Enzymatic Conversion of Prostaglandin H₂ to Prostaglandin F₂₀ by Aldehyde Reductase from Human Liver: Comparison to the Prostaglandin F Synthetase from Bovine Lung*

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The primary structure of prostaglandin (PG) F synthetase from bovine lung shows 62% similarity with that of human liver aldehyde reductase (EC 1.1.1.2) (Watanabe, K., Fujii, Y., Nakayama, K., Ohkubo, H., Kuramitsu, S., Kagiyama, H., Nakani, S., and Hayaishi, O. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 11–15). We therefore purified human liver aldehyde reductase to homogeneity and compared the immunological and catalytic properties of aldehyde reductase and PGF synthetase. Although both enzymes belong to a group of aldoketoreductases and their molecular weights are essentially identical, aldehyde reductase had no cross-reactivity to anti-PGF synthetase serum. Furthermore, there was a difference in the substrate specificity for reduction of PGs between the two enzymes. Aldehyde reductase catalyzed the reduction of PGJ₃, ∆¹²-PGJ₂, PGH₂, or PGA₂, but not that of PGB₂, PGD₂, or PGE₂, whereas PGF synthetase reduced PGD₂. The optimum pH, Kₘ value for PGH₂, and the turnover number were 6.5, 100 μM, and 3.1 min⁻¹, respectively. The PGH₂ 9,11-endoperoxide reductase activity of aldehyde reductase was not affected in the presence of a substrate such as p-nitrobenzaldehyde, DL-glyceraldehyde, or 9,10-phenanthrenequinone, suggesting that PGH₂ 9,11-endoperoxide and other substrates are reduced at different active site(s). The reaction product formed from PGH₂ by this enzyme was identified as PGF₂₀, by gas chromatography/mass spectrometry; TMS, trimethylsilyl; HPLC, high performance liquid chromatography.

Prostaglandin (PG)¹ F synthetase has been purified to homogeneity from bovine lung, and its properties have been studied in detail (1). The enzyme exhibits a broad substrate specificity and catalyzes the reduction of PGH₂ to PGF₂₀ and that of PGD₂ to 9α,11β-PGF₂₀, which is a stereoisomer of PGF₂₀ (2), at different active sites on the same molecule (1, 2). Recently, cDNA sequences specific for PGF synthetase have been isolated from a cDNA library of bovine lung mRNA sequences, and the primary structure of this enzyme has been determined (3). Comparison of the amino acid sequence of PGF synthetase revealed 62% similarity to that of human liver aldehyde reductase (4). However, little information is available about the catalytic properties of aldehyde reductase concerning the reduction of PGs such as PGH₂, PGB₂, and PGE₂ (5, 6). To examine the role of aldehyde reductase in PG metabolism, we purified this enzyme from human liver and characterized its catalytic properties with respect to various PGs. In this paper, we report that aldehyde reductase as well as PGF synthetase catalyzes the reduction of PGH₂ to PGF₂₀, although this enzyme is biochemically and immunologically different from PGF synthetase.

EXPERIMENTAL PROCEDURES

Reagents—[5,6,8,9,12,14,15-²H]PGD₂ (100 Ci/mmol) and [5,6,8,11,12,14,15-²H]PGE₂ (165 Ci/mmol) were obtained from Du Pont-New England Nuclear. [¹⁴C]PGH₂ was prepared as described previously (7), using acetone powder of sheep vesicular gland microsomes (Ran Biochemicals, Tel Aviv) as a source of PG endoperoxide synthetase. Authentic PGs were generous gifts of Ono Pharmaceutical Company, Osaka, Japan. Other materials and commercial sources were as follows: oxidase, from P-L Biochemicals; NADP (Grade III), NADPH, glucose 6-phosphate, glucose-6-phosphate dehydrogenase from baker’s yeast (Type IX), n-erythrose, succinic semialdehyde, and daunorubicin, from Sigma; p-nitrobenzaldehyde, 9,10-phenanthrenequinone, and pyruvate, from Wako Pure Chemicals; n-butyraldehyde, dt-glyceraldehyde, menadione, methylglyoxal, and pyrozole, from Nakarai Chemicals; phenylglyoxal, from Aldrich; 5-carboxybenzaldehyde, from Tokyo Kasei Kogyo Co; precoated silica gel glass plates (F₂₅₄), from Merck; Electrophoresis Calibration Kit (low molecular weight), DEAE-Sepharose, Sephadex G-100, and Red-Sepharose, from Pharmacia; DEAE-cellulose, from Whatman. PGF synthetase from bovine lung was purified by the method as described previously (1). All solvents used were HPLC grade from Nakarai Chemicals, Kyoto, Japan. Other chemicals were at least of reagent grade.

