BIOSYNTHESIS OF PROSTAGLANDINS IN THE RENAL MEDULLA OF RABBIT

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1. Introduction

An enzyme system present in the vesicular gland of sheep catalyzes the conversion of certain unsaturated fatty acids into prostaglandins [1–3]. The yields of PGE_1 and PGE_2* from 8,11,14-eicosatrienoic acid and arachidonic acid, respectively, are very high (about 70%). Considerably lower yields of prostaglandins (2–5%) have been reported for homogenates of sheep intestine [2], guinea pig lung [4] and rat stomach [5], whereas preparations of pig eye iris [6] and sheep thymus and sheep uterus [2] give very low yields (< 1%) of prostaglandins. The present paper reports the efficient conversion of arachidonic acid into prostaglandins by the renal medulla of rabbit.

2. Materials and Methods

5,6,8,9,11,12,14,15-octatritio-arachidonic acid was prepared by reduction of 5,8,11,14-eicosatetraynoic acid (a generous gift of Dr. U.Gloor, Hoffman-La Roche & Co., Basle, Switzerland) with tritium gas and Lindlar catalyst. Part of the product was diluted with unlabeled arachidonic acid and was purified by silicic acid chromatography. No impurities could be detected by gas-liquid radiochromatographic analysis of the methyl ester. The specific activity of this preparation was 44 $\mu C/\mu mole$.

Crystalline PGE_2 was a generous gift of Dr. J. Pike, The Upjohn Company, Kalamazoo, Michigan.

The renal medullas were separated from cortex by scissor dissection [7]. The tissue was minced and homogenized in 4 volumes of 0.1 M potassium phosphate buffer pH 7.4 containing 20 mM EDTA, 1 mM reduced glutathione and 5,6,8,9,11,12,14,15-octatritio-arachidonic acid. The homogenates were incubated at 37° for 30 min. Then ethanol was added and the solution was filtered, acidified and extracted with diethyl ether. The ether was evaporated and the residue, which contained 85–90% of the incubated radioactivity, was subjected to silicic acid chromatography. The columns (1 g of silicic acid) were eluted with ethyl acetate – benzene 1:9, ethyl acetate – benzene 2:8, ethyl acetate, and methanol (30 ml of each eluent). The material present in the fraction eluted with ethyl acetate was treated with diazomethane and then subjected to thin layer chromatography. The solvent system used was the organic layer of ethyl acetate – methanol – water 16:1:10. A Berthold Dünnschichtscanner II was used for the assay of radioactivity of the plates. The

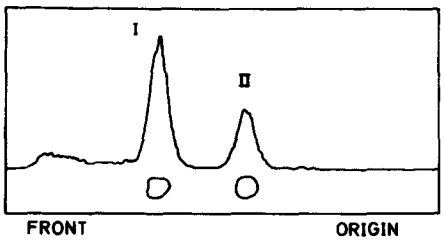

Fig. 1. Thin layer radiochromatogram of material eluted with ethyl acetate during silicic acid chromatography as described in the text. Full ordinate scale represents 6.000 counts/min.

* Abbreviations: PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGA_2, prostaglandin A_2; PGB_2, prostaglandin B_2; methoxime, O-methyl-oxime; TMSi, trimethylsilyl.

