*Proc. Natl. Acad. Sci. USA*
Vol. 83, pp. 1583–1587, March 1986
Biochemistry

# Stereospecific conversion of prostaglandin $D_2$ to (5*Z*,13*E*)-(15*S*)-9α,-11β,15-trihydroxyprosta-5,13-dien-1-oic acid (9α,11β-prostaglandin $F_2$) and of prostaglandin $H_2$ to prostaglandin $F_{2\alpha}$ by bovine lung prostaglandin F synthase

(prostaglandin-$D_2$ 11-ketoreductase/11-epi-prostaglandin $F_{2\alpha}$/bovine lung)

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**ABSTRACT** **A prostaglandin F (PGF) synthase was recently purified from bovine lung that catalyzed the reduction of both $PGH_2$ and $PGD_2$ but at different active sites on the enzyme. In view of the recent finding that $PGD_2$ is stereospecifically reduced to a unique biologically active compound, (5*Z*,13*E*)-(15*S*)-9α,11β,15-trihydroxyprosta-5,13-dien-1-oic acid (9α,11β-$PGF_2$ or 11-epi-$PGF_{2\alpha}$), by a human liver cytosolic enzyme, detailed characterization of the products formed from $PGH_2$ and $PGD_2$ by the bovine lung PGF synthase was carried out. Chromatographic characteristics of the products formed and stereochemical analysis procedures using mass spectrometry indicated that the enzyme stereospecifically reduces $PGH_2$ to $PGF_{2\alpha}$, whereas $PGD_2$ is stereospecifically converted to 9α,11β-$PGF_2$. The finding that this enzyme catalyzes the formation of both C-11 hydroxy epimers of $PGF_2$, albeit from different substrates, is of interest in that these two compounds may exert different biological actions.**

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; also called 9α,11α-$PGF_2$) is produced by a number of mammalian organs (1, 2) and exerts a variety of biological actions (3–6). Three different biosynthetic pathways have been described for the formation of $PGF_{2\alpha}$: (*i*) 9,11-endoperoxide reduction of $PGH_2$ (7–9), (*ii*) 11-ketoreduction of $PGD_2$ (10, 11), and (*iii*) 9-ketoreduction of $PGE_2$ (12, 13). Recently a cytosolic enzyme with 11-ketoreductase activity was purified to apparent homogeneity from bovine lung (14). Interestingly, it was found that this enzyme catalyzed the reduction of both $PGD_2$ and $PGH_2$ but at two different active sites on the enzyme. Thus, this enzyme has been called "PGF synthetase" (14), herein called "PGF synthase."

Recently it was reported that $PGD_2$ is stereospecifically transformed *in vitro* to a unique biologically active compound, (5*Z*,13*E*)-(15*S*)-9α,11β,15-trihydroxyprosta-5,13-dien-1-oic acid (9α,11β-$PGF_2$; also called 11-epi-$PGF_{2\alpha}$) by a cytosolic enzyme present in human liver (15). Furthermore, evidence was presented that the formation of 9α,11β-$PGF_2$ also occurs *in vivo* in humans. The $PGF_2$ products formed from $PGD_2$ and $PGH_2$ by the bovine lung PGF synthase had been tentatively identified as $PGF_{2\alpha}$. However, in view of the above finding that $PGD_2$ is stereospecifically reduced to 9α,11β-$PGF_2$ by a human liver enzyme, detailed characterization of the products formed from $PGD_2$ and $PGH_2$ by the bovine lung PGF synthase was performed. We report the finding that this enzyme stereospecifically reduces $PGH_2$ to $PGF_{2\alpha}$, whereas $PGD_2$ is stereospecifically converted to 9α,11β-$PGF_2$.

## MATERIALS AND METHODS

**Materials.** [5,6,8,9,12,14,15-$^3H_7$(N)]$PGD_2$ (100 Ci/mmol) and [1-$^{14}$C]arachidonic acid (60 mCi/mmol; 1 Ci = 37 GBq) were obtained from New England Nuclear. [1-$^{14}$C]$PGH_2$ was prepared from [1-$^{14}$C]arachidonic acid as described (14). 9α,11β-$PGF_2$ was synthesized as described by Iguchi *et al.* (16). [3,3,4,4-$^2H_4$]$PGF_{2\alpha}$ was a gift of John Pike (Upjohn Company). Other PGs were kindly donated by Ono Pharmaceutical Company. Other materials and commercial sources were as follows: NADP, NADPH, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase from Baker's yeast (type IX), from Sigma; precoated silica gel glass plates (F254), from Merck. The boric acid-impregnated silica gel plates were prepared by the method of Lincoln *et al.* (17). PGF synthetase was purified from bovine lung to apparent homogeneity as described (14).