Enzyme Assay—The aldehyde reductase activity was determined according to the method of Wernuth et al. (5). The standard reaction mixture contained the following in a total volume of 1 ml: 0.1 M sodium phosphate buffer (pH 6.5), 0.08 mM NADPH, and enzyme. The reaction was started by the addition of substrate. For measurements during the earlier three steps of purification, the assay mixture contained 10 mM pyrazole to inhibit alcohol dehydrogenase activity. The enzyme activity was measured spectrophotometrically at 37 °C by following the decrease in absorbance at 340 nm. One unit of enzyme was defined as the amount that caused the oxidation of 1 μmol of NADPH/min.

The PGH₂ 9,11-endoperoxide reductase activity was determined as described previously (1). The standard reaction mixture contained 0.1 mM sodium phosphate buffer (pH 6.5), 500 μM NADP, 5 mM glucose
6-phosphate, glucose-6-phosphate dehydrogenase (1 unit), 80 μM 1-[(13)C]PGH₂ (0.1 μCi), and enzyme in a total volume of 0.05 ml. Incubation was carried out at 37 °C for 2 min. The reaction was started by the addition of enzyme and terminated by 0.25 ml of diethyl ether, methanol, 0.2 M citric acid (30:4:1). The mixture of PGD₃, PGE₂, and PGF₂α (20 μg each) was added to the solution as autolysis was performed. The reaction mixture was subjected to thin layer chromatography (TLC) in a solvent system of diethyl ether/methanol/acetic acid (90:20:1). The positions of the PGs on the silica gel plate were visualized with iodine vapor. Silica gel was scraped off in sections corresponding to PGF₂α, PGE₂, PGD₂, PGH₂, and others, and the radioactivity of each section was measured in a Triton-toluene scintillator by a Beckman liquid scintillation spectrometer model LS 2800.

The PGD₃ 11-ketoreductase and PGE₂ 9-ketoreductase activities were assayed under the same conditions as those for the PGF₂α, 9,11-endoperoxide reductase activity except that 1.5 mM [3H]PGD₂ or [3H]PGE₂ (0.14 μCi each) was used as a substrate in place of 80 μM [1-[(13)C]PGH₂ (0.1 μCi) and that the incubation time was 30 min.

The spontaneous decomposition of [1,14C]PGH₂ and [3H]PGD₂ or [3H]PGE₂ to [1,14C]PGF₃α (0.9-1.1%/2 min) and [3H]PGF₃α (2.2-4.0%/30 min), respectively, was taken into account to obtain the actual activities of PGH₂, 9,11-endoperoxide reductase, PGD₃, 11-ketoreductase, and PGE₂ 9-ketoreductase, respectively. One unit of enzyme activity was defined as the amount which produced 1 μmol of PGF₃α/min at 37 °C. Specific activity was expressed as the number of units per milligram of protein.

Protein concentration was determined according to the method of Lowry et al. (8), using bovine serum albumin as a standard.

