FORMATION OF PROSTAGLANDIN E_1 FROM 15-KETOPROSTAGLANDIN E_1 BY GUINEA PIG LUNG 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE

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Summary —— Partially purified NAD^+-linked 15-hydroxyprostaglandin dehydrogenase from guinea pig lung was found to catalyze the backward reaction, i.e. reduction of 15-ketoprostaglandin E_1 to physiologically active prostaglandin E_1. The backward reaction was dependent on the presence of NADH and showed an optimum pH at 5.5 while the optimum pH of the forward reaction was 8.8 or higher. The apparent Km for 15-keto-PGE_1 was 52 μM while that for PGE_1 was 10 μM. The forward and backward reaction rates were lowered to the same extents by partial heat inactivation of the enzyme or by the addition of p-chloromercuribenzoate.

The first step in the metabolism of prostaglandin E and F compounds is the oxidation of the secondary alcohol group at C-15 to ketone (1, 2). This reaction, catalyzed by 15-hydroxyprostaglandin dehydrogenase, yields a product having greatly reduced biological activity (3). 15-Hydroxyprostaglandin dehydrogenase was partially purified from various tissues (4, 5) and some of its kinetic properties were analyzed. However, the reversibility of this enzyme reaction still remains to be clarified (5-7).

The present communication reports the backward reaction by partially purified NAD^+-linked 15-hydroxyprostaglandin dehydrogenase from guinea pig lung. The backward reaction proceeded at a comparable rate to the forward reaction when followed by measuring the formation of [^{3}H]PGE_1 from 15-keto-[^{3}H]PGE_1. Some of the properties of the backward reaction were analyzed and compared with those of the forward reaction.

MATERIALS AND METHODS

[5, 6-^{3}H]PGE_1 was purchased from New England Nuclear (Boston, Mass.), and unlabeled PGE_1 was from Fuji Chemical Industries (Tokyo, Japan). 15-Keto-[^{3}H]PGE_1 was prepared by incubation of [^{3}H]PGE_1 with partially purified swine lung 15-hydroxyprostaglandin dehydrogenase. The swine lung 15-hydroxyprostaglandin dehydrogenase was purified essentially according to the method of Änggård and Samuelsson (4). Precoated silica gel glass plates 60 for thin-layer chromatography were obtained from E. Merck, (Darmstadt, Germany).

Measurement of Enzymatic Activity. 15-Hydroxyprostaglandin dehydrogenase (forward reaction) was assayed as follows: The standard assay mixture I contained 30 μM PGE_1, 1.2 mM NAD^+, 80 mM phosphate buffer (pH 7.3), and the enzyme in a total volume of 0.50 ml. Incubation was performed at 37° for 20 min and terminated by dipping the tubes in ice-water. The amount of 15-keto-PGE_1 formed was determined from the absorption at 500 nm after the addition of 30 μl of 2 N NaOH (4). Alternatively, the formation of NADH was followed by measuring the absorption at 340 nm.

The backward reaction of 15-hydroxyprostaglandin dehydrogenase was assayed by measuring the formation of [^{3}H]PGE_1 from 15-keto-[^{3}H]PGE_1. The standard assay mixture II contained 30 μM 15-keto-[^{3}H]PGE_1 (0.3 μCi), 1 mM NADH, 100 mM McIlvaine buffer (pH 5.5), and the enzyme in a total volume of 0.50 ml. The reaction was performed at 37° for 20 min and terminated by the addition of 0.5 ml of 0.1 M citric acid. The mixture was then extracted with ethyl ether. The solvent was evaporated under reduced pressure and the residue was dissolved in a small volume of acetone. This solution was applied onto a silica gel plate and developed with the solvent system, ethyl acetate-acetone-acetic acid (90:10:1) (8). The radioactivity on the silica gel plate was monitored with a radio-chromatogram scanner. Then, the silica gel was scraped off and counted for radioactivity in toluene-ethanol (50 : 50) scintillation fluid with a Packard liquid scintillation spectrometer.

The enzymatic activity (unit) is expressed as nanomoles of product generated per minute under the assay conditions described above. Protein was determined by the method of Lowry et al (9).

Enzyme Purification. Guinea pig lungs (20 g) were homogenized in a Waring blendor for 2 min with 80 ml of 0.1 M phosphate buffer (pH 7.3) containing 1 mM dithiothreitol. The homogenate was centrifuged at 10,000 g for 20 min, and the supernatant solution was further centrifuged at 105,000 g for 60 min. Solid ammonium sulfate was added to the supernatant fluid with stirring to 20% saturation. The precipitate was removed by centrifugation, and the supernatant solution was then brought to 40% saturation of ammonium sulfate and allowed to stand for 60 min. The precipitate was collected by centrifugation and was dissolved in 0.01 M phosphate buffer (pH 7.3) containing 0.1 mM dithiothreitol. This solution was passed through a Sephadex G-25 column (5 x 35 cm) that had been equilibrated with the same buffer. The fractions containing the enzyme activity were combined and applied onto a TEAE-cellulose column (4 x 17 cm) that had been equilibrated with 0.01 M phosphate buffer (pH 7.3) containing 0.1 mM dithiothreitol. The column was washed with 0.01 M phosphate buffer (pH 7.3) containing 0.2 M NaCl and 0.1 mM dithiothreitol. Then, the enzyme was eluted with 0.01 M phosphate buffer (pH 7.3) containing 0.4 M NaCl and 0.1 mM dithiothreitol. The active fractions were pooled