**Enzyme Incubation Conditions and Isolation of Products Formed.** The reaction mixture for $PGD_2$ contained 0.1 M potassium phosphate (pH 6.5), 1.5 mM [$^3$H]$PGD_2$ (0.1 μCi), 0.5 mM NADP, 5 mM glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, and purified enzyme in a total volume of 0.1 ml. Incubation was carried out at 37°C for 1 hr. Approximately 80% of $PGD_2$ was converted to a single more polar product. The reaction mixture for $PGH_2$ contained 0.1 M potassium phosphate (pH 6.5), 70 μM [1-$^{14}$C]$PGH_2$ (0.37 μCi), 0.5 mM NADPH, and purified enzyme in a total volume of 1 ml. Incubations were carried out at 37°C for 2 min. About 30% of $PGH_2$ was metabolized by the enzyme during a 2-min incubation. Under these conditions, $PGH_2$ was degraded nonenzymatically to $PGE_2$ (22%), $PGD_2$ (16%), 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (2%), and $PGF_{2\alpha}$ (<1%). Reactions were terminated, and products were extracted with 3 vol of diethyl ether/methanol/0.2 M citric acid, 30:4:1 (vol/vol), to the reaction mixture. The organic phase was evaporated, and the residue was subjected to TLC with the solvent system A (see below). The major enzymatic product formed from both $PGH_2$ and $PGD_2$ that migrated in the region of $PGF_{2\alpha}$ ($R_f$

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Abbreviations: PG, prostaglandin; 9α,11β-$PGF_2$ (11-epi-$PGF_{2\alpha}$), (5*Z*,13*E*)-(15*S*)-9α,11β,15-trihydroxyprosta-5,13-dien-1-oic acid; RP, reversed phase; $Me_3Si$, trimethylsilyl; BA-TLC, boric acid-impregnated TLC; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide.

approximately 0.27) was scraped and extracted from the silica gel with ethyl acetate/acetic acid, 100:1 (vol/vol).

**Identification of Products Formed by PGF Synthase.** Further analysis of the $PGF_2$ products formed from $PGD_2$ and $PGH_2$ was carried out by using boric acid-impregnated TLC (BA-TLC), reversed-phase (RP)-HPLC, and GC/MS.

Three different solvent systems (A, B, and C) were used for BA-TLC: A, benzene/dioxane/acetic acid, 20:20:1 (vol/vol); B, diethyl ether/methanol/acetic acid, 90:2:0.1 (vol/vol); and C, ethyl acetate/acetic acid, 99:1 (vol/vol). TLC plates subjected to chromatography with solvent systems A and B were developed twice, and the plate chromatographed with solvent system C was developed three times.

RP-HPLC was performed on a 5-$\mu$m Alltech $C_{18}$ column with the solvent system acetonitrile/water/acetic acid, 27:73:0.1 (vol/vol) at 1-ml/min in 1-ml fractions. Characteristic elution volumes for 9$\alpha$,11$\beta$-$PGF_2$ and $PGF_{2\alpha}$ were approximately 41–43 ml and 53–56 ml, respectively.

Methyl esters of compounds were formed by treatment with excess ethereal diazomethane. Trimethylsilyl ($Me_3Si$) ethers were formed by treatment with 20 $\mu$l of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Regis, Morton Grove, IL) and 20 $\mu$l of dimethylformamide at room temperature for 15 min. Butylboronate derivatives were formed by treatment with 50 $\mu$l of butylboronic acid (Aldrich) in pyridine (10 mg/ml) at 60°C for 45 min.