Identification of the Reaction Product by Gas Chromatography/Mass Spectrometry (GC/MS)—The purified enzyme (10 μg) was incubated with [1,14C]PGH₂ (80 μM, 0.1 μCi) in the presence of the NADPH-generating system at 37 °C for 2 min, and the organic extract was subjected to TLC in a solvent system of diethyl ether/methanol/acetic acid (90:20:1). The major product with an Rₜ value of 0.66 was extracted from a silica gel plate with ethyl acetate/acetonic acid (89:11). The combined extracts of 10 samples (equivalent to about 3.5 μg of PGF₃α) were evaporated under reduced pressure and the residue was dissolved in 20 ml of 15% (v/v) ethanol. The sample was adjusted to pH 3.0 with acetic acid and applied to a SEP-PAK C₁₈ cartridge (11). The ethyl acetate eluate was pooled and evaporated in vacuo. For analysis by GC/MS, the product was converted to a methyl ester using diazomethane prepared from N-methyl-N-nitrosoniumylurea. The methyl ester was further converted to a trimethylsilyl (TMS) derivative and analyzed by a JEOL combined gas chromatography mass spectrometer model JMS DX-300.

RESULTS

Properties of Aldehyde Reductase—The molecular weight of aldehyde reductase calculated from the amino acid sequence (4) is 36,359, which is practically the same as that of PGF synthetase (Mₚ, 36,517). Moreover, the primary structure of aldehyde reductase shows 62% similarity with that of PGF synthetase (9). To compare the immunological properties of the two enzymes, we first examined the relative mobility on SDS-PAGE and cross-reactivity to rabbit anti-PGF synthetase antiserum. On the Coomassie Brilliant Blue-stained gel shown in Fig. 1A, PGF synthetase and aldehyde reductase each showed a single band of protein with a molecular weight of approximately 35,000 and 36,500, respectively. Since PGF synthetase employed here had the N and C termini deduced from a specific cDNA sequence for the molecule (3), underestimation of the molecular weight on SDS-PAGE appeared to be due to its physicochemical properties. Furthermore, aldehyde reductase did not cross-react with anti-PGF synthetase antiserum at all (Fig. 1B), indicating that the antigenicity of aldehyde reductase is different from that of PGF synthetase.

The pH dependence of the reaction is shown in Fig. 2A. p-Nitrobenzaldehyde, DL-glyceraldehyde, and 9,10-phenanthrenequinone are routinely used as the substrates for monitoring aldehyde, aldose, and carboxyl reductases, respectively. As shown in Fig. 2A, the pH optimum with all three substrates was 6.5. In addition, we found that aldehyde reductase had PGH₂, 9,11-endoperoxide reductase activity with optimal pH also at 6.5 (Fig. 2A, inset).

Substrate Specificity—The substrate specificity of the purified aldehyde reductase was examined spectrophotometrically at 37 °C (Table I). As demonstrated in a previous report (5, 6), the enzyme had a broad substrate specificity for a number of aromatic aldehydes and aldoses in the presence of NADPH. Among them, p-nitrobenzaldehyde was the best substrate. Sodium glucuronate, phenylglyoxal, and methylglyoxal were reduced, but the Kₐ values were 3.7, 5.2, and 1.7 mM, respectively, or about 10-fold higher than that for p-nitrobenzaldehyde. The reaction rates for p-carboxybenzaldehyde and succinic semialdehyde were 56 and 50% of that observed with p-nitrobenzaldehyde. Furthermore, aldoses such as D-erythrose and Dl-glyceraldehyde were also reduced. In contrast to the PGF synthetase (1), carbonyl compounds (9,10-phenanthrenequinone and menadione) were relatively poor substrates of this enzyme.
parent p-nitrobenzaldehyde, DL-glyceraldehyde, and 9,10-phenanthrenequinone with NADH at 37 °C consumed when the coenzyme, indicating that the reaction is enzymic. The activity was estimated to be 0.80. NADPH and NADH were examined for their capacity to serve as a cofactor under the standard assay conditions using various substrates (Table I). In the absence of NADPH (D), or with enzyme boiled for 5 min (O). Values are corrected for spontaneous conversion.

Among PGs tested here, PGJ2, Δ12-PGJ2, PGH2, and PGA2 served as substrates; the reaction rates were 1.3-0.5% of that observed with p-nitrobenzaldehyde. However, neither PGB2, PGD2, nor PGE2 was acted upon by this enzyme under the present experimental conditions.