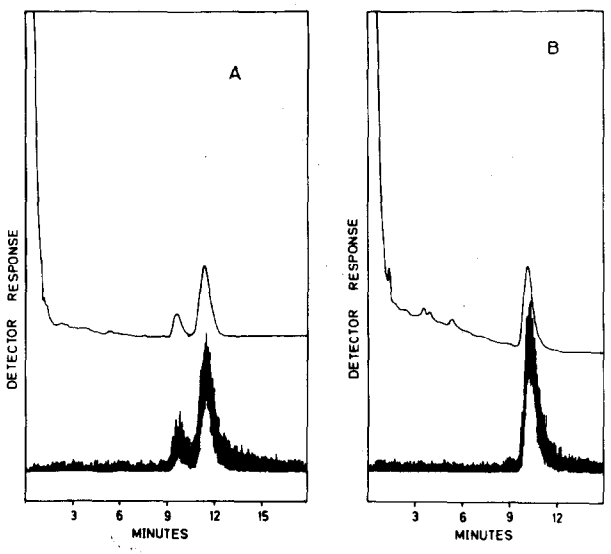

Fig. 2. (A). Gas-liquid radiochromatogram of the methoxime and TMSi ether derivative of Compound I. (B). Gas-liquid radiochromatogram of the TMSi ether derivative of Compound II. Upper traces, mass detection; lower traces, radioactivity.

radioactive zones were scraped off, eluted with ethyl acetate and the radioactivity was determined using a Packard Tri-Carb model 3375 liquid scintillation counter.

3. Results and discussion

Preliminary experiments with whole homogenates of rabbit kidney medullas indicated a good conversion of octatritio-arachidonic acid into compounds having chromatographic properties resembling those of prostaglandins. In order to obtain material for identification, 0.6 mg of octatritio-arachidonic acid was incubated with a homogenate of 6 g of renal medulla. Two peaks of radioactivity appeared on silicic acid chromatography of the diethyl ether extract of the incubation mixture. One peak (62% of the recovered radioactivity) was eluted with ethyl acetate – benzene 1:9, and another peak (30%) was eluted from the column with ethyl acetate. The latter material was esterified by treatment with diazomethane and was analyzed by thin layer radiochromatography. Two major peaks of radioactivity appeared (fig. 1). The radioactive compounds were provisionally designated Compound I (65% of the radioactivity applied to the plate, R_F = 0.65; reference methyl ester of PGE_2, R_F = 0.65) and Compound II (25% of the radioactivity, R_F = 0.45; reference methyl ester of $PGF_{2\alpha}$, R_F = 0.45).

Compound I was diluted with unlabeled methyl ester of PGE_2 and part of the mixture was subjected to thin layer radiochromatography using solvent system M I [8]. The peak of radioactivity coincided with the spot of the added methyl ester of PGE_2 (R_F = 0.55). Another part was converted into the methoxime and TMSi ether derivative* [9] and analyzed by gas-liquid radiochromatography (column 1% SE 30). The two peaks of radioactivity coincided with the peaks of the *syn-anti*-isomers of the methoxime and TMSi ether derivative of the added methyl ester of PGE_2 (retention times corresponding to C-23.9 and C-24.5, c.f. ref. 9). Finally, part of the labeled material was treated with 0.5 M NaOH in ethanol – water 1:1. The product was re-esterified and then subjected to thin layer radiochromatography (solvent system: organic layer of ethyl acetate – 2,2,4-trimethylpentane – water 3:3:4. The peak of radioactivity coincided with the spot of the methyl ester of PGB_2 (R_F = 0.40).

In another experiment Compund I was analyzed without the addition of unlabeled methyl ester of PGE_2. Gas-liquid radiochromatographic analysis of the methoxime – TMSi ether derivative showed two peaks of radioactivity coinciding with two mass peaks (C-23.9 and C-24.5, see fig. 2). The mass spectra recorded on these peaks were indistinguishable from those recorded on the corresponding peaks of the methoxime – TMSi ether derivative of the methyl ester of PGE_2 (c.f. ref. 9).

Compund II was isolated after the original thin layer chromatography (fig. 1) and part of the material was subjected to a second thin layer chromatography using solvent system M I [8]. The peak of radioactivity coincided with the spot of the reference $PGF_{2\alpha}$ methyl ester (R_F = 0.34). Another part was converted into the TMSi ether derivative and subjected to gas-liquid radiochromatography. The peak of radioactivity coincided with a mass peak which had a retention time identical with that of the TMSi ether derivative of the methyl

Table 1
Composition of labeled material formed on incubation of 5,6,8,9,11,12,14,15-octatritio-arachidonic acid with whole homogenates of rabbit renal medulla. The radioactivity of PGE_2 has been corrected for loss of tritium from C-9 of the precursor which occurs during the conversion [3].