GC/MS analysis was performed by using a Nermag R10-10C gas chromatograph/mass spectrometer interfaced with a DEC PDP-11/23 plus computer system. GC was performed as a six-meter DB-1 fused silica capillary column programmed from 190–325°C at 25°C/min. Helium was used as the carrier gas at a flow rate of 1 ml/min. Ion-source temperature was 200°C, electron energy was 87 eV, and filament current was 0.25 mA.

## RESULTS

After incubation of $PGH_2$ and $PGD_2$ with the purified bovine lung PGF synthase, products formed were extracted and subjected to TLC using solvent system A. The major enzymatic products formed from both $PGH_2$ and $PGD_2$, which migrated in the region of $PGF_{2\alpha}$ ($R_f$ approximately 0.27), were isolated and further analyzed by BA-TLC, GC/MS, and HPLC.

To assess the stereochemical configuration of the C-9 and C-11 PGF-ring hydroxyl groups, the ability of the products to form a butylboronate derivative when treated with *n*-butylboronic acid was determined. A butylboronate derivative will form bridging the oxygens at C-9 and C-11 only if both hydroxyls are *cis* in the prostane ring (18).

The enzymatic products formed from $PGD_2$ and $PGH_2$ were initially converted to a methyl ester and then sequentially treated with *n*-butylboronic acid and BSTFA and subsequently analyzed by GC/MS. $[^2H_4]PGF_{2\alpha}$ was coderivatized with each sample to provide a means to determine the completion of the boronation reaction when analyzed by GC/MS.

**Identification of the Product Formed from $PGD_2$.** Figs. 1 and 2 show the mass spectra obtained from analysis of the product formed from $PGD_2$. The mass spectrum in Fig. 1 is characteristic for the methyl ester–$Me_3Si$ ether derivative of both 9$\alpha$,11$\beta$-$PGF_2$ (15) and $PGF_{2\alpha}$ (19), both of which have essentially identical mass spectra except for minor variation in relative ion abundances. The mass spectrum in Fig. 2 is essentially identical to that previously reported for the methyl ester–butylboronate–$Me_3Si$ ether derivative of $[^2H_4]PGF_{2\alpha}$ (15). Importantly, there are no doublet ion peaks in either mass spectrum four atomic mass units apart, indicating that the $[^2H_4]PGF_{2\alpha}$ that was added to the sample was quantitatively converted to a butylboronate derivative, whereas none