Reduction of PGH2 by the Purified Enzyme—As shown in Fig. 2B, the PGH2 reduction was dependent on the enzyme amount. The purified enzyme in the present study was inactivated by boiling for 5 min and required NADPH as a coenzyme, indicating that the reaction is enzymic. The apparent Km value for PGH2 was calculated to be about 100 μM (Fig. 3). Under the standard assay conditions for PGH2 9,11-endoperoxide reductase except with 0.14 μM [1-14C]PGH2 and 80 μM NADPH, approximately 0.273 nmol of NADPH was consumed when 0.340 nmol of PGF2α was produced. The stoichiometry of consumed NADPH to the produced PGF2α was estimated to be 0.80. NADPH and NADH were examined for their capacity to serve as a cofactor under the standard assay conditions using various substrates (Table II). In the case of ketoaldehyde and aldose substrates, the reaction proceeded at less than 3% of the rates observed with NADPH when the same concentration of NADH was used as coenzyme. The purified enzyme exhibited high specificity for NADPH, and the Km values for NADPH and NADH were 5 and 50 μM, respectively (Table I). However, there was a difference in cofactor requirement between the reduction of ketoaldehyde and aldose compounds and that of PGH2 9,11-endoperoxide and carbonyl compounds. The reductase activities toward PGH2, 9,10-phenanthrenequinone, and menadione with NADH at 80 μM were 30, 1160, and 393% of those with NADPH at the same concentration.

To obtain information on the active site(s) of this enzyme, we examined the inhibitory effect of three substrates such as p-nitrobenzaldehyde, DL-glyceraldehyde, and 9,10-phenanthrenequinone on the PGH2 9,11-endoperoxide reductase activity. These substrates had no effect on the reduction of PGH2 (80 μM) up to 0.5 mM p-nitrobenzaldehyde (2-fold the Km value for this substrate), 12.5 mM DL-glyceraldehyde (3-fold the Km value), and 0.04 mM 9,10-phenanthrenequinone (2-fold the Km value) concentration ranges. These results taken together suggest that PGH2 and the three substrates

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**Table I**

| Substrate Concentration Relative activity Km (mM) |
|-----------------|-----------------|-----------------|
| p-Nitrobenzaldehyde | 0.5 | 100 | 0.24 |
| Sodium gluturonate | 10 | 96 | 3.7 |
| Phenylglyoxal | 3.5 | 78 | 2.2 |
| Methylglyoxal | 5.0 | 60 | 1.7 |
| p-Carboxybenzaldehyde | 0.5 | 56 | 0.03 |
| Succinic semialdehyde | 10 | 50 | 0.22 |
| p-Erythrose | 100 | 45 | 6.7 |
| DL-Glyceraldehyde | 12.5 | 19 | 3.7 |
| n-Butyraldehyde | 10 | 5 | 0.9 |
| 9,10-Phenanthrenequinone | 0.05 | 2.5 | 0.02 |
| Menadione | 0.25 | 1.6 | 0.3 |
| Daunorubicin | 0.25 | N.D.* | 0.005 |
| Pyruvate | 10 | N.D. | 0.05 |
| PGJ2 | 1.0 | 1.3 | 0.50 |
| Δ12-PGJ2 | 1.0 | 0.8 | 0.3 |
| PGH2 | 0.08 | 0.6 | 0.10 |
| PGA2 | 1.0 | 0.5 | 0.71 |
| PGB2 | 1.0 | N.D. | 0.5 |
| PGD2 | 1.0 | N.D. | 1.5 |
| PGE2 | 1.0 | N.D. | 1.5 |
| NADPH* | 0.005 | 1.0 | 0.05 |

*Not detected.

*The PGH2 9,11-endoperoxide reductase activity was determined by using [1-14C]PGH2 (80 μM, 0.1 μCi) as described under “Experimental Procedures.”

Both PGD2 12-ketoreductase and PGE2 9-ketoreductase activities were measured by the radiochemical method as described under “Experimental Procedures.”

*For the determination of Km value for NADPH, p-nitrobenzaldehyde and PGH2 were held constant at 0.5 mM and 80 μM, respectively.

*For the determination of Km value for NADH, p-nitrobenzaldehyde was held constant at 0.5 mM.