and concentrated to about 6 ml by ultrafiltration (TEAE-cellulose enzyme). When necessary, this preparation was further purified by gel filtration on a Sephadex G-100 column (4 x 85 cm) that had been equilibrated with 0.01 M phosphate buffer (pH 7.3) containing 0.4 M NaCl and 0.1 mM dithiothreitol. The enzyme preparations were stored at -70°. For most of the experiments the TEAE-cellulose enzyme was used. This preparation was free of prostaglandin Δ^{13} reductase and prostaglandin E 9-ketoreductase.

RESULTS AND DISCUSSION

When 15-keto-[^{3}H]PGE_1 was incubated with the partially

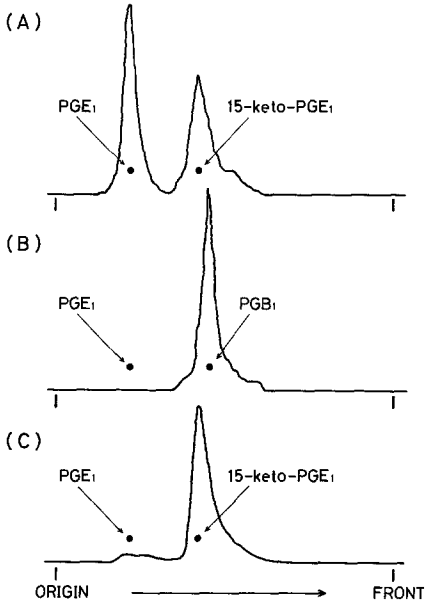

Fig. 1. Thin-layer chromatogram of the product derived from 15-keto-PGE_1 on incubation with 15-hydroxyprostaglandin dehydrogenase in the presence of NADH. (A) 15-Keto-[^{3}H]PGE_1 in the standard assay mixture II was incubated with the TEAE-cellulose enzyme (0.18 mg) at 37° for 20 min. Extraction and thin-layer chromatography were carried out as described in the text. (B) The silica gel of PGE_1 region in the experiment A was scraped off and extracted with methanol. The solvent was evaporated and then the residue was heated at 100° for 5 min in 0.02 N NaOH. The treated sample was subjected to thin-layer chromatography as above. (C) The silica gel of PGE_1 region in the experiment A was scraped off and extracted with methanol. The solvent was evaporated and the residue obtained was incubated with 15-hydroxyprostaglandin dehydrogenase and NAD^+ under the standard assay conditions I for 90 min. Thin-layer chromatography was carried out as above. The points indicate the positions where reference standards were located.

purified 15-hydroxyprostaglandin dehydrogenase preparation in the presence of NADH, the product was located at the position of PGE_1 on thin-layer chromatography (Fig. 1A). This product was converted to PGB_1 upon treatment with alkali (Fig. 1B) or to 15-keto-PGE_1 on incubation with the above 15-hydroxyprostaglandin dehydrogenase preparation and NAD^+ (Fig. 1C). These facts indicate the identity of the product with PGE_1. PGE_1 produced from 15-keto-PGE_1 obviously possesses 15(S)-hydroxy group because 15-hydroxyprostaglandin dehydrogenase is stereospecific with regard to the configuration at C-15 (10). Formation of PGE_1 as the product of the backward reaction of 15-hydroxyprostaglandin dehydrogenase was also confirmed by reversed phase partition chromatography (Fig. 2). The time course of the conversion of 15-keto-PGE_1 to PGE_1 is shown in Fig. 3. More than 80% of 15-keto-PGE_1 was converted to PGE_1 after the incubation for 180 min under the conditions employed.

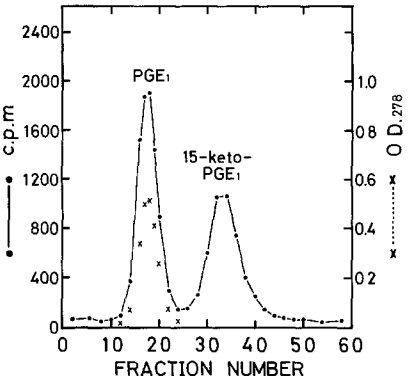

Fig. 2. Reversed phase partition chromatogram of the product derived from 15-keto-PGE_1 on incubation with 15-hydroxyprostaglandin dehydrogenase in the presence of NADH. Incubation conditions and extraction procedure were the same as Fig. 1A. The column consisted of 4.5 g of hydrophobic Super-Cel with the solvent system of C-47 supplemented with acetic acid. The moving phase was methanol-water-acetic acid (141 : 159 : 2) and the stationary phase was chloroform-isooctanol (15 : 15) (11). Fraction volume, 8 ml. The unlabeled reference PGE_1 was detected by measuring the absorption at 278 nm after treatment with alcoholic 0.5 N KOH.