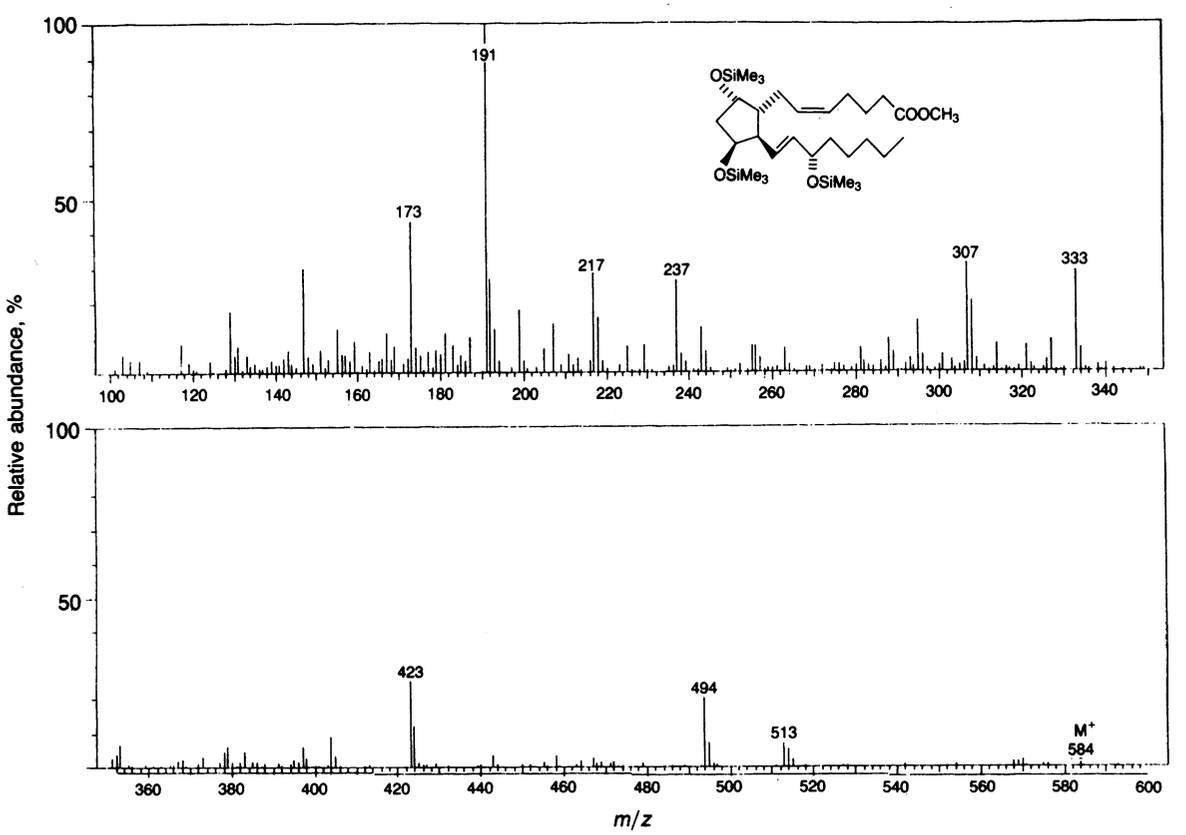


FIG. 1. Mass spectrum of the $PGF_2$ product formed from $PGD_2$ by the PGF synthase after coderivatization with $[^2H_4]PGF_{2\alpha}$ by treatment sequentially with diazomethane, *n*-butylboronic acid, and BSTFA.