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**Fig. 3.** PGH2 dependence of PGF2α synthesis catalyzed by aldehyde reductase. The purified enzyme (5.8 μg) was incubated with various concentrations of PGH2 at 37 °C for 2 min. The enzyme activity was corrected from nonenzymic formation of PGF2α. Inset, the Lineweaver-Burk plot.
The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 6.5), enzyme, 0.08 mM NADH or NADPH, and substrates at various concentrations as indicated in a total volume of 1.0 ml. The reaction was initiated by the addition of substrate, and the enzyme activity was measured spectrophotometrically at 37 °C as described under “Experimental Procedures.”

| Substrate                          | Concentration | NADH | NADPH | NADH/NADPH |
|------------------------------------|---------------|------|-------|------------|
| Phenylglyoxal                      | 3.5           | 0.175| 11.4  | 0.02       |
| DL-Glyceraldehyde                  | 10.0          | 0.073| 2.76  | 0.03       |
| 9,10-Phenanthrenequinone           | 0.05          | 4.23 | 0.365 | 11.60      |
| Menadione                          | 0.25          | 0.920| 0.234 | 3.99       |
| PGD<sub>2</sub>                     | 1.0           | N.D. | N.D.  |            |
| PGE<sub>2</sub>                     | 1.0           | N.D. | N.D.  |            |

*The PGHz 9,11-endoperoxide reductase activity was measured under the standard assay conditions except with 80 μM NADH or NADPH.

*a Not detected.

**Table II**
Cofactor requirement for various substrates of human liver aldehyde reductase

On the basis of GC/MS analysis, the reaction product formed from PGHz was identified as PGE<sub>2</sub>.

**DISCUSSION**

PGE<sub>2</sub> is produced by various tissues including liver (12) and exerts a variety of biological activities (13). However, its biological origin is yet to be elucidated. Two pathways producing PGE<sub>2</sub> were previously reported: 9-ketoreduction of PGE<sub>2</sub> (14, 15), and 9,11-endoperoxide reduction of PGHz (16, 17).

In 1981, Watanabe et al. (18) in our laboratory found PGD 11-ketoreductase, which converted PGD<sub>2</sub> to PGE<sub>2</sub>, in the cytosol fraction of various rat tissues. The highest specific activity was observed in the lung. They further purified this enzyme from bovine lung to homogeneity and characterized it in detail (1, 2). As a result this enzyme was found to catalyze the conversion of PGHz to PGE<sub>2</sub>, and that of PGD<sub>2</sub> to 9α,11β-PGF<sub>2α</sub>, at different active sites on the same molecule, and was later named PGE synthetase (1). The molecular weight and substrate specificity of PGE synthetase are reportedly similar to those of human brain carbonyl reductase (19). On the other hand, Wermuth (19) has reported that carbonyl reductase purified from human brain also reduced PGD<sub>2</sub>, but PGHz 9,11-endoperoxide and PGD<sub>2</sub> reductase activities have not yet been studied. In the present study, we demonstrated that human liver aldehyde reductase, which belongs to the aldotetoreductase family, catalyzed the conversion of PGHz to PGE<sub>2</sub> with NADPH as a cofactor (Figs. 2-4) and that the reduction of PGHz 9,11-endoperoxide proceeded at 30% of the rate observed with NADPH when the same concentration of NADH was used as coenzyme (Table II). Furthermore, we showed that the active site of PGHz reduction was different from that used for the reduction of other aldotetone compounds. Under standard assay conditions containing 80 μM [1-<sup>14</sup>C]PGHz, the formation of [1-<sup>14</sup>C]PGE<sub>2</sub> was not affected by the addition of PGD<sub>2</sub> or PGE<sub>2</sub> up to 1.5 mM (data not shown). Therefore, the 9,11-endoperoxide of PGHz appears to be reduced directly to PGE<sub>2</sub>, but not via PGD<sub>2</sub> or PGE<sub>2</sub>. These findings are in agreement with the results on PGE synthetase (1).