Arachidonic acid incubated (μg/g of tissue)	Radioactivity (% of recovered radioactivity)			
	Arachidonic acid	Monohydroxy-acids	PGE_2	$PGF_{2\alpha}$
15	29	7	47	5
48	52	7	24	6
98	58	6	22	7

ester of $PGF_{2\alpha}$ (C-24.0, see fig. 2). The mass spectrum of the TMSi ether derivative of Compound II was indistinguishable from that of the TMSi ether derivative of the methyl ester of $PGF_{2\alpha}$. Ions of high intensity were present at m/e 513 (M-71; loss of $\cdot C_5H_{11}$), 494 (M-90; loss of TMSiOH), 423 (M-(71 + 90)), 404 (M-2 X 90), 397, 333 (M-(71 + 2 X 90)), 307, 217 and 191.

The radioactive material present near the solvent front of the original thin layer chromatography (fig. 1) was shown to be heterogenous when analyzed by thin layer chromatography using a less polar solvent system (organic layer of ethyl acetate – 2,2,4-trimethylpentane – water 3:3:4). At least two peaks of radioactivity appeared. One of these coincided with the reference spot of the methyl ester of PGA_2 (R_F = 0.40) and another, broad peak appeared further down (R_F = 0.30). These compounds, which together never constituted more than 10% of the radioactivity applied to the original thin layer plate, were not further characterized.

The material eluted with ethyl acetate – benzene 1:9 from the silicic acid column was treated with diazomethane and then separated into two fractions by a second silicic acid chromatography. One peak of radioactivity (91%, eluted with diethyl ether – hexane 5:95) was formed by methyl octatritio-arachidonate as judged by gas-liquid radiochromatography. Another peak (9%, eluted with diethyl ether – hexane 15:85) consisted of methyl esters of monohydroxyacids as indicated by gas-liquid radiochromatographic analysis of the TMSi ether derivatives. Two peaks appeared, one having a retention time characterisitic for the TMSi ether derivative of a C_{20} hydroxyester, and one having a retention time typical for the TMSi ether derivative of a C_{17} hydroxyester. The positions of the oxygen function of these hydroxyesters were determined by mass spectrometric analysis of the derived saturated ketoesters [10]. The mass spectrum of the C_{20} ketoester was similar to that of methyl 11-keto-arachidate, but also contained ions typical for methyl 15-ketoarachidate (c.f. ref. 10). The mass spectrum of the C_{17} ketoester was identical with that of methyl 12-ketoheptadecanoate [10]. The formation of C_{20} monohydroxyacids oxygenated at C-11 and C-15 as well as a C_{17} hydroxyacid oxygeneated at C-12 is in agreement with the previously reported formation of 11-hydroxy-8,12,14-eicosatrienoic acid, 15-hydroxy-8,11,13-eicosatrienoic acid and 12-hydroxy-8,10-heptadecadienoic acid from 8,11,14-eicosatrienoic acid in the vesicular gland of sheep [10, 2].

In other experiments the conversion of different amounts of octatritio-arachidonic acid into PGE_2, $PGF_{2\alpha}$ and monohydroxyacids was studied. The results are given in table 1.

The present work demonstrates the formation of PGE_2 and $PGF_{2\alpha}$ from arachidonic acid by homogenates of rabbit renomedullary tissue. PGE_2 and $PGF_{2\alpha}$ have earlier been isolated from this tissue by Daniels et al. [11] and Lee et al. [12]. The latter group also obtained PGA_2, most part of which, however, was considered to be formed non-enzymatically from PGE_2 during the extraction procedures. In the present work only traces of a labeled compound tentatively identified as PGA_2 could be isolated after incubations of octatritio-arachidonic acid.

Studies on the biosynthesis of prostaglandins in the renomedullary tissue of other species and studies on the further metabolism of prostaglandins in renal tissue are in progress in our laboratory and will be reported later.

Acknowledgements

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