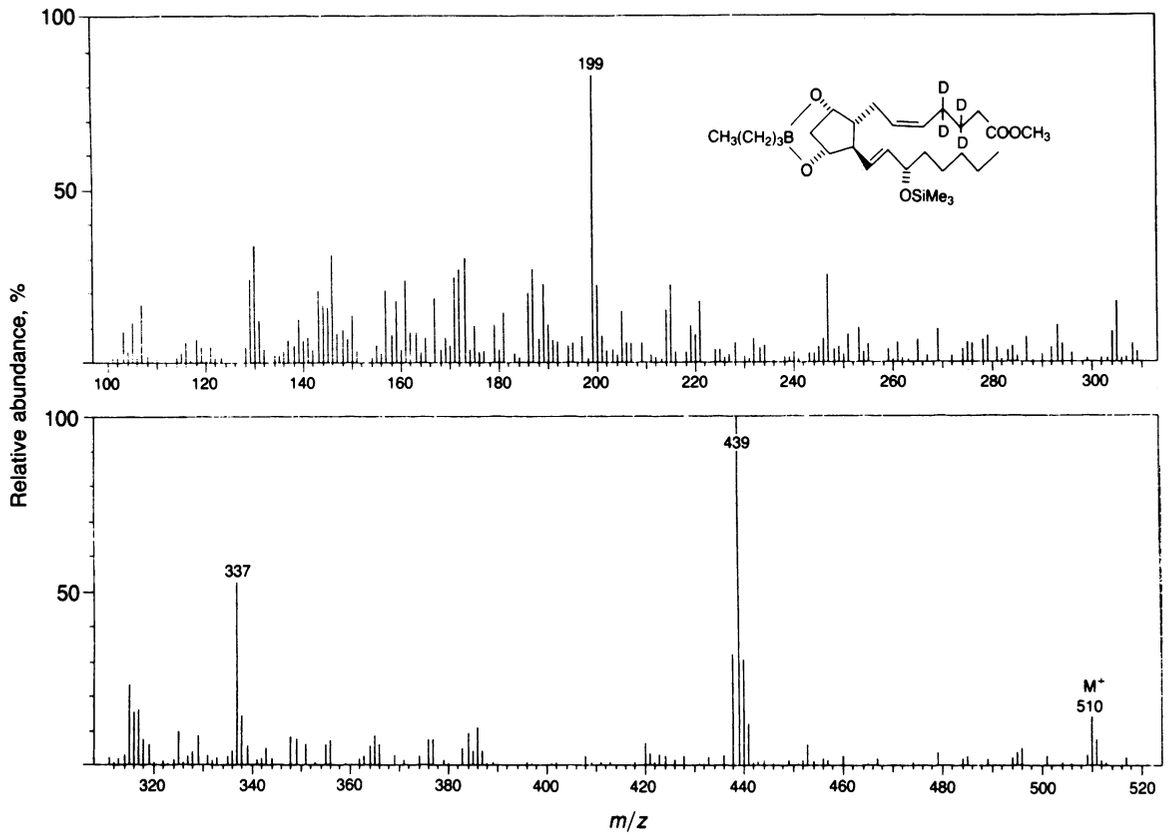


FIG. 2. Mass spectrum of $[^2H_4]PGF_{2\alpha}$ coderivatized with the $PGF_2$ product formed from $PGD_2$ by the PGF synthase after treatment sequentially with diazomethane, *n*-butylboronic acid, and BSTFA.

of the enzymatic product formed from $PGD_2$ reacted with *n*-butylboronic acid.

The failure of the $PGF_2$ product formed from $PGD_2$ to form a butylboronate derivative provided evidence that $PGD_2$ was stereospecifically converted by the enzyme to $9\alpha,11\beta$-$PGF_2$. This was further supported by BA-TLC (data not shown) and RP-HPLC analysis of the product. $PGF_{2\alpha}$ and $9\alpha,11\beta$-$PGF_2$ are widely separated on RP-HPLC (15). RP-HPLC analysis of the product formed from $PGD_2$ revealed only a single radioactive peak with an elution volume characteristic of that of $9\alpha,11\beta$-$PGF_2$, 40–42 ml (Fig. 3). Virtually no radioactive peak was detected at the elution volume characteristic for $PGF_{2\alpha}$ (approximately 53–56 ml).

**Identification of the Product Formed from $PGH_2$.** When the methyl esters of the product formed from $PGH_2$ and added $[^2H_4]PGF_{2\alpha}$ were sequentially treated with *n*-butylboronic acid and BSTFA and were analyzed by GC/MS, a mass spectrum essentially identical to that shown in Fig. 1 was obtained. Again, there were no doublet ion peaks in this mass spectrum four atomic mass units apart, indicating that the compound did not react with *n*-butylboronic acid and that $[^2H_4]PGF_{2\alpha}$ was quantitatively converted to a butylboronate derivative. However, analysis of the mass spectrum obtained for the $[^2H_4]PGF_{2\alpha}$ (Fig. 4) revealed doublet ion peaks four atomic mass units apart, indicating the presence of a mixture of unlabeled $PGF_{2\alpha}$ and $[^2H_4]PGF_{2\alpha}$. Doublet ion peaks were present for ions retaining the deuterium atoms on C-3 and C-4 of the $[^2H_4]PGF_{2\alpha}$, such as the molecular ions at $m/z$ 506 and 510 and fragmentation ions at $m/z$ 435 and 439 [$M^+ - 71$, loss of $\cdot(CH_2)_4CH_3$] and at $m/z$ 333 and 337 [$M^+ - 173$, loss of $\cdot CH(OSiMe_3)(CH_2)_4CH_3$]. However, the ion at $m/z$ 199, $[\cdot CH{=}CHCH(OSiMe_3)(CH_2)_4CH_3]^+$, comprising the lower side chain (C-13 to C-20) did not retain the deuterium atoms at C-3 and C-4 and, thus, was present in the mass spectrum of both unlabeled $PGF_{2\alpha}$ and $[^2H_4]PGF_{2\alpha}$. For this reason, also, the base ion of this mixed mass spectrum is $m/z$ 199 instead of $m/z$ 439 (compare Fig. 2).

The above data indicated that both $9\alpha,11\beta$-$PGF_2$ and $PGF_{2\alpha}$ were formed during incubation of $PGH_2$ with the enzyme. This was further supported by RP-HPLC analysis (Fig. 5). A radioactive peak representing approximately 23%