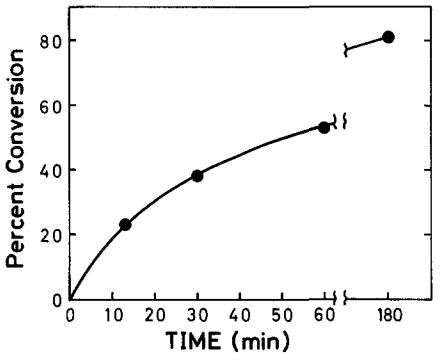

Fig. 3. Conversion of 15-keto-PGE_1 to PGE_1 by the backward reaction of 15-hydroxyprostaglandin dehydrogenase. Incubation conditions were the same as Fig. 1A except that 0.09 mg of the enzyme was used.

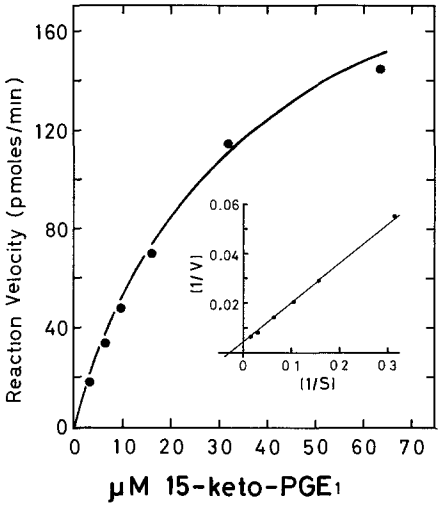

Fig. 4. Effect of 15-keto-PGE_1 concentration on the backward reaction of 15-hydroxyprostaglandin dehydrogenase. The standard assay conditions II were used except that 15-keto-PGE_1 concentrations were varied as indicated.

The backward reaction of 15-hydroxyprostaglandin dehydrogenase was dependent on the presence of NADH. NADPH was ineffective. Therefore, the reaction is expressed as follows: $PGE_1 + NAD^+ \rightleftharpoons$ 15-keto-PGE_1 + NADH + H^+. The backward reaction

was inhibited 43% by 4 mM NAD^+ but not by 4 mM $NADP^+$ while the forward reaction was inhibited 70% by 1 mM NADH under the standard assay conditions. The apparent Km value for 15-keto-PGE_1 was calculated to be 52 μM from the double reciprocal plots of Lineweaver and Burk (Fig. 4). On the other hand, the apparent Km value for PGE_1 was 10 μM under the standard assay conditions. The optimum pH for the backward reaction was 5.5 while that for the forward reaction was 8.8 or higher (Fig. 5).

The specific activities of the enzyme preparation obtained by Sephadex G-100 gel filtration were 36 and 14 units per mg of protein for the forward and backward reaction, respectively, under the standard assay conditions. Partial heat denaturation of the enzyme as well as the addition of p-chloromercuribenzoate decreased the forward and backward reaction activities to the same extents, suggesting that the single enzyme is responsible for both reactions (Table I).

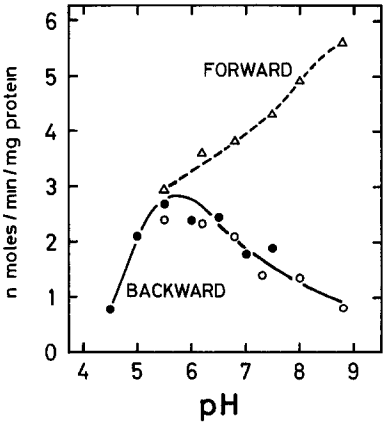

Fig. 5. Effect of pH on the forward and backward reaction of 15-hydroxyprostaglandin dehydrogenase. The standard assay conditions were used except that the following buffers were employed: 0.1 M phosphate buffer (o, △) and 0.1 M McIlvaine buffer (●). The backward reaction (——) and the forward reaction (△---△).

Table I. Effects of partial heat inactivation and p-chloromercuribenzoate on the forward and backward reaction of 15-hydroxyprostaglandin dehydrogenase

	Remaining activity	
	Forward reaction	Backward reaction
Heat treatment [a]		
0 min at 50°	100%	100%
5	43	37
10	23	20
15	16	14
p-Chloromercuribenzoate [b]		
0 μM	100%	100%
6	33	41
60	0	0

a/ The enzyme (TEAE-cellulose enzyme, 1.4 mg) was heated at 50° in 1.0 ml of 0.05 M potassium phosphate buffer (pH 7.3). At indicated times, aliquots (50 μl and 100 μl) were withdrawn and assayed immediately for the forward and backward reaction, respectively, under the standard assay conditions.

b/ p-Chloromercuribenzoate was added into the standard assay mixture.

The backward reaction was also observed with the partially purified preparation of swine lung 15-hydroxyprostaglandin dehydrogenase.

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