and PGH_2 standards were prepared using ram seminal vesicles microsomes according to Hecker *et al.* with the exception that *p*-hydroxy-mercurybenzoate was omitted. See: Hecker, M.; Hatzelmann, A.; Ullrich, V. *Biochem. Pharmacol.* **1987**, 36, 851.
7. Conditions for preparative HPLC: Zorbax Sil column (9,2×250 mm); mobile phase: freshly dried and distilled hexane/isopropanol/acetic acid (93:7:0.1); flow rate: 5.6 mL/min; room temperature; detection: 206 nm; retention time: 6 min.
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9. Prostaglandin standards were a generous gift from Kevelt Co., Tallinn, Estonia.
10. ^{13}C NMR of PGG_2 (125,7 MHz, in acetone-d_6 at 4°C, Me_4Si) δ 178.03 (s, C-1), 134.40 (d, C-13), 131.24 (d, C-14), 130.41 (d, C-5), 129.04 (d, C-6), 86.16 (d, C-15), 82.35 (d, C-11), 80.00 (d, C-9), 51.48 (d,C-12), 48.62 (d, C-8), 42.68 (t, C-10), 33.29 (t, C-16), 32.95 (t, C-2), 32.14 (t, C-18), 27.68 (t, C-7), 26.89 (t, C-4), 25.39 (t, C-17), 25.25 (t, C-3), 22.95 (t, C-19), 14.00 (q, C-20).
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13. For analogous reduction in plasma and further references, see: Morrow, J.D.; Harris, T.M.; Roberts, L.J. *Anal. Biochem.* **1990**, 184, 1.
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