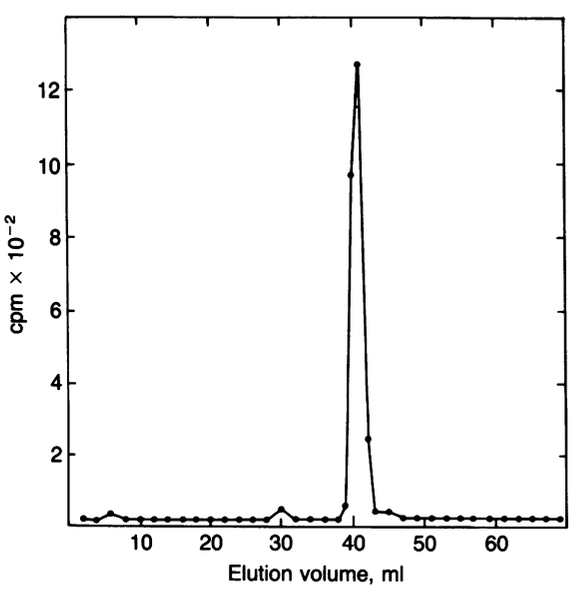


FIG. 3. RP-HPLC analysis of the product formed from incubation of $[^3H]PGD_2$ with the PGF synthase. The solvent system was acetonitrile/water/acetic acid, 27:73:0.1, run isocratically at 1 ml/min; 1-ml fractions were collected. $9\alpha,11\beta$-$PGF_2$ is characteristically eluted at approximately 40–43 ml and $PGF_{2\alpha}$ at 53–56 ml.

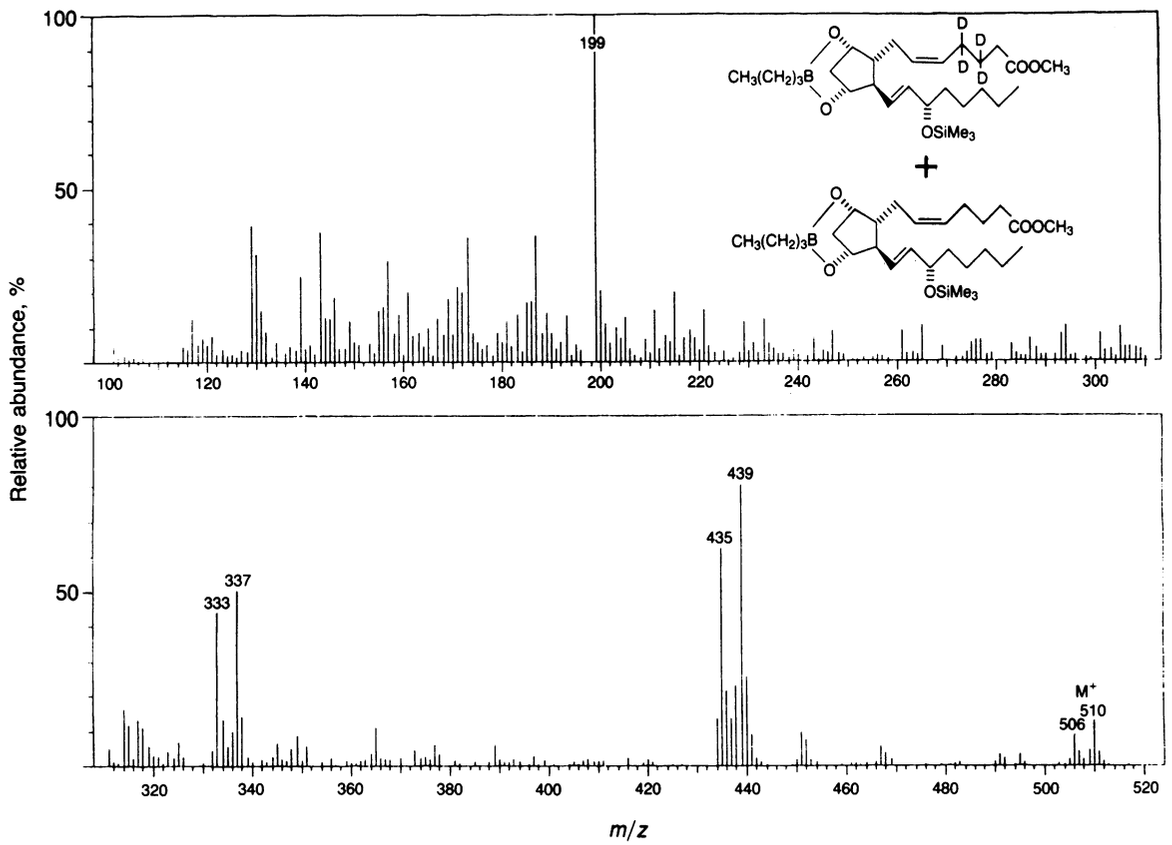


FIG. 4. Mass spectrum obtained for $[^2H_4]PGF_{2\alpha}$ coderivatized with the $PGF_2$ products formed from $PGH_2$ during incubation with the PGF synthase after treatment sequentially with diazomethane, *n*-butylboronic acid, and BSTFA.

of the total recovered radioactivity was eluted with a retention volume characteristic of 9α,11β-$PGF_2$ at 41–43 ml in addition to a less-polar peak (77%), which was eluted with a retention volume characteristic for $PGF_{2\alpha}$ at 53–56 ml.

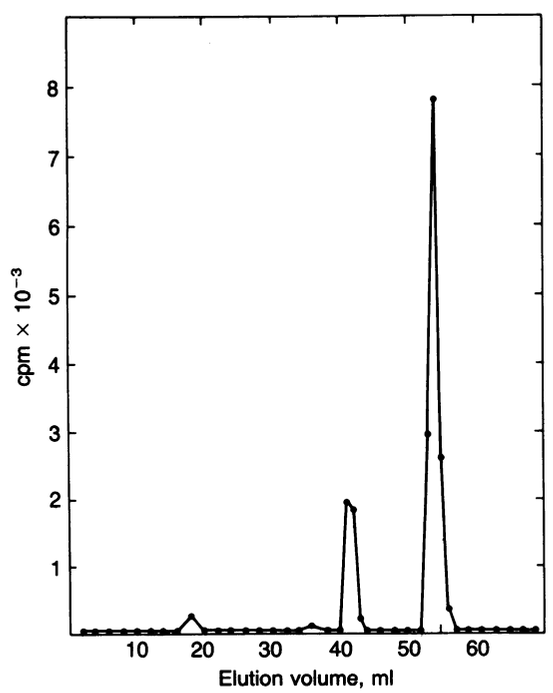


FIG. 5. RP-HPLC analysis of the products formed during incubation of $[^{14}C]PGH_2$ with the PGF synthase. The solvent system was acetonitrile/water/acetic acid, 27:73:0.1, run isocratically at 1 ml/min; 1-ml fractions were collected. 9α,11β-$PGF_2$ is characteristically eluted at approximately 40–43 ml and $PGF_{2\alpha}$ at 53–56 ml.

**Origin of the Formation of 9α,11β-$PGF_2$ During Incubation of $PGH_2$ with PGF Synthase.** Since the C-11 oxygen of $PGH_2$ is oriented α, it is difficult to envision how the enzyme could reduce $PGH_2$ to 9α,11β-$PGF_2$ with inversion of the configuration of C-11. However, as previously reported, $PGH_2$ is nonenzymatically degraded in part to $PGD_2$ during the incubation of $PGH_2$ with the PGF synthase (14). Therefore, an attractive explanation for the formation of 9α,11β-$PGF_2$ during incubation of $PGH_2$ with this enzyme is that $PGH_2$ is not directly transformed by the enzyme to 9α,11β-$PGF_2$ but that the $PGD_2$ which is formed from nonenzymatic degradation of $PGH_2$ during the incubation is reduced by the enzyme to 9α,11β-$PGF_2$.

The above hypothesis was confirmed by demonstrating that incubation of radiolabeled $PGH_2$ with the enzyme in the presence of a concentration of unlabeled $PGD_2$ that was approximately 40-fold greater than the $K_m$ of the enzyme for $PGD_2$ prevented the formation of radiolabeled 9α,11β-$PGF_2$ (Table 1). Likewise, phenanthrenequinone, which competitively inhibits the reduction of $PGD_2$ by this enzyme, almost completely abolished the formation of 9α,11β-$PGF_2$.

## DISCUSSION

We recently reported that PGF synthase purified from bovine lung catalyzed the reduction of both $PGH_2$ and $PGD_2$ but at different active sites on the enzyme (14). The products formed by the enzyme from $PGH_2$ and $PGD_2$ were tentatively identified as $PGF_{2\alpha}$. However, more detailed characterization of these products has now revealed that the enzyme stereospecifically reduces $PGH_2$ to $PGF_{2\alpha}$ but stereospecifically converts $PGD_2$ to 9α,11β-$PGF_2$.

Therefore, the 11-ketoreductase activity of the purified bovine lung PGF synthase is similar to the cytosolic enzyme with 11-ketoreductase activity in human liver, which stereospecifically reduces $PGD_2$ to 9α,11β-$PGF_2$ (15).

Table 1. Effects of $PGD_2$ and phenanthrenequinone (PQ) on the product formation from $PGH_2$ by PGF synthase

| Addition | Products from $PGH_2$, nmol/min | |
|---|---|---|
| | $9\alpha,11\beta$-$PGF_2$ | $PGF_{2\alpha}$ |
| None | 0.14 | 0.20 |
| 5 mM $PGD_2$ | 0.02 | 0.18 |
| 28 $\mu$M PQ | 0.03 | 0.31 |

The reaction mixture contained 0.1 M potassium phosphate (pH 6.5), 80 $\mu$M [1-$^{14}$C]$PGH_2$ (0.42 $\mu$Ci), 0.5 mM NADP, 5 mM glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase and enzyme (6 $\mu$g), in a total volume of 0.1 ml in the absence or presence of 5 mM $PGD_2$ or 28 $\mu$M PQ. Incubation was carried out at 37°C for 2 min. Reactions were terminated by addition of 3 vol of diethyl ether/methanol/0.2 M citric acid as described. The organic phase was evaporated, and the sample was analyzed by RP-HPLC with the solvent system acetonitrile/water/acetic acid, 30:70:0.1 (vol/vol). In the absence of enzyme, 0.018 and 0.044 nmol of $9\alpha,11\beta$-$PGF_2$ and $PGF_{2\alpha}$, respectively, were produced under these conditions. Numbers are corrected for the nonenzymatic formation of each product.

Whether the human liver enzyme with 11-ketoreductase activity also catalyzes the reduction of $PGH_2$ has not been examined.

Thus, at this time it is not possible to generalize whether 11-ketoreductase and 9,11-endoperoxide reductase activities are linked to a single enzyme in all organs and tissues and in all species. Although the bovine lung PGF synthase and the human liver enzyme with 11-ketoreductase activity stereospecifically reduce $PGD_2$ to $9\alpha,11\beta$-$PGF_2$, 11-ketoreductase activity has been reported in sheep blood that stereospecifically reduces $PGD_2$ to $PGF_{2\alpha}$ (20). Although 11-ketoreductase activity also has been described in rabbit liver (11, 21) and a variety of organs in the rat (10), stereochemical analysis of the C-11 hydroxyl in the $PGF_2$ products formed was not carried out. Therefore, it is uncertain whether the sheep blood enzyme with 11-ketoreductase activity is unique in that it converts $PGD_2$ to $PGF_{2\alpha}$, whether there may be species differences in the 11-ketoreductase, or whether different 11-ketoreductases are distributed in certain organs within a species.

The full spectrum of biological activity of $9\alpha,11\beta$-$PGF_2$ remains to be elucidated. $PGF_{2\alpha}$ and $9\alpha,11\beta$-$PGF_2$ in some systems may exert similar biological effects, such as raising blood pressure in the rat (15) and contraction of human coronary arteries *in vitro* (22). However, $9\alpha,11\beta$-$PGF_2$ has been shown to inhibit ADP-induced human platelet aggregation, whereas $PGF_{2\alpha}$ is devoid of an inhibitory effect on ADP-induced platelet aggregation (23). Thus, the finding that this single enzyme purified from bovine lung catalyzes the formation of both $PGF_{2\alpha}$ and $9\alpha,11\beta$-$PGF_2$, albeit from different substrates, is of interest in that these two PGs may in some systems exert different biological actions.

We thank Drs. S. Ito, Y. Watanabe, and Y. Urade for their critical reading of this manuscript. The technical assistance of C. H. Kelly was greatly appreciated. This work was supported in part by Grant GM 15431 from the National Institutes of Health. L.J.R. is a Burroughs Wellcome Scholar in Clinical Pharmacology.

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