Aldehyde reductase purified from human liver also reduced PGA<sub>2</sub>, PGJ<sub>2</sub>, and Δ<sup>12</sup>-PGJ<sub>2</sub>, but the reduction of PGF<sub>2α</sub>, PGE<sub>2</sub>, or PGD<sub>2</sub> was undetectable up to 1.0-1.5 mM concentration ranges (Table I). These results suggest that the substrate specificity of aldehyde reductase on PG reduction is similar to that of PGE synthetase except for PGD<sub>2</sub>. Although the Km value of aldehyde reductase for PGHz was greater than that of enzymes such as bovine lung PGF synthetase (1), rat brain PGD synthetase (20), bovine vesicular gland PGE synthetase (21), and rabbit aorta PGI synthetase (22), its value was in the same order as that for PGs of human brain (23), rat spleen PG synthetase (24), PGD 11-ketoreductase (25), and PGE 9-ketoreductase (26). The specific activity of aldehyde reductase for PGHz (88 milliunits/mg of protein) was about 1.5-fold higher than that of previously purified PGE synthetase (1). As judged from both the specific activity and the Km value for PGHz, it is possible that aldehyde reductase also, at least in part, contributes to the formation of PGE<sub>2α</sub> in human liver.

The enzymes responsible for the formation of PGE<sub>2α</sub> from PGHz have been demonstrated in various tissues (16, 17). Several isozymes of glutathione S-transferase purified from rat liver catalyzes the direct reduction of PGHz to PGE<sub>2α</sub> with reduced glutathione as a specific electron donor (26-28). On the other hand, Wong (25) reported that PGD<sub>2</sub> 11-ketoreductase purified from rabbit liver catalyzed the conversion of

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**Fig. 4. Identification by GC/MS of the reaction product formed from PGHz.** Preparation and derivatization were performed as described under "Experimental Procedures." The derivatized authentic PGE<sub>2α</sub> (A) and reaction product (B) were subjected to GC (1% SE-30, 2 m x 3 mm, 250 °C). The peaks with a retention time of 3.5 min was analyzed by MS. Operation conditions were as follows: ion source temperature, 270 °C; ionization voltage, 70 eV; and ionization current, 200 μA.

mentioned above are metabolized at different active site(s).

**Identification by GC/MS of the Reaction Product Formed from PGHz.**—The enzymatic product formed from PGHz was initially converted to a methyl ester, sequentially treated with a silylating reagent, and then subjected to GC/MS. The derivatized compound appeared as a single peak on GC with a retention time of 3.5 min and gave a mass spectrum (Fig. 4B) essentially identical to that obtained with the corresponding derivative of authentic PGE<sub>2α</sub> (Fig. 4A). Ions were observed at the following m/z: 584 (M<sup>+</sup>); 569 (M<sup>+</sup> - 15), loss of -CH<sub>2</sub> = CH; 513 (M<sup>+</sup> - 71), loss of -(CH<sub>2</sub>)<sub>2</sub>CH; 494 (M<sup>+</sup> - 90), loss of TMSOH; 423 [(M<sup>+</sup> - (71 + 90)], combined loss of -(CH<sub>2</sub>)<sub>2</sub>CH and TMSOH; 404 (M<sup>+</sup> - 2 x 90), loss of 2TMSOH; 353 [(M<sup>+</sup> - (71 + 2 x 90)], combined loss of -(CH<sub>2</sub>)<sub>2</sub>CH and 2TMSOH.
PGD$_2$ to PGE$_2$. Recently, PGD 11-ketoreductase activity has also been found in human liver, and the enzymatic product has been identified as 9α,11β-PGF$_2$, which is a stereoisomer of PGE$_2$ (29). Therefore, the formation of PGE$_2$, and 9α,11β-PGF$_2$, is catalyzed by different enzymes, for example, aldehyde reductase, glutathione S-transferase, and PGD 11-ketoreductase.

Recently the primary structure of PGF synthetase has been established. Watanabe et al. (3) found 62% similarity in the amino acid sequences between PGF synthetase and human liver aldose reductase, both members of aldoketoreductase family. Moreover, Carper et al. (30) also discovered that rat lens aldose reductase has 50% identity with human liver aldose reductase. Thus, the sequence information gives a better understanding of structural relationship among the functionally similar members of the aldoketoreductase family